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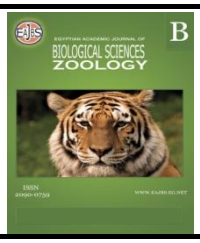

Stress Responses and Gonadosomatic Index of Red Tilapia (*Oreochromis* Spp.) Subjected To Visual And Physical Interactions

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ABSTRACT

Red tilapia is a popular cultured species due to its attractive color and excellent growth and feed conversion rates. In this study, the effect of visual and physical interactions on the ventilation rate (VR), eye color pattern (ECP), and Gonadosomatic Index (GSI) of red tilapia was evaluated. There were four treatments in the study: T1 (No Interaction); T2 (Visual Interaction); T3 (Visual and Physical Interactions) and; T4 (Physical Interaction). Changes in VR and ECP were monitored for 15 days, while GSI was computed at the end of the study. The mean VR and ECP of the red tilapia exposed to visual and/or physical interactions were significantly higher compared to those in the control ($P<0.05$). However, the fastest VR and darkest ECP were observed in T3. This conforms to the results of GSI wherein T3 had the lowest GSI which may be due to the higher level of stress experienced by fish in T3 compared to other treatments. On the other hand, T4 had the highest female GSI as opposite sex fish were exposed to physical interaction. Communication between sexual partners modulates reproductive behavior.

INTRODUCTION

Red tilapia (*Oreochromis* spp.) is one of the aquaculture's most adaptive species. It is abundantly found in the wild and known to be cultured in several parts of the world (Rahman *et al.,* 2013). It has excellent growth and feed conversion rates in freshwater and has become interesting to aquaculturists due to its resemblance to some marine high-valued species (Liao & Chen, 1983).

Stress is an inherent component of the life of all vertebrates, including fishes. 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). Social interaction is stressful and subordinate individuals are often subjected to chronic stress, which greatly affects both their behavior and physiology (Backström & Winberg, 2017). It has been documented also that stressful conditions brought about by social ranking might affect the quality of reproduction in fish. Since reproduction is crucial in all animals, and rank typically regulates access to reproductive opportunities, understanding the mechanisms that regulate socially-induced reproductive processes is extremely important (Maruska, 2014). The influence of social interactions on the stress response in fishes has received much recent

interest (Gilmour, *et al.,* 2005). Vision is well developed in the cichlid fish species and visual communication is an important part of their reproductive interaction (Castro *et al.,* 2009).

Gonadosomatic index (GSI) which is an index of gonad size relative to fish size is a good indicator of gonadal development in fish. The percentage of body weight of fish that is used for the production of eggs is determined by the GSI (Khan *et al.,* 2013). The analysis of GSI values, which provide a measure of gonad size relative to body weight (Wootton, 1990), can provide a quantitative assessment of the degree of gonadal development, the breeding season, and the reproductive cycle (Gutiérrez-Estrada *et al.,* 2000).

In this study, the effects of social interaction and visual communication in the GSI and behavioral responses (ventilation rate and eye color pattern) of red tilapia were determined. This information will contribute to the possible effect of visual communication and social interactions in the reproduction of red tilapia.

MATERIALS AND METHODS

Experimental Fish:

Eighty (80) male and female red tilapia (*Oreochromis* spp.) weighing 100-120 grams were obtained from the Freshwater Aquaculture Center (FAC), Central Luzon State University, Science City of Muñoz, Nueva Ecija. The fish were acclimated to the rectangular concrete tank with a continuous flow of water for 7 days. The water level of the tank was maintained at 0.5m. The fish were fed twice a day at 3% of the fish body weight. After the acclimation period, forty-eight (48) fish were selected for use in the study. The fish were relatively similar by weight and length so that there were no phenotypic variations that may affect the GSI of the fish.

Experimental Treatments:

The experimental treatments in this study are shown in Table 1. Every treatment had three replicates. In Treatment 1, red tilapia (1 male and 1 female) were isolated. In Treatment 2, two fish (1 male and 1 female) were stocked in an aquarium and the two fish were separated by a glass divider. Visual Interaction was observed in this treatment. In Treatment 3, four fish (2 males and 2 females) were stocked in an aquarium with a glass divider separating males among females. This treatment was considered as visual and physical interactions. In Treatment 4, two-male and two-female tilapia were stocked in an aquarium. Physical interaction was the purpose of this treatment. Among the treatments, Treatment 1 was the control where there was complete isolation among others so that there was no physical or visual interaction. The ventilation rate and eye color pattern were monitored at 8:00am- 9:00am daily and GSI was computed after the 15 days observation period.

Table 1. Experimental treatments.

TREATMENT	DESCRIPTION
1 (Control)	No Interaction (male & female in 2 separate aquaria)
2	Visual Interaction (male & female in an aquarium separated by a glass divider)
3	Visual & Physical Interactions (2 males & 2 females in an aquarium; males separated from females by a glass divider)
4	Physical Interaction (2 males & 2 females in an aquarium)

Eye Color Pattern (ECP):

The ECP was quantified as a darkened area of both iris and sclera. The circular area of the eye was divided into 8 equal parts using 4 imaginary diameter lines (see Fig. 1). The ECP values observed ranged from zero (no darkening) to eight (total darkening) (adopted from Volpato et *al.*, 2003)

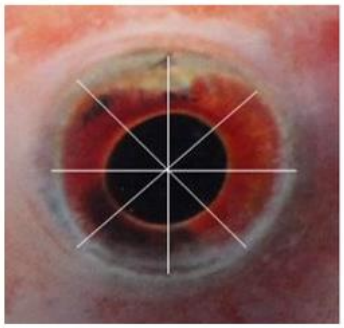

Fig. 1. Eye color pattern (ECP) of red tilapia with eye divided into eight equal parts using four imaginary lines

Ventilation Rate (VR):

The VR was visually estimated by counting the time (i.e. seconds) for 20 successive opercular or buccal movements (adopted from Alvarenga & Volpato, 1995). It was measured three times per fish in a period of 3 consecutive minutes and the average was computed.

Gonadosomatic Index (GSI):

The gonadosomatic index is an indicator of the state of gonadal development. Bodyweight and total gonad weights of the fish were measured. The GSI value was computed by following the formula by Islam *et al.* (2012) (Equation 1).

$$\text{GSI } (\%) = \left(\frac{\text{Gonad weight}}{\text{Body weight}}\right) \times 100 \qquad (1)$$

Statistical Analysis:

Data were analyzed using one-way Analysis of Variance and when significant differences among treatments were found ($P<0.05$), the mean values were compared using Duncan's Multiple Range Test. The statistical analysis was performed using SPSS Version 16 for windows.

RESULTS AND DISCUSSION

Ventilation Rate (VR):

Significantly higher mean VRs were observed in T2, T3, and T4 compared to those in T1, from Day 4 until the end of the study (Table 2). According to Volpato *et al.* (2003), VR is a very sensitive parameter because a rapid visual perception of a predator was sufficient to evoke a sudden VR increase. Furthermore, during the same period of the experiment, T3 had a significantly higher mean VRs compared to those in T2 and T4 indicating that fish in T3 were still experiencing stress, and the increasing VR values with time shows that the fish continued to respond to the stressor. VR is proven to be an indicator of stress in fish since it changes quickly in response to any disturbances imposed (Volpato *et al.*, 1989).

The highest mean VR recorded in the whole study was monitored at T3 which is 1.55±0.08 beats/sec noted on 14th day. According to Barreto *et al.* (2004), an increase in VR is closely associated with tilapia visual detection. Moreover, the presence of another fish in the same environment would further alert the fish since they may sense potential predators or competitors. Hence, if the fish did not find the other fish harmless, an increase in VR can be observed as it is one of the responses of the fish to a stressor. Based on Barreto & Volpato

(2011), the VR can adjust rapidly in response to alert stimuli.

Table 2. The VR (beats/sec) of the fish subjected to visual and physical interactions.

PERIOD (DAYS)	TREATMENT			
	T1	T2	T3	T4
	no interaction	visual interaction	visual and physical interactions	physical interaction
0	1.08±0.03^{a}	1.08±0.03^{a}	1.11±0.02^{a}	1.08±0.03^{a}
1	1.10±0.03^{b}	1.14±0.04^{ab}	1.20±0.04^{ab}	1.23±0.10^{a}
2	1.13±0.05^{b}	1.20±0.06^{b}	1.30±0.03^{a}	1.20±0.05^{b}
3	1.14±0.08^{b}	1.14±0.08^{b}	1.29±0.05^{a}	1.23±0.04^{ab}
4	1.11±0.08^{c}	1.25±0.09^{b}	1.45±0.05^{a}	1.29±0.04^{b}
5	1.11±0.03^{c}	1.30±0.03^{b}	1.52±0.02^{a}	1.26±0.10^{b}
6	1.14±0.06^{c}	1.30±0.03^{b}	1.43±0.05^{a}	1.25±0.09^{ab}
7	1.10±0.08^{c}	1.32±0.04^{b}	1.48±0.04^{a}	1.27±0.04^{b}
8	1.18±0.03^{c}	1.30±0.08^{b}	1.48±0.05^{a}	1.27±0.05^{ab}
9	1.10±0.08^{c}	1.34±0.01^{b}	1.49±0.04^{a}	1.28±0.11^{b}
10	1.10±0.06^{c}	1.36±0.07^{b}	1.45±0.03^{a}	1.28±0.00^{b}
11	1.07±0.04^{c}	1.35±0.04^{b}	1.52±0.05^{a}	1.29±0.05^{b}
12	1.10±0.08^{c}	1.34±0.01^{b}	1.49±0.06^{a}	1.28±0.05^{b}
13	1.07±0.05^{c}	1.32±0.02^{b}	1.50±0.03^{a}	1.28±0.05^{b}
14	1.11±0.06^{c}	1.31±0.05^{b}	1.55±0.08^{a}	1.33±0.06^{b}
15	1.11±0.08^{c}	1.26±0.08^{b}	1.53±0.09^{a}	1.38±0.04^{b}

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

Eye Color Pattern (ECP):

The ECP in all treatments increased after subjecting the fish to stressors. However, significant differences were only observed on the 3rd day onwards. Significantly higher mean ECPs can be observed in T3 and T4 compared to those in T1 and T2. This indicates that fish in T3 and T4 experienced more stressful condition as compared to those in T1 and T2 (Table 3). According to Miyai *et al.* (2011), eye darkening in fish can be a consequence of the stress. It was also reported by O'Connor *et al.* (1999) that changes in body color have a connection with eye darkening in salmonids. Since body color changes when fish are stressed, it is therefore concluded that eye color changes also when fish are introduced to stressors.

The highest mean ECP (Fig. 2) recorded in the whole study was observed at T3 which is 7.33±0.29 and was recorded on 15th day. According to Vera Cruz & Tauli (2015), the eye color may also indicate the level of stress, with more stress as the color becomes darker. Hence, high ECP recorded in T3 means higher level of stress in fish. This conforms to the VR observation wherein the fastest VR was observed in T3. Mean ECP in all treatments increased throughout the study, showing that the fish continuously respond to the stressors. Agreeing to Vera Cruz & Brown (2007), ECP can be classified as a detectable physiological response to social stress in the fish. Henceforth, an increase in the mean ECP of fish indicates stressful condition to the fish.

Table 3. The ECP of the fish subjected to visual and physical interactions.

PERIOD (DAYS)	TREATMENT			
	T1	T2	T3	T4
	no interaction	visual interaction	visual and physical interactions	physical interaction
0	0.17±0.29^{a}	0.50±0.50^{a}	0.33±0.58^{a}	0.33±0.29^{a}
1	0.33±0.29^{a}	0.67±0.58^{a}	0.50±0.50^{a}	0.78±0.39^{a}
2	0.83±0.29^{a}	1.00±0.50^{a}	0.83±0.29^{a}	0.89±0.19^{a}
3	1.00±0.00^{b}	1.33±0.29^{b}	1.83±0.29^{a}	1.81±0.17^{a}
4	1.50±0.50^{b}	1.83±0.29^{b}	2.67±0.58^{a}	2.72±0.25^{a}
5	2.00±0.00^{b}	2.00±0.00^{b}	3.17±0.29^{a}	3.06±0.42^{a}
6	2.00±0.00^{b}	2.00±0.00^{b}	3.50±0.50^{a}	3.36±0.13^{a}
7	2.33±0.58^{b}	2.50±0.50^{b}	4.00±0.00^{a}	3.53±0.21^{a}
8	2.50±0.50^{b}	2.83±0.29^{b}	4.33±0.58^{a}	3.81±0.17^{a}
9	2.83±0.29^{c}	3.00±0.00^{c}	4.67±0.29^{a}	4.08±0.14^{b}
10	2.83±0.29^{d}	3.50±0.50^{c}	5.33±0.29^{a}	4.44±0.10^{b}
11	3.00±0.00^{d}	3.67±0.58^{c}	5.67±0.29^{a}	4.89±0.19^{b}
12	3.00±0.00^{d}	3.83±0.76^{c}	6.17±0.29^{a}	5.08±0.14^{b}
13	3.17±0.29^{d}	4.33±0.29^{c}	6.33±0.29^{a}	5.36±0.38^{b}
14	3.33±0.29^{c}	4.50±0.50^{b}	6.67±0.76^{a}	6.08±0.14^{a}
15	3.50±0.00^{d}	4.83±0.76^{c}	7.33±0.29^{a}	6.36±0.00^{b}

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

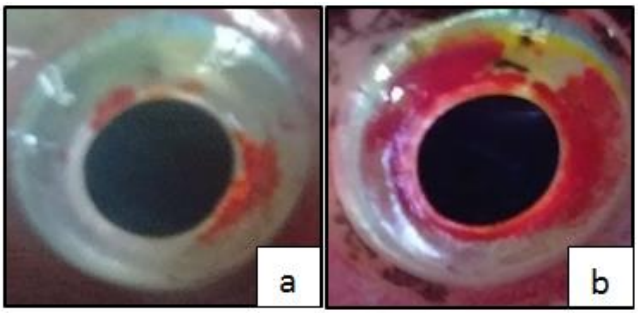

Fig. 2. ECP of the same fish subjected to visual and physical interaction. (a) before introduction to a stressor; (b) after introduction to a stressor

Gonadosomatic Index:

No significant difference in the mean male GSI can be observed among the treatments (Table 4). However, T4 mean male GSI is noticeably numerically higher than those of the other treatments. On the other hand, significant differences in the mean female GSI can be observed, wherein mean GSI in T4 (where physical interaction occurred) was significantly higher compared to those in T1, T2, and T3 (Table 4). According to Castro *et al.* (2009), communication between sexual partners modulates the reproductive behavior of fish stimulating gonad development. Physical interaction occured in T4 where fish were free to perform courtship that may led to the increase in the GSI of the fish.

Table 4. The GSI of the male fish subjected to visual and physical interactions.

Sex	TREATMENT			
	T1	T2	T3	T4
	no interaction	visual interaction	visual and physical interactions	physical interaction
Male	0.88±0.37[a]	0.77±0.13[a]	0.89±0.27[a]	1.13±0.05[a]
Female	1.22±0.15[b]	1.17±0.29[b]	0.85±0.09[b]	2.57±1.08[a]

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

It was observed that the highest VR and ECP recorded were in T3, at the same time, the lowest female GSI was observed also in T3 where physical interaction among the same sex and visual interaction among the opposite sex occured. Results on morphological responses showed that the fish in T3 were the most stressed as compared to the fish in the other treatments because the fastest VR and highest ECP were observed in T3. Hence, the stress condition of the fish and the GSI of the fish have an inverse relationship. Territorial behavior is a known social stress that influences reproduction and growth of the fish (Pfenning *et al.*, 2012). Agreeing to Louiz *et al.* (2009), low GSI is often linked to the response of the fish to stress. This study ascertains that stress may affect the GSI of the fish.

On the other hand, T4 which was the second-highest recorded in VR and ECP had the highest GSI recorded. In T4, although same sex fish were present, it was observed that the opposite sex fish grouped together and separated from the other group of male and female. The physical interaction of opposite sex maybe the factor that increased the GSI of the fish. According to Castro *et al.* (2009), reproductive fish behavior is affected by male-female interactions that stimulate physiological responses such as hormonal release and gonad development. Hence, physical interaction that occured between opposite sex in T4 affected the gonadal development of the fish which resulted in higher GSI.

Conclusion

Based on the results of the study, fish subjected to visual interaction, visual and physical interactions and physical interaction showed higher ECP and exhibit faster VR compared to fish not exposed to interaction. However, the darkest ECP, fastest VR and lowest GSI were observed in T3 where visual (between opposite sex) and physical (between same sex) interactions occurred. This shows that the most stressed fish were in T3 and that stress can be a hindrance to the gonadal development of the fish. On the other hand, T4, where physical interaction between same and opposite sex occurred, had the highest recorded GSI which clearly shows that the physical interaction between opposite sex may contribute to the gonadal development of the fish.

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