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Body darkening as thermal stress indicator for Nile tilapia (*Oreochromis niloticus* L.)

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Abstract Increasing water temperature due to global warming has decreased the aquaculture production of tilapia in the Philippines. Stress is the initial effect of increasing temperature, but when stress prolongs and not predicted nor prevented, fish health is suppressed and may lead to mortality. Thus, stress indication is of premium importance. In order to provide a more convenient and reliable stress indication, this study had reported a morphological response parameter (body darkening) as a thermal stress indicator and used other established parameters to determine their associations and reliability. Results suggested that body darkening and all other stress indictors were positively and significantly related to water temperature. Particularly, 80.50% of the variations in body darkening scores were brought by the changes in water temperature. More so, body darkening revealed highly significant positive relationships with the darkening of the eye ($\rho = 0.902$, $p < 0.000$, $n = 22$), ventilation rates ($\rho = 0.902$, $p < 0.001$, $n = 22$), and cortisol levels ($\rho = 0.868$, $p < 0.001$, $n = 22$). Similar to other known stress indicators for fish, body darkening is likewise a realible, convenient, sensitive, and a non-invasive stress indicator.

Keywords: Ventilation, Indication, Cortisol, Eye color pattern

Introduction

Global warming has been a great problem of this century, as it alters the natural environment's conditions (Rani, 2016). Tilapia is the second most cultured fish in ponds, cages, and pens in the Philippines (BFAR, 2019). However, recently, its production seems to suffer considerably from the detrimental effects of increasing water temperature. According to Guerrero (2019), average annual production rate for tilapia in the Philippines from 2007 to 2016 was only at 0.7% which greatly declined from the 240% average rate in 2001 to 2011. Moreover, Guerrero (2019) pointed out that 68% of the farmers identified high water temperature as the primary cause of decreasing production.

In fact, several studies have pointed that increasing water temperature can cause stress among fishes (Harper and Wolf, 2009; Van Ham *et al*., 2003)

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and to some extent, mortality. To deal with environmental change, fish respond by altering its physiological functions which is mediated by the neuro-endocrine system and includes the release of hormones such as cortisol and adrenaline (Barton and Iwama, 1991). Hence, cortisol level has been widely used indicator of stress in fishes (Odore *et al*., 2004; Barton, 2002; Mommsen *et al*., 1999). Aside from cortisol level, quite a few stress indicators have been proven and utilized in fish health monitoring in relation to various stressors, including water temperature. These include fish ventilation rate and eye color pattern. Ventilation rate is known as a sensitive indicator (Barreto and Volpato, 2011; Bell *et al.*, 2010; Gibson and Mathis, 2006), while, eye color pattern is a potentially easy, inexpensive, and non-invasive way to measure level of fish stress (Frietas *et al*., 2014).

Given the availability of many inexpensive stress indicators, its practicality is still less pleasing due mostly to difficulty of the methods. In the case of eye color pattern, one should look and come closely to fish in order to visualize the four imaginary lines along its eye to assess the level of stress, while observation of breathing rate so as requires keen observation of the buccal movements which is extra difficult when fish is in motion and much more prone to human error.

Therefore, screening of any potential stress indicators, most particularly those of more practical use, is an important approach to further understand the biological profile of an organism and serve as effective tool for stress indication particularly in farmed Nile tilapia. Withal, this study tried to determine whether or not the body darkening (BD) in Nile tilapia (*O. niloticus*) is one of its stress coping styles, thus effective thermal stress indicator.

Materials and Methods

Twenty-four (24) Nile tilapia of similar age (100 days old) and weight groups (90-100 grams) were used in this study. A total of 24 experimental units were used. Fish samples were conditioned for 30 days using rectangular concrete tanks (2.5 x 1 x 1.2m) receiving continuous flow of water. During conditioning, each unit was provided with aeration system to generate sufficient dissolved oxygen for the fish. Also, fish were fed twice daily at 3 percent of its body weight, but fish were fasted two hours prior to isolation. Glass aquaria measuring 12 x 12 x 12 cm were used as holding units for the fish during isolation and exposure test. Only one fish per aquarium was stocked in order to avoid any potential stressor which social interaction may cause. Water temperatures were then modulated gradually using electric water heater to attain desired temperature levels (Normal or no heater (27.0 - 30.0 ˚C), 32.0 ˚C, 34.0 ˚C, and 36.0 ˚C). Completely Randomized Design (CRD) was used. Each treatment was replicated six times. Randomization was pre-determined using scientific calculator (Casio-fx-350MS, Casio Comp. Co. Ltd.). All sides except the front (for monitoring purposes) of each glass aquarium were covered with white clean paper to avoid visual interaction among experimental fish.

During temperature exposure, changes in the eye color pattern (ECP), ventilation rate (VR) and body darkening (BD) of the fish were monitored at 0, 1, 2, 6, 12, 24, 48, 72, and 96 hours. Body darkening was quantified using a color chart where scores can range from 0 to 9 (Fig. 1).

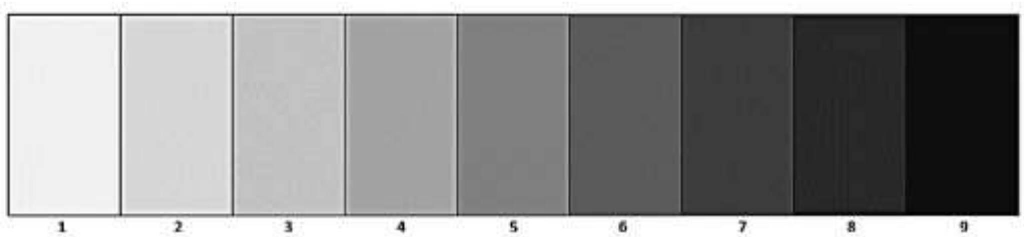

Figure 1. Body darkening scoring chart (Dela Cruz, 2018)

For ECP quantification, the circular area of the eye was divided into 8 equal parts using 4 imaginary diameter lines (Volpato *et al*., 2003). The ECP value observed ranged from zero (no darkening) to eight (total darkening) (Fig. 2). Ventilation rates were monitored through recording the time required for the fish to do a specified number of buccal movements (adapted from Alvarenga and Volpato, 1995). Blood plasma cortisol was analyzed after the 96-hour exposure. This was done using the collected blood thru cardiac puncturing of sedated fish. Sedation was carried out using MS-222 (tricaine methanesulfonate). Cortisol analysis followed the electrochemiluminescence immunoassay (ECLIA) protocol.

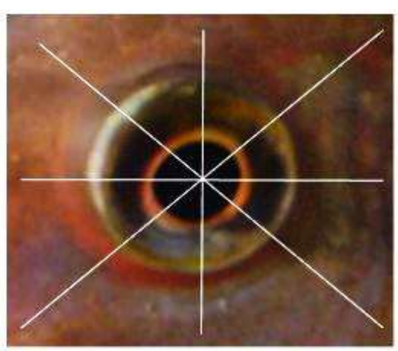

Figure 2. Eye color pattern of the fish

Relationships between parameters and temperature and among parameters were analyzed using multiple Pearson correlation (two-tailed test) and Regression test. All statistical analyses were carried out using SPSS software version 21.

Results

Regression analysis revealed that all parameters were largely related to water temperature (Fig. 3). These parameters showed the increasing scores and/or levels in response to rising water temperature. Body darkening (BD) recorded the second highest value for coefficient in determination (R^2) at 0.805, while highest was observed from the other morphological tool (eye color pattern) with R^2 = 0.831.

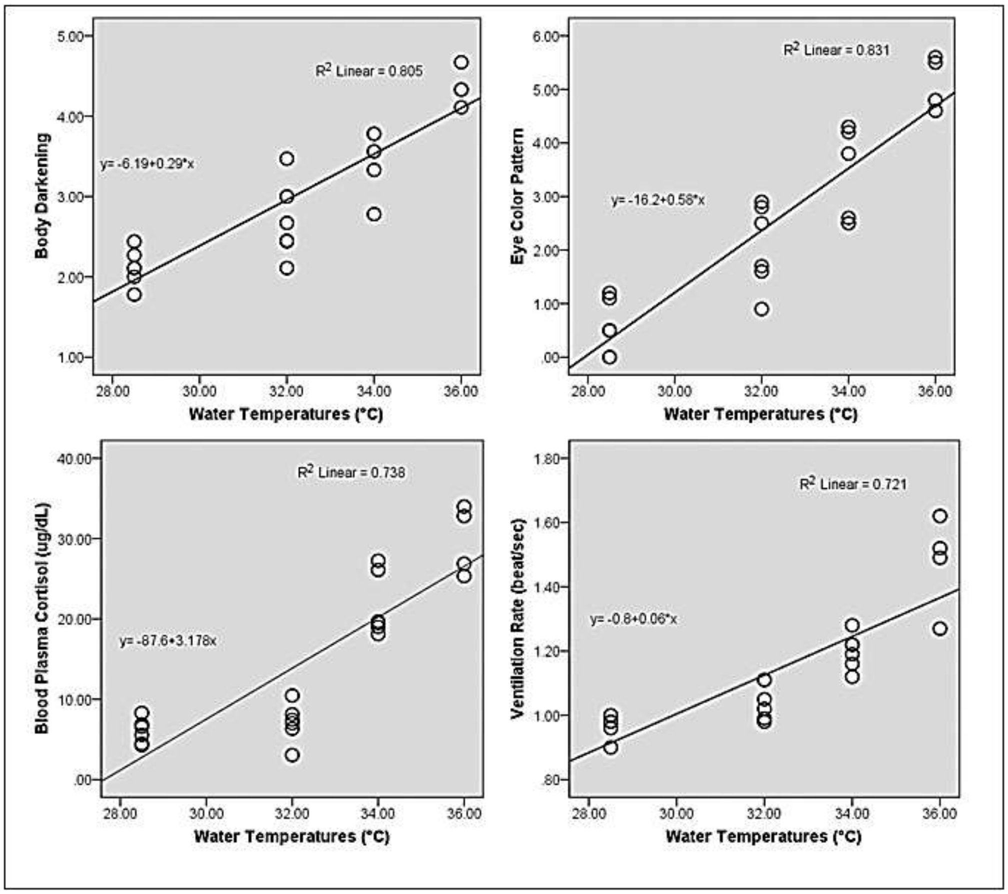

Figure 3. Linear regression plots for all parameters versus water temperature

As presented in Figure 3, the equation of the line for body darkening indicates that for every one unit change (1 ˚C) in water temperature, fish body will darken at level equivalent to 0.58 point in the body darkening scoring chart (Fig. 1). Moreover, the value of its R^2 specifies that 80.50% in the variability in the score of BD is brought by the changes in water temperature (Fig. 4).

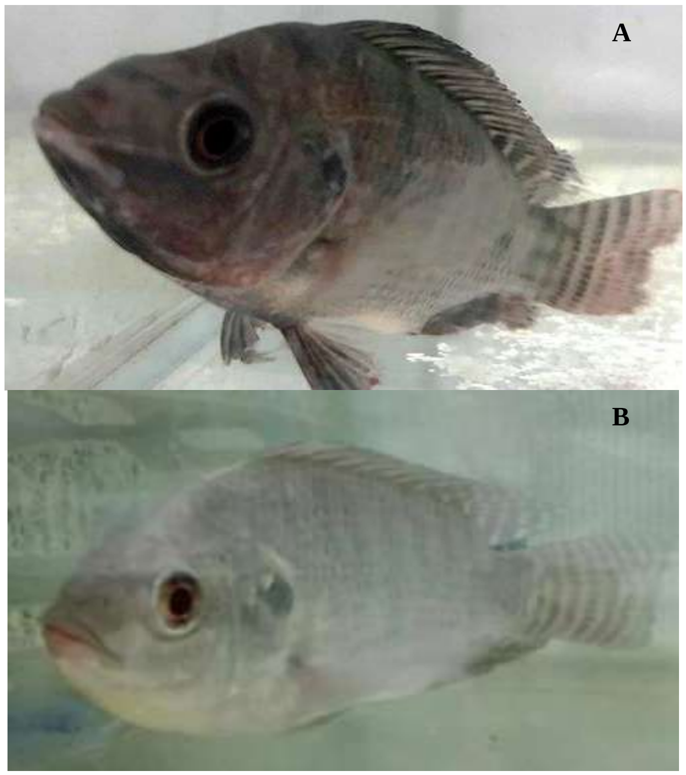

Figure 4. Nile tilapia with different body color response; (a) fish under heated water; (b) fish under normal water temperature

In view of the relationship among parameters (Table 1), body darkening (BD) revealed highly significant positive relationships with all other established stress indicators in this study (eye color pattern, ventilation rate, and blood plasma cortisol). Body darkening was associated with the darkening of the eye (ρ = 0.902, p <0.000, n =22), increase of ventilation rates (ρ = 0.902, p <0.001, n =22), and levels of stress hormone (cortisol) (ρ = 0.868, p <0.001, n =22).

Table 1. Correlation between body darkening (BD) and other parameters

		Established Stress Indicators		
Body Darkening		Eye Color Pattern	Ventilation Rate	Plasma Cortsiol
(BD)	P	0.902**	0.902**	0.868**
	Significance	0.000	0.000	0.000
	N	22	22	22

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

Discussion

The increase in water temperature had largely stimulated the darkening in Nile tilapia body. More so, the darkening of the body was in parallel response to eye color pattern, ventilation rate, and level of stress hormone (blood plasma cortisol) which are all known stress indicators in Nile tilapia. According to Szisch *et al*. (2012), fish can alter their coloration in response to environmental condition, physiological challenges and stressful stimuli. Similar to Högland *et al*. (2000) who found out that social stress could induce skin darkening in Arctic charr (*Salvelinus alpinus*), this study also revealed that changes in the water temperature affected the color of Nile tilapia, that the higher the temperature, the darker its body color.

Similar to all other established stress indicators used in this study, it is concluded that body darkening can also effectively indicate thermal stress in Nile tilapia. While other indicators are difficult to use and more prone to human errors, body darkening is a sensitive, potentially easy, inexpensive, non-invasive, and less prone to error stress indicator for thermally-stressed Nile tilapia. Further studies on body darkening as a stress indicator for other stressors are recommended.

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