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RESPONSES OF RED NILE TILAPIA (*Oreochromis niloticus L.*) SUBJECTED TO SOCIAL AND CONFINEMENT STRESSES

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ABSTRACT

Red tilapia is gaining popularity nowadays in the market but only limited studies on its behavioral and physiological response to stress are available. In this study, 27 red Nile tilapia (RNT) were subjected to confinement and social stresses. This experiment monitored their physiological (plasma cortisol) and morphological (ventilation rate [VR] and skin color) responses to stress. The plasma cortisol and VR of the fish before exposure to stressors were significantly lower compared to their values after the introduction of stressor. For fish subjected to social stress, there was also a significantly higher cortisol level and VR in the subordinate fish compared to those in the dominant fish. The skin color of red tilapia was also intensified when the fish was subjected to confinement and social stresses. Higher plasma cortisol levels, faster VRs, and darker skin color were exhibited more by fish subjected to social stress compared to fish subjected to confinement stress. There was also positive correlation between mean plasma cortisol concentration and mean VR (r=0.945; $P<0.05$).

Keywords: *Confinement stress, plasma cortisol, Red Nile tilapia, skin color, social stress, ventilation rate*

INTRODUCTION

Red tilapia, *Oreochromis* spp., is becoming popular for its enticing color which leads to higher marketability. Its high salinity tolerance makes it possible to be raised in brackish water and even in seawater (Watanabe et al. 1990). Well-pigmented red tilapia produced in saline water could also achieve higher prices than other farmed tilapia in local markets (i.e. restaurants) due to its physical resemblance to some high-valued marine species (Thodesen et al. 2013). Although red tilapia may not be popular in in Chinese aquaculture today, domestic demand in China for this tilapia variety may increase in the future. Red is traditionally a favorable color in Chinese culture because it represents beauty, reunion, good fortune, happiness, success, and harmony.

Since red tilapia has a big potential market, it is important to establish good management practices to ensure good quality and quantity production. Stress is the response or complex adaptive reactions of fish to cope with stressors (Barton 2002). When fish are stressed, it results to the stimulation of the hypothalamus, which activates the neuroendocrine system and therefore, a subsequent cascade of metabolic and physiological changes happen (Wedemeyer 1990). Determining stress in fish has been reported in several ways such as elevation of plasma cortisol (Corrêa 2003) and changes in ventilation rate and skin color (Vera Cruz and Brown 2007).

Physiological, behavioral, and morphological responses due to stress are important in considering the maintenance of homeostatic condition in fish well-being. In this study, effects of different stressors such as confinement and social stressors on the plasma cortisol level and morphological/behavioral characteristics (ventilation rate and skin color) of red Nile tilapia (*Oreochromis niloticus*) were observed. The stimuli used were confinement (Volpato and Barreto 2001) and social stressors (Corrêa et al. 2003) since they are well-recognized potent stressors to fish.

MATERIALS AND METHODS

Experimental Fish

The fish stock population was 90 adult red Nile tilapia (RNT) males which were acclimated for 10 days in concrete indoor tanks supplied with well-aerated tap water before the start of the experiment. The RNT was considered a different breed of red tilapia since it was purely developed from Nile Tilapia. The source of fish was from Philfish Gen, Freshwater Aquaculture Center (FAC), Central Luzon State University (CLSU), Science City of Muñoz, Nueva Ecija, Philippines. It was first transferred to Swansea, United Kingdom, then eventually to FAC, CLSU. Feeding rate was 4% of their body weight using pellet feed (Feedmix Nutrition Specialist Inc.®) containing 50% crude protein, 6% crude fat, and 7% crude fiber. Feeding was administered twice: one in the morning (0800 h) and another in the afternoon (1400 h).

Experimental Compartment

There were eighteen units of rectangular aquaria used throughout the experiment. All glass aquaria have the same size that measures 30 x 15 x 30 cm. Three sides of each isolation aquarium were covered with white paper to avoid the isolated fish from seeing other fish during the isolation period.

Confinement Stressor

Confinement was performed by displacing the fish to a small side of the aquarium by means of an opaque partition, allowing the fish to move only in the 25% of the total area of the aquarium for 60-90 minutes.

Social Stressor

Social stress was provided by pairing the focal fish with a larger resident fish. Each resident fish was larger than the respective focal animal because larger body size is also strongly associated with dominance on fish. The procedure guaranteed a clear dominance-subordinance relationship, thus characterizing a typical situation of social stress to the focal fish (Volpato and Fernandes 1994).

Experimental Design

For the confinement experiment, nine RNT weighing 45-100 grams were randomly selected and used. On the other hand, 18 RNT were also used for social experiment. Dominant fish which weighed 50.0-90.6 grams were used as social stressors and were selected to be heavier than the subordinate fish, which weighed 45-81.2 grams. Isolation was imposed 10 days before the test. The fish were fed daily except during blood collection days. In each experiment, physiological (plasma cortisol) and morphological (ventilation rate [VR] and skin color) changes were monitored. The pre-stressor measurement of plasma cortisol was performed 5 days after the beginning of isolation. Five days later, the nine fish in the confinement stress experiment were divided in three groups and the three fish in each group were exposed individually to confinement stressors for 60 minutes (T60) before blood collection, or 90 minutes (T90) before blood collection, or were only observed for their physical changes during stress period and there was no blood collection. The morphological changes (VR and skin color) were monitored 4 minutes and 2 minutes before introduction of stressors (pre-stress): immediately after introduction of stressor and 2, 4, 6, 8, 10, 30, 60, and 90 minutes later. In the social stress experiment, the same procedure was employed but the fish were exposed to social interaction.

Ventilation rate (VR) was measured visually by counting 20 opercular or buccal movements and concomitantly registering the time elapsed. Skin color of fish was monitored by taking pictures and recording videos using Canon EOS-D60. All the morphological changes were observed visually about 1 meter away from the fish. These observations continued for 4 days.

Plasma Cortisol Isolation

Fish sample was sedated using methanetricane sulfonate (MS-222). The anesthetic was diluted in water with the concentration of 150-200 mg/L for rapid anesthesia. Blood was collected through intra-cardiac route in the gill cavity. The blood was transferred into EDTA tubes to avoid coagulation. It was centrifuged in a 12000 rpm with a temperature of 4°C for plasma isolation. Extracted plasma from blood was transferred into new tubes for ELISA reading. Quantitative determination of cortisol concentration was done by using the protocol of the Adaltis EIAgen cortisol kit (Adaltis®, Milano, Italy).

Statistical Analyses

The correlation between plasma cortisol level and ECP was assessed using Pearson correlation coefficient. Means were compared using T-test. Analyses were carried out using Predictive Analysis Software version 18.

RESULTS AND DISCUSSION

Plasma Cortisol

Elevation in plasma cortisol is the most widely used indicator of stress in fish (Bonga 1997). In this study, plasma cortisol levels were quantified using competitive ELISA. Blood collection for plasma isolation was done before the introduction of stressor (pre-stress) and after the introduction of stressor (post-stress [i.e. T60 and T90]). Plasma cortisol value during pre-stress period was lesser as expected compared to its amount after the fish were subjected to stressors.

In fish subjected to confinement stressor, mean plasma cortisol value for pre-stress period were 22.60±21.72 ng/ml for T60 and 15.75±6.23 ng/ml for T90. It was observed that after exposing the fish to confinement stressor, its mean plasma cortisol value significantly (*P*<0.01) increased to 50.62±50.39 ng/ml after 60 minutes and 42.58±20.78 ng/ml after 90 minutes (post-stressor).

Many studies have proven the effectiveness of determining the cortisol level to detect stress in fish. In the study of Vijayan et al. (1997), confinement of Mozambique tilapia (*Oreochromis mossambicus*) for 2 or 24 hours resulted in significantly higher plasma cortisol. Breves et al. (2010) also have the same results when they studied the ionoregulatory, endocrine responses and cortisol elevation of Mozambique tilapia in disturbed salt and water balance exposed to confinement and handling stresses.

Higher cortisol levels also resulted when there is a social interaction among fishes. In fish subjected to social stress, it was seen that plasma cortisol levels increased also after the introduction of stressor. Both the subordinate and dominant fish had increased cortisol levels but greater increase was observed in the subordinate ones. The mean cortisol level in subordinate fish during the post-stress (170.89±78.91 ng/ml; mean of T60 and T90) period was significantly higher (*P*<0.01) compared to that during pre-stress (47.88±43.90 ng/ml; mean of T60 and T90) period. There was also a significantly higher (*P*<0.05) mean cortisol levels in the subordinate (195.87±98.36 ng/ml for T60; 145.92±76.67 ng/ml for T90) than in the dominant (67.96±34.26 ng/ml for T60; 74.96±55.52 ng/ml for T90) fish after the establishment of social hierarchy.

Subordinate fish have greater cortisol levels due to their fighting encounters with the dominant fish (Barcellos et. al. 1999). High values of plasma cortisol have always been attributed to the severity and duration of the stressor (Barton and Schreck 1987). In the study of Barreto and Volpato (2006), cortisol concentration in Nile tilapia subjected to social stressors increased from 30-50ng/ml (pre-stress period) to 120-200ng/ml (post-stress period).

Ventilation Rate

It was observed that VR changes abruptly as soon as the fish were stressed. A gradual decline in the VR was observed and may be due to the adaptation of the fish to the stressful condition. It was proven by Øverli et al. (2004) that coping techniques are present on teleost fishes, and these coping characteristics influence both social rank and levels of aggression. There was only a slight difference on VR values in the T60, T90, and no blood collection treatments.

Before the introduction of confinement stressor, the red Nile tilapia has an average VR of 1.29 beats/sec. It increased rapidly to 1.47 beats/sec just after the stressor was introduced. Increased ventilatory activity is commonly used as a sign of stress and poor welfare in fish. It is one of the rapid morphological responses of fish due to problems in water oxygenation and other environmental changes (Martins et al. 2012). The VR rate increases up to a certain critical low oxygenation level after which it again decreases and the fish suffocate after a while. In contrast, the frequency of ventilation decreases when oxygen is in excess (Xu et al. 2006).

It was also proven that fish were stressed when introduced to an environment with a larger resident fish by observing its VR. In this study, the VR of the smaller fish increased from 1.42 to 1.82 beats/sec in T60. The VR of larger fish also increased a little after 0-4 mins since there were new fish introduced in their environment but not as much as the increase in the VR of the smaller fish. The VR values of the dominant-resident fish also decreased as time goes by (after 8-10 mins, 30 mins, 60 mins, and 90 mins) and were almost the same as the VR recorded before the introduction of the smaller fish, indicating that they were not really stressed since they knew that they were stronger than the small ones. The mean VR of smaller fish (1.73 beats/sec) was significantly (*P*<0.01) higher compared to that of the larger fish (1.41 beats/sec). The same trends on the change in VR of smaller and larger fish were observed in the T90 and no blood collection treatment. Fish tend to increase their VR if they sensed predators in their environment. Predators place considerable stress on their prey both physiologically and psychologically which includes rapid and increasing heartbeat and VRs (Brown et al. 2005).

Skin Color

Questions about the adaptive nature of optical signals and the role that color signals play in the generation and maintenance of biodiversity has been tested by the measurement of animal coloration in many studies (e.g., Gray and McKinnon 2007; Price et al. 2008). Color alteration is controlled by the brain through the pigment called chromatophore (Skold et al. 2012).

In fish subjected to confinement stressors, it was observed that skin color intensified a bit compared to its color during the isolation period. The skin of the fish turned into a redder color since red is the primary body pigment of RNT.

Skin color of the subordinate red tilapia subjected to social stress also became more intense. The skin color of the dominant was paler compared to that of the subordinate (Figure 1). This skin color variation was observed on the third day after the start of social interaction. The same pattern is also seen in other cichlid species including the Nile tilapia when stressed (Beeching 1995).

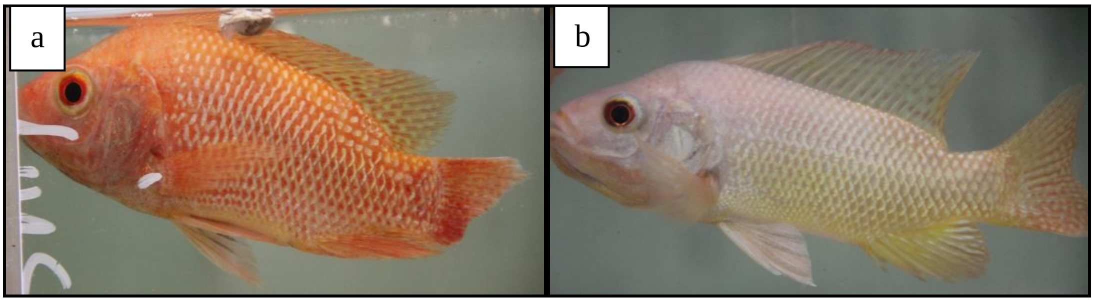

Figure 1. Body color of the (a) subordinate fish and (b) dominant fish

Relationship between Cortisol Concentration, VR, and Skin Color

There was a positive correlation (Figure 2) between mean VR and mean cortisol concentration in T60, T90, and no blood collection (r=0.945; P<0.05). This indicates that as the cortisol concentration increases, the VR also increases. In the study of Barreto and Volpato (2004), VR was recorded to determine the stress in Nile tilapia. Confined fish showed higher VR and plasma cortisol levels. One of the advantages of using VR as stress indicator in fish is it changes quickly in response to the disturbances imposed to the fish.

Ventilation rate and skin color also has positive relationship. As VR increases, skin color also becomes more intense (i.e. red-orange). Dominant fish had lower VR and skin color was pale, while in subordinate fish the skin color was orange which indicates a clear social hierarchy. In the same way, skin color and cortisol concentration had a positive relationship. Subordinate fish with intense red-orange color had higher cortisol concentration compared to that of paler dominant fish.

In both of the pre-stress and post-stress periods, it was deduced that there was no significant difference between the plasma cortisol levels, VRs, and skin color of T60 and T90. This indicates that fish that were exposed to confinement and social stress for 60 minutes has almost the same level of stress to fish which were exposed to stressor for 90 minutes.

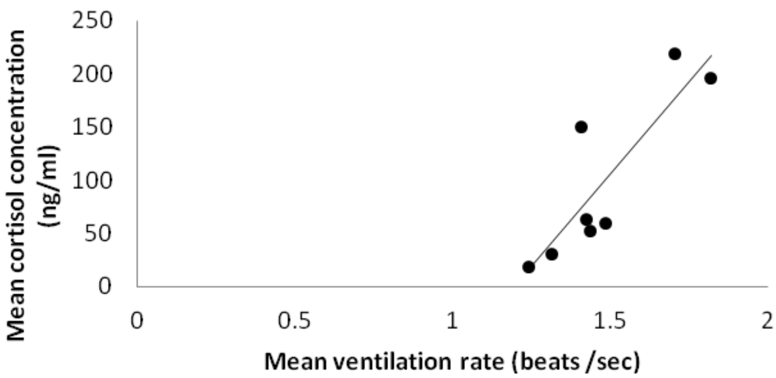

Figure 2. Relationship between mean ventilation rate and mean cortisol concentration (r=0.945; P<0.05)

Higher cortisol levels and VRs were seen on fish that were subjected to social stressor than those that were exposed to confinement stressor. In the same way, RNT subjected to confinement stress had paler skin color compared to that of the fish subjected to social stress. This means that social interaction is more stressful to the fish than confinement condition since it resulted to higher plasma cortisol concentration, faster VRs, and greater intensification of the skin color which is a known response to stress. Subordinate RNT had undergone scale losses, skin lesions, and destruction of fins due to the attacks received from the dominant fish. Amount of these morphological and physiological changes in fish due to stress depends on the severity of stress itself. This only suggests that social interaction is a more stressful condition in fish than the confinement stress.

Since cortisol concentration has a direct relationship with VR and skin color, then it can be concluded that morphological changes in red tilapia are an effective and a cheaper way to determine and quantify stress. Usually, plasma cortisol quantification is more laborious and an expensive way to determine stress.

Considering that redder color of fish attracts more consumers especially Chinese, then it can also be deduced that farmers can stress the fish during conditioning before travelling and selling to consumers. This technique must be studied further for appropriate protocol that the fish must be subjected to stress at a level which is tolerable for their health and con dition.

Seeing that culturing of red tilapia has a big potential in the local and international market, investors might engage themselves into red tilapia farming. This may also lead the farmers to further intensify the culture of this species. When that happens, it would be a great help in maintaining food security globally. It may also encourage people to eat and appreciate red tilapia meat considering its good texture, flavor, aroma, and high nutrition content. Since it is cheaper than other meats, it may serve as the primary source of protein especially in poor countries.

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REFERENCES

Barcellos, L.J.G., S. Nicolaiewsky, S.M.G. De Souza, and F. Lulhier. 1999. “Plasmatic Levels of Cortisol in the Response to Acute Stress in Nile Tilapia, *Oreochromis niloticus* (L.), Previously Exposed to Chronic Stress.” *Aquaculture Research* 30:437-444.

Barton, B.A. 2002. “Stress in Fishes: A Diversity of Responses with Particular Reference to Changes in Circulating Corticosteroids.” *Integrated and Comparative Biology* 42:517-525.

Barton, B.A. and C.B. Schreck. 1987. “Influence of Acclimation Temperature on Interrenal and Carbohydrate Stress Response in Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*).” *Aquaculture* 62:299-310.

Barreto, R.E. and G. L. Volpato. 2004. “Caution for Using Ventilatory Frequency as an Indicator of Stress in Fish.” *Behavioural Processes* 66:43-51.

Barreto, R.E. and G.L.. 2006. “Stress Responses of the Fish Nile Tilapia Subjected to Electroshock and Social Stressors.” *Brazil Journal of Medical and Biological Research* 39:1605-1612.

Beeching, S.C. 1995. “Colour Pattern and Inhibition of Aggression in the Cichlid Fish *Astronotus ocellatus*.” Journal of Fish Biology 47:50-58.

Bonga, S.E.W. 1997. “The Stress Response in Fish.” *Physiology Review* 77:591-625.

Breves J.P., T. Hirano, and E.G. Grau. 2010. “Ionoregulatory and Endocrine Responses to Disturbed Salt and Water Balance in Mozambique Tilapia Exposed to Confinement and Handling Stress.” *Comparative Biochemistry and Physiology A* 155: 294-300.

Brown, C., C. Gardner, and V.A. Braithwaite. 2005. Differential Stress Responses in Fish from Areas of High- and Low-predation Pressure.” *Journal of Comparative Physiology B* 175:305-312.

Corrêa, S.A., M.O. Fernandes, and K.K. Iseki. 2003. “Effect of the Establishment of Dominance Relationships on Cortisol and Other Metabolic Parameters in Nile Tilapia (*Oreochromis niloticus*).” *Brazilian Journal of Medical and Biological Research.* 36:1725-1731.

Gray, S.M. and Mckinnon, J.S. 2007. “Linking Color Polymorphism Maintenance and Speciation.” *Trends in Ecology and Evolution* 22(2):71-79.

Martins, C.I.M., L. Galhardo, C. Noble, B. Damsgård, M.T. Spedicato, W. Zupa, M. Beauchaud, E. Kulczykowska, J.C. Massabuau, T. Carter, S.R. Planellas, and T. Kristiansen. 2012. “Behavioural Indicators of Welfare in Farmed Fish.” *Fish Physiology and Biochemistry* 38(1):17-41.

Øverli Ø., W.J Korzan, E. Höglund, S. Winberg, H. Bollig, M. Watt, G.L. Forster, B.A. Barton, E. Øverli, K.J. Renner, and C.H. Summers. 2004. “Stress Coping Style Predicts Aggression and Social Dominance in Rainbow Trout.” *Hormones and Behavior* 45:235-241.

Price, A.C., C.J. Weadick, J. Shim, and F.H. Rodd. 2008. “Pigments, Patterns, and Fish Behavior.” *Zebrafish* 5(4):297-307.

Skold, H.N., S. Aspengren, and M. Wallin. 2012. "Rapid Color Change in Fish and Amphibians–Function, Regulation, and Emerging Applications." *Pigment Cell Melanoma Research* 26:29-38.

Thodesen, J., M. Rye, Y.X. Wang, S.J. Li, H.B. Bentsen, M.H. Yazdi, and T. Gjedrem. 2013. Genetic Improvement of Tilapias in China: Genetic Parameters and Selection Responses in Growth, Survival, and External Color Traits of Red Tilapia (*Oreochromis spp.*) after Four Generations of Multi-trait Selection." *Aquaculture* 416 (417):354-366.

Vera Cruz, E.M. and C.L. Brown. 2007. "The Influence of Social Status on the Rate of Growth, Eye Color Pattern, and Insulin-like Growth Factor-I Gene Expression in Nile Tilapia, *Oreochromis niloticus*." *Hormones and Behavior* 51:611-619.

Vijayan, M.M., C. Pereira, E.G. Grau, and G.K. Iwama. 1997. Metabolic Responses Associated with Confinement Stress in Tilapia: The Role of Cortisol." *Comparative Biochemistry and Physiology C* 116:89-95.

Volpato, G.L. and M.O. Fernandes. 1994. "Social Control of Growth in Fish." *Brazilian Journal of Medical and Biological Research* 27:797-810.

Volpato, G.L. and R.E. Barreto. 2001. "Environmental Blue Light Prevents Stress in the Fish Nile Tilapia." *Brazilian Journal of Medical and Biological Research* 34:1041-1045.

Watanabe, W.O., L.J. Ellingson, B.L. Olla, D.H. Ernst, and R.I. Wicklund. 1990. "Salinity Tolerance and Seawater Survival Vary Ontogenetically in Florida Red Tilapia." *Aquaculture* 87:311-321.

Wedemeyer, G.A., B.A. Barton, and D.J. Mcleay. 1990. "Stress and Acclimation." In *Methods for Fish Biology*, edited by C.B. Schreck and P.B. Moyle, 451-489. Bethesda, Maryland: American Fisheries Society.

Xu, J., Y. Liu, S. Cui, and X. Miao. 2006. "Behavioral Responses of Tilapia (*Oreochromis niloticus*) to Acute Fluctuations in Dissolved Oxygen Levels as Monitored by Computer Vision." *Aquacultural Engineering* 35:207-217.