E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2020; 8(1): 287-290

www.fisheriesjournal.com
Received: 14-11-2019
Accepted: 18-12-2019

Jericho T Bernandino
College of Fisheries- Freshwater Aquaculture Center, Central Luzon State University, Science City of Muñoz, Nueva Ecija 3120, Philippines

Rea Mae C Templonuevo
College of Fisheries- Freshwater Aquaculture Center, Central Luzon State University, Science City of Muñoz, Nueva Ecija 3120, Philippines

Emmanuel M Vera Cruz
College of Fisheries- Freshwater Aquaculture Center, Central Luzon State University, Science City of Muñoz, Nueva Ecija 3120, Philippines

Corresponding Author:
Jericho T Bernandino
College of Fisheries- Freshwater Aquaculture Center, Central Luzon State University, Science City of Muñoz, Nueva Ecija 3120, Philippines

Responses of red tilapia (*Oreochromis* spp.) subjected to electric shock and handling stress

Jericho T Bernandino, Rea Mae C Templonuevo and Emmanuel M Vera Cruz

Abstract
Stress in fish can be caused by any disturbance in their environment which may include electric shock and handling. In this study, stress responses of Red tilapia subjected to electric shock (T2) and handling stress (T3) were determined. Changes in ventilation rate (VR), eye color pattern (ECP) and body color were observed 2, 4, 6, 8, 10, 30, 60 and 90 minutes after subjecting the fish to stressors. Results showed that mean VR, ECP and body color of fish increased immediately after introduction to stressors. Fish in T2 had significantly faster VR, darker ECP and body color as compared to the control group (T1), after 6, 2 and 10 minutes of exposure to electric shock. Fish in T3 had significantly darker ECP than the control group (T1), 6 minutes after subjecting to handling stress. Generally, fish subjected to electric shock (T2) had the highest stress level among the treatments.

Keywords: Red tilapia, stress, electric shock, handling

Introduction
Stress is a generalized response frequency in fish which can be modulated by specific stressor conditions [1]. It has been an important factor in the ability of vertebrates, including fishes, to perform necessary life functions [2]. Fish exposed to stress will undergo series of biochemical and physiological changes in an attempt to adapt to the stressful condition [3]. Their body responds under condition of stress which depends on the duration or magnitude of stress on fish [4].

Stunning and slaughter methods used in fish causes high stress level most of the time which includes asphyxia in air or ice, gills cut, decapitation, gutting (with live animals), salt bath and thermal shock in ice and water [5]. Electro stunning is considered as one of the less stressful and humane fish stunning methods since it causes rapid stunning and less animal suffering. It is a clean and potentially efficient procedure used to provoke loss of consciousness prior to killing or slaughtering of animal [6]. Electric current passes through the water or directly passed to fish until complete loss of consciousness. High shock might provoke immobilization and serious injuries to the fish in few seconds of exposure [7]. Thus, even low electroshock (20v) induced a fast increased in plasma cortisol and glucose level [1]. Moreover, handling in fish also induces perturbations of various biological parameters which have been investigated or reviewed in an attempt to analyze and quantify the resulting stress [8]. In the study of Fatemeh *et al.* [9], fish subjected to handling stress for 30 seconds significantly increased its plasma cortisol level. Disturbances in the environment could elicit stress responses in fish species.

Red tilapia (*Oreochromis* spp.) is one of aquaculture's most adaptive species. It is abundantly found in the wild and known to be cultured in several parts of the world [10]. 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 [11]. In this study, effects of electric shock and handling stress in the physiology and morphology of red tilapia such as ventilation rate (VR), eye color pattern (ECP) and skin color were observed.

Methodology
Experimental fish and units
Twenty red tilapia (50-60 g) obtained from the Freshwater Aquaculture Center, Central Luzon State University, Science City of Muñoz, Nueva Ecija, were acclimated for one week in

concrete indoor tanks supplied with well-aerated tap water. Then, fish were isolated for 10 days in 10 x 10 inches aquaria containing approximately 12 liters of water and ECP was observed daily. Fish were fed daily at the rate of 3% of their body weight. After isolation, nine proactive fish (~those with mean ECP values of 0-3.5) were selected for the experiment.

Experimental design and procedures

The experiment was performed using the Completely Randomized Design. There were three treatments in the study (Table 1) and each having three replicates. The fish were stocked at one fish per aquarium and then the fish were subjected to the stressor (electric shock or handling stress). The VR, ECP and body color (BC) were monitored before the introduction of stressors (pre-stress); immediately after introduction of stressor and 2, 4, 6, 8, 10, 30, 60 and 90 minutes later [12]. The VR, ECP and BC changes were observed 1 meter away from the aquarium.

Table 1: Treatments in the study.

Treatment	Description
Treatment 1	No stressors introduced
Treatment 2	Electric shock for 5 sec. (12 V dc, 64 ± 34 mA)
Treatment 3	Fish handled for 5 sec.

Electric shock and handling stress

Electric shock was applied with an electric stimulator having two electrodes; both electrodes were dipped in the aquarium. Electricity (12 V dc, 64 ± 34 mA) was emitted for 5 seconds in the water [13] with the use of 12 V AVR. Handling stress was administered by moving out the fish from the water and handled for 5 seconds.

Ventilation rate

Ventilation rate was measured visually by counting 20 opercular or buccal movements and concomitantly registering the time elapsed.

Eye color pattern

Eye color pattern was quantified through observation of the darkened area of both iris and sclera [14]. The ECP value was determined by dividing the eye into eight equal parts using four imaginary diameter lines (Figure 1). Darkening of each division of the iris and sclera around the pupil was observed and for each measurement, a value ranging from 0 (no darkening) to 8 (total darkening) was recorded [15].

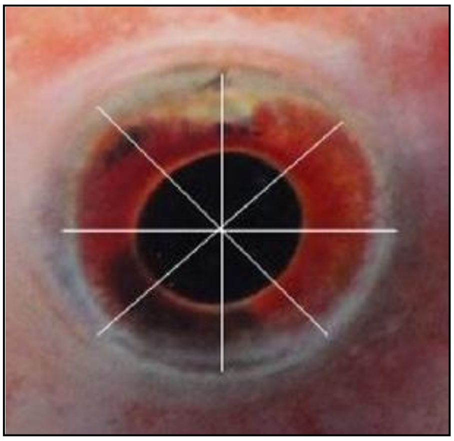

Fig 1: Eye color pattern of red Nile tilapia divided into eight imaginary parts

Body color

Body color of the fish receiving different treatments was compared by using a color chart, from pale pink (1) to intense orange (13) (Figure 2). Since the fish did not have fair color in each part of its body, the caudal fin, dorsal fin and the belly part of the fish were used to observe any changes in the color of the fish.

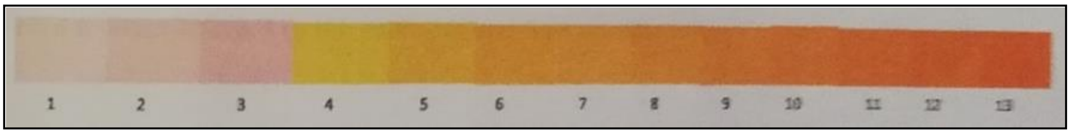

Fig 2: Color chart used in the study

Statistical analysis

Data were expressed as means plus or minus standard deviation (SD) and were analyzed using one-way analysis of variance (ANOVA). Differences between means were analyzed using Duncan' multiple range test. SPSS Version 16 for windows was used in data analysis.

Results and Discussion

Ventilation rate

The VR observed before the introduction of stressor showed no significant differences among the three treatments. Then, immediately after, 2, 4, 6, 8, 10, 30, and 60 minutes after the stressor, the mean VR increased (Table 2). Significant difference between mean VRs of the control (T1) and electric shock (T2) treatments was observed 6 minutes after the exposure of the fish to the stressor. The VR is a sympathetic response that quickly increases in response to stressors [16]. Sixty minutes after the exposure fish to the stressor, the mean VR of the electric shock (T2) treatment was significantly higher compared to those of the handling stress treatment (T3) and T1 which indicates that fish were stressed more due to electric shock than handling. Moreover, fish exposed to electric shock took a longer time to cope-up with stress as compared to fish subjected to handling stress as evidenced by increasing mean VR with time up to 60 minutes in T2 and only up to 6 minutes in T3. According to Roques *et al.* [13], fish exposed to electric shock has a slower recovery compared to handled-only fish. The VR can also be linked to metabolic rates [17]. Stress responses can induce catabolic responses and consequently modulate metabolism [18], therefore causing variation in the VR. Individual differences in metabolic rates have recently been proposed to be a physiological background of coping styles [19].

Table 2: The VR (beats/second) of the fish subjected to stressors.

Time (min)	Control (no stressors)	Electric shock	Handling stress
Pre stress (0 min)	1.45±0.11[a]	1.72±0.27[a]	1.51±0.12[a]
2	1.43±0.21[a]	1.89±0.45[a]	1.74±0.14[a]
4	1.43±0.19[a]	1.88±0.32[a]	1.69±0.15[a]
6	1.44±0.19[b]	2.03±0.25[a]	1.72±0.26[ab]
8	1.45±0.18[b]	2.02±0.26[a]	1.68±0.21[ab]
10	1.44±0.17[b]	1.99±0.25[a]	1.65±0.20[ab]
30	1.43±0.17[b]	2.17±0.04[a]	1.58±0.17[b]
60	1.43±0.16[b]	2.23±0.01[a]	1.52±0.17[b]
90	1.41±0.17[b]	2.03±0.03[a]	1.49±0.17[b]

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

Eye color pattern

The ECP of red tilapia was significantly increased after introduction to electric shock and 6 minutes after handling stress. Immediately 2 minutes after exposure to stressor, more than half of their eyes were covered by red or black pigment. However, there was only a slight difference between the ECP of fish that received electric shock (T2) and handling stress (T3), and the difference was not statistically significant. Results also conform to the results on VR but ECP seemed to be more sensitive stress indicator than VR as significant difference with that of the control was observed 2 minutes and 6 minutes after exposure to stressor in T2 and T3, respectively. Fish is said to be more stressed as the total darkened area of the eye became higher. The ECP in fish has been proven to be a potentially easy, inexpensive, and non-invasive way to measure conditions of fish stress [20]. It was also reported by O'Connor *et al.* [21], 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.

Table 3: The ECP of the fish subjected to stressors.

Time (min)	Control (no stressors)	Electric shock	Handling stress
Pre stress (0 min)	0.52±0.30[a]	1.05±0.36[a]	1.10±0.59[a]
2	1.43±0.29[b]	5.38±2.51[a]	4.62±1.57[ab]
4	1.19±0.16[b]	5.43±2.44[a]	4.62±1.84[ab]
6	1.19±0.16[b]	5.43±2.44[a]	4.90±2.03[a]
8	1.10±0.22[b]	5.43±2.44[a]	4.95±1.81[a]
10	1.14±0.14[b]	5.43±2.44[a]	4.86±1.51[a]
30	0.76±0.08[b]	5.43±2.44[a]	4.71±1.62[a]
60	0.48±0.22[b]	5.48±2.32[a]	4.48±1.80[a]
90	0.48±0.22[b]	5.26±2.12[a]	4.33±1.70[a]

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

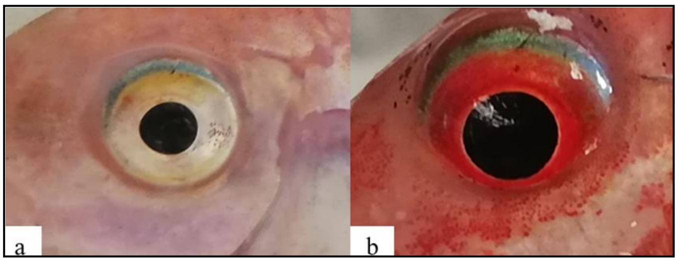

Fig 3: ECP of fish (a) before and (b) after subjecting to electric shock

Body Color

Body color of red tilapia intensified immediately 2 minutes after exposure to both electric shock (T2) and handling stress (T3) (Table 4). However, significantly darker shade of red was only observed in the bodies of fish in T2 as compared to that in T1, 10 minutes after exposure to electric shock stressor. Fish subjected to electric shock displayed more intense color than fish in the control treatment.

In most of the animals, rapid change in color is associated with social signaling and breeding [22]. Moreover, in teleost fish, both external and internal factors (physical, nutritional, genetic, neuro-hormonal) have been known to influence the chromatic state of fish [23]. Their skin color can change in response to environmental conditions, physiological challenges, stressful stimuli [24] and cultural condition (in some fish such as red porgy, *Pagrus pagrus*) [25].

Evaluating all the stress indicators, generally, fish subjected to electric shock (T2) had the highest stress level among the treatments. However, no mortality was recorded all throughout the study but, fish displayed changes on some of its body parts such as arising of fins after subjecting to stressors. In the study of Templonuevo and Vera Cruz [12], subordinate red Nile tilapia had darker body color compared to the dominant fish. Subordinate fish was more stressed since it had undergone scale losses, skin lesions, and destruction of fins due to the attacks received from the dominant fish.

Table 4: Body color of the fish subjected to stressors.

Time (min)	Control (no stressors)	Electric shock	Handling stress
Pre stress (0 min)	3.48±0.68[a]	5.00±1.36[a]	3.62±0.08[a]
2	4.38±0.70[a]	6.10±1.90[a]	4.67±0.30[a]
4	3.57±0.62[a]	6.05±1.94[a]	4.57±0.38[a]
6	3.57±0.62[a]	6.00±1.98[a]	4.67±0.36[a]
8	3.48±0.79[a]	6.00±1.98[a]	4.48±0.22[a]
10	3.43±0.74[b]	6.10±1.90[a]	4.48±0.22[ab]
30	2.95±0.70[b]	5.95±1.90[a]	4.48±0.33[ab]
60	2.62±0.79[b]	5.76±1.69[a]	4.29±0.14[ab]
90	2.57±0.76[b]	5.71±1.61[a]	4.00±0.14[ab]

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

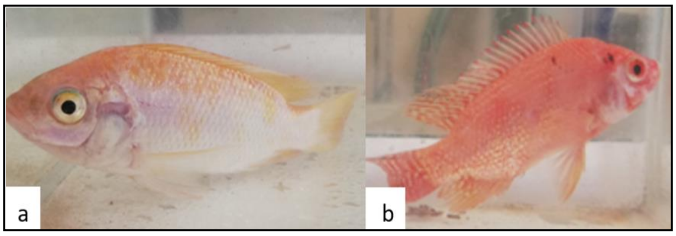

Fig 4: Body color of fish (a) before and (b) after subjecting to electric shock

Conclusion

The VR, ECP, and BC were proven to be effective and inexpensive stress indicators in red tilapia. Electric shock at 12 V dc, 64 ± 34 mA and handling stress (both imposed for 5 sec) greatly contributed to the stressed condition of red tilapia but they were not lethal to the fish since no mortality was recorded. However, electric shock with an intensity of 12 volts had been observed to be more stressful to fish compared to handling stress. Stress responses of fish increased immediately after exposure of fish to stressor and gradually decreased with time, which was a result of the fish's coping-style. Fish exposed to electric shock took a longer time to cope-up with stress as compared to fish subjected to handling stress.

References

1. Barreto RE, Volpato GL. Ventilatory frequency of Nile tilapia subjected to different stressors. Journal of Experimental Animal Science. 2006; 43:189-196.
2. Schreck CB. Stress and compensation in teleostean fishes: response to social and physical factors. *In*: Pickering, A.D. (Ed.), Stress and Fish. Academic Press, London, 1981, 295-321.
3. Costas B, Aragão C, Mancera JM, Dinis MT, Conceição LEC. High stocking density induces crowding stress and affects amino acid metabolism in Senegalese sole *Solea senegalensis* (Kaup 1858) juveniles. Aquaculture Research. 2008; 39:1-9.
4. Adams SM. (ed.) Biological indicators of stress in fish. American Fisheries Society Symposium Series 8, Bethesda, Maryland, 1990.
5. Oliveira Filho PRC, Girao PJM, Lapa-Guimarães J, Natori MM, Vargas SC, Viegas EMM *et al*. Impact of electrical stunning on fish behavior and meat quality of pacu (*Piaractus mesopotamicus*). Acta Scientiarum Technology. 2016; 38(1):81-88.
6. Van de Vis H, Kestin SC, Robb D, Oehlenschlager J, Lambooi B, Munkner W *et al.* Is humane slaughter of fish possible for industry? Aquaculture Research. 2003; 34:211-220.
7. Dwyer WP, Shepard BB, White RG. Effect of backpack electroshock on west slope cutthroat trout injury and growth 110- and 250-days post-treatment. North American Journal of Fisheries Management. 2001; 21:646-650.
8. Mazeaud MM, Mazeaud F, Donaldson EM. Primary and Secondary Effects of Stress in Fish: Some New Data with a General Review. Transactions of the American Fisheries Society. 2011; 106:1977.
9. Fatemeh A, Sanaz G, Shahla J. Plasma Cortisol Changes and Body Composition in Stizostedion lucioperca Exposed to Handling Stress. Pakistan Journal of Biological Sciences. 2008; 11:623-627.
10. Rahman SA, Zambry H, Basha S, Kamarzaman S, Chowdhury AJK. The potential role of Red tilapia (*Oreochromis niloticus*) scales: allergic reaction test in mice. Journal of Applied Pharmaceutical Science. 2013; 3(10):45-50.
11. Thodesen J, Rye M, Wang YX, Li SJ, Bentsen HB, Yazdi MH *et al.* 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. 1990; 416(417):354-366.
12. Templonuevo RMC, Vera Cruz EM. Responses of Red Nile Tilapia (*Oreochromis niloticus* L.) Subjected to Social and Confinement Stresses. The Central Luzon State University International Journal of Science & Technology. 2016; 1(2):7-14.
13. Roques JAC, Abbink W, Chereau G, Fourneyron A, Spanings T, Burggraaf D *et al.* Physiological and behavioral responses to an electrical stimulus in Mozambique tilapia (*Oreochromis mossambicus*). Fish Physiology and Biochemical. 2011; 38:1019-1028.
14. Volpato GL, Luchiari AC, Duarte CRA, Barreto RE, Ramanzini GC. Eye color as an indicator of social rank in the fish Nile tilapia. Brazilian Journal of Medical and Biological Research. 2003; 36:1659-1663.
15. Vera Cruz EM, Tauli MP. Eye Color Pattern During Isolation Indicates Stress-Coping Style in Nile Tilapia (*Oreochromis niloticus* L). International Journal of Scientific Research in Knowledge. 2015; 3(7):0181-186.
16. Priede IG. Metabolic scope in fishes: in Fish energetics: new perspectives (eds) P Tytler and P Calow (Baltimore: Johns Hopkins University Press, 1985, 33-64.
17. Alvarenga CDM and Volpato GL. Agonistic profile and metabolism in alevins of the Nile tilapia. Physiology and Behavior. 1995; 57:75-80.
18. Mommsen TP, Vijayan MM, Moon TW. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries. 1999; 9:211-268.
19. Careau V, Thomas D, Humphries MM, Reale D. Energy metabolism and animal personality. Oikos. 2008; 117:641-653.
20. Freitas RHA, Negrão CA, Felício AKC, Volpato GL. Eye darkening as a reliable, easy and inexpensive indicator of stress in fish. Zoology. 2014; 117(3):179-84.
21. O'connor KI, Metcalfe BN, Taylor AC. Familiarity influences body darkening in territorial disputes between juvenile salmon. Animal Behaviour. 2000; 59:1095-1101.
22. Kindermann C, Narayan E, Wild C, Wild F, Hero JM. The effect of stress and stress hormones on dynamic colour-change in a sexually dichromatic Australian frog. Comparative Biochemistry and Physiology – A, 2013; 165:223-227.
23. Fujii R. Coloration and chromatophores. *In*: The Physiology of Fishes. (Ed by D.H. Evans). CRC Press, Boca Raton, 1993, 535-562.
24. Szisch V, Van Der Salm AL, Wendelaar Bonga SE, Pavlidis M. Physiological colour changes in the red porgy, *Pagrus pagrus*, following adaptation to blue lighting spectrum. Fish Physiology and Biochemistry. 2002; 27:1-8.
25. Rotllant J, Tort L, Montero D, Pavlidis M, Martinez M, Wendelaar Bonga SE *et al*. Background colour influence on the stress response in cultured red porgy *Pargus pargus*. Aquaculture. 2003; 223:129-139.