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Original Article

Effects of different background colors on adult spawning, egg hatchability, and larval survival of zebrafish (*Danio rerio*)

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Abstract

Background color has significant effects on the reproductive and survival performance of fish species. While zebrafish (*Danio rerio*) is a popular species in laboratory and recreational aquaculture, very little has been known about its background color preference. Thus, this study evaluated the effects of different colors of aquaria on the spawning, hatching, and survival of this species. The study found that zebrafish do not have color preference for spawning. Though red and black backgrounds are statistically comparable, these colors showed significantly higher ($P < 0.05$) hatching and survival rates than other colors used in this study. Furthermore, performance of red was higher than black but it was not statistically significant. This study reveals that, among all colors evaluated, red is the most suitable for hatching of zebrafish eggs and survival of its larvae whereas, yellow and transparent should be avoided.

Keywords: Breeding; environment color; hatching rate; ornamental fish; spawn

1 | INTRODUCTION

In aquaculture, many considerations are given to the designs of facilities, the emphasis being on its size and shape, so does main attention is certain to the hydrodynamic attribute of the rearing systems (Kesbic *et al.* 2017). In common practice, background color which may affect fish growth performance (Strand *et al.* 2007; Banan *et al.* 2011; Elnwishy *et al.* 2012; Kesbic *et al.* 2017), feed intake (Stand *et al.* 2007; Kesbic *et al.* 2017), survival (Pedreira and Sipauba-Tavares 2001; Takeshita and Soyano 2008; Brian 2015), stress level (Volpato and Barreto 2001; Barcellos *et al.* 2009; Imanpoor and Abdullahi 2011) and reproduction (Volpato *et al.* 2004; Brian 2015) is mostly disregarded when setting-up an aquaculture facility (Brian 2015; Kesbic *et al.* 2017).

According to Sabri *et al.* (2012), 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 (Volpato and Barreto 2001). 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. Since different fish species require diverse environmental factors during their life stages, selection of the right background color may offer the fish a less stressful culture environment (Banan *et al.* 2011;

Kesbic *et al.* 2017,). Thus, in aquaculture, environmental conditions should be well considered to guarantee improved fish welfare (Sabri *et al.* 2012).

The zebrafish (*Danio rerio*) is a popular aquarium fish worldwide (Galib and Mohsin 2011; Kucharczyk *et al.* 2018) and an important laboratory model species in aquaculture research (Lawrence 2007), developmental genetics, neurophysiology, and biomedicine (Amsterdam and Hopkins 2006; Tavares-Lopes 2013). Given the relatively replete biological literatures concerning this species, little is known about its natural ecology (Liew *et al.* 2012) and husbandry (Lawrence 2007) especially in the aspects of its reproduction (Kucharczyk *et al.* 2018). Surprisingly, there exists no literature specific to the effect of different background colors in its breeding other than photoperiod. Therefore, this study explored the particular effect of background colors on the reproductive performance of zebra fish, the hatchability of its eggs, and survival of its larvae.

2 | METHODOLOGY

2.1 Experimental fish and unit

A total of 30 zebra fish (*Danio rerio*) individuals of the same weight group (0.62 ± 0.07 g) were used in this study. Individuals were obtained from the Wet Laboratory of the Central Luzon State University, Philippines. Fifteen 4-liter plastic containers were filled with tap water and were used as holding units. Each unit was aerated (DO: 4.5 – 5.0 g L^{-1}) and covered with different colors of cellophane as experimental treatments. Temperature (28 – 30°C), salinity (0 ppt), photoperiod (12 h light and 12 h dark – natural rhythm), and pH (7.0 – 7.5) were maintained during experimental periods following ZFIN protocol for zebrafish breeding (Westerfield 2000). Fish were fed to satiation twice a day with live newly- hatched *Artemia salina* in morning at 0800 and in the evening at 1700 hours. All water quality parameters were found similar across groups ($P > 0.05$) throughout the 7-day (24 February to 1 March, 2017) study period.

2.2 Experimental design

Five groups of background colors *viz.* no color (control), black (treatment 2), blue (treatment 3), yellow (treatment 4), and red (treatment 5) were used in this study. The study was replicated thrice and carried out under Complete Randomized Design (CRD).

2.3 Experimental procedures

Fish individuals were conditioned for seven days prior to mating experiment. Conditioning of male and female individuals was carried out using separate holding units. Aeration system and commercial feeds were provided during conditioning. After conditioning, pairing (1 male : 1 female) and stocking was done. Spawning was monitored every hour after the first 16 hours of pairing. Parent fish (pair), after spawning, were removed immediately from the holding unit. The time taken for each pair to spawn was recorded. Viability of eggs was examined using 4X-stereo microscope (Amscope, USA). A total of 50 viable fertilized eggs were collected from each unit and subjected to 36 hours post-fertilization hatching test under respective background color. Twenty (20) newly hatched larvae from each unit were further subjected to 96-hour survival test.

2.4 Data analysis

Normality test and homogeneity of variances (Levene's test) were carried out before analyzing the data. Analysis of Variance (ANOVA) for spawning and hatching rate followed by Tukey HSD test were performed to interpret the values. While, percent survival was analyzed using Brown-Forsythe ANOVA followed by Games-Howell posthoc test. Means were compared employing an α level of significance of 0.05.

3 | RESULTS

All zebrafish pairs were spawned after 22 to 30 hours of pairing and it did not vary significantly among groups ($P > 0.05$). However, the fastest spawning was observed in tanks with black background and followed by red and while whereas the slowest was in tank with yellow background (Table 1).

TABLE 1 Spawning and hatching rate of zebrafish (*Danio rerio*) in tanks with different background colors ($n = 3$).

Background color	Spawning (h)	Hatching rate (%)
No color	25.00 (±1.00)^{a}	70.67 (±8.08)^{abc}
Black	23.33 (±1.53)^{a}	84.67 (±5.03)^{ab}
Blue	27.00 (±2.65)^{a}	66.33 (±9.61)^{bc}
Yellow	27.67 (±0.58)^{a}	64.00 (±6.00)^{c}
Red	24.00 (±1.73)^{a}	87.33 (±5.03)^{a}

Means in columns with the same superscript letter are not significantly different at $P < 0.05$.

While hatching rates in red, black, and transparent (control) backgrounds were not significantly different (Table 1), significantly higher rates ($P < 0.05$) were observed in tanks with red (87.33%) and black (84.67%) backgrounds compared to yellow (64%). Red also varied significantly from blue background (66.33% hatching rate).

Survival rate of fish in tanks with all background colors had decreased as time progressed from 24 h to 96 h. Survival rates in tanks with blue and yellow backgrounds declined drastically (Figure 1). Evidently, both red and black backgrounds consistently presented higher percent of survival from 24 h to 96 h of the test. Both red and black backgrounds, after the 96-h test, have shown significantly higher ($P < 0.05$) percent of survival, 93.33% and 88.33%

respectively, than control tanks (73.33%).

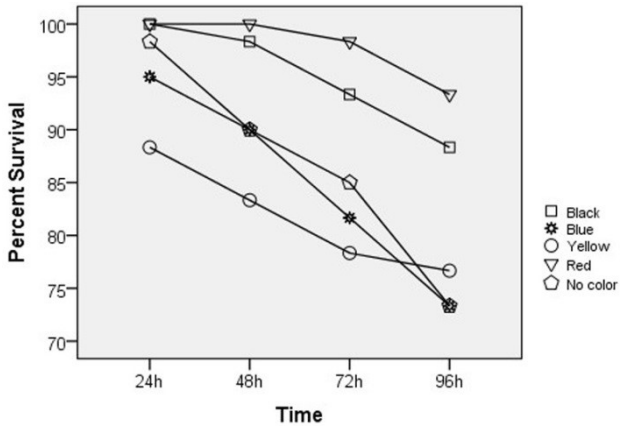

Figure 1 Survival trend of zebrafish (*Danio rerio*) for 96-h exposure test under different background colors.

4 | DISCUSSION

Environmental color can improve and/or disrupt fish welfare (Volpato and Barreto 2001). Yet, little is known about the color preference of zebrafish with regards to its spawning, hatching, and survival performance. In support, the present study shows that background color may play a significant role in this regard in zebrafish. Zebrafish held in tanks with red and black backgrounds presented improved performance. Under typical aquaculture condition, tank color has always been largely neglected and has been chosen almost by chance (Volpato and Barreto 2001). Although spawning performance of zebrafish under different background colors did not vary significantly, a different trend was observed for hatching rates. The higher hatching rates were found for red and black backgrounds. In Nile tilapia (*Oreochromis niloticus*) relationship between background color and spawning (Volpato *et al.* 2004) and hatchability (Brian 2015) were examined and it has been revealed that the blue lights can positively affect the reproductive motivation of males, exhibited by larger nest area and reproduction frequency (Volpato *et al.* 2004). Tilapia egg hatchability, in contrast, was not significantly affected by background color, but numerically higher in tanks with blue background (Brian 2015). Such results were not observed for zebrafish in this study.

Percent survival of zebrafish larvae was likewise positively affected by colored background in this study than control. The use of red and black backgrounds improved its survival rate. To my knowledge, there have been no studies that compares zebrafish larvae survival rate in tanks with different background colors. However, few studies have been focused on Nile tilapia *O. niloticus* (McLean *et al.* 2008; Brian 2015; Manliclic *et al.* 2018), summer flounder *Paralichthys dentatus* (McLean *et al.* 2008) and Eurasian perch *Perca fluviatilis* L. (Tamazouzt *et al.* 2000). In Nile tilapia, light blue to blue background can significantly improve its survival rate (McLean *et al.* 2008; Brian 2015; Manliclic *et al.* 2018), while the use of red background should be avoided (Brian 2015). For Eurasian perch, greyish background was found to be the best for its survival (Tamazouzt *et al.* 2000); whereas, red fits well for summer flounder (McLean *et al.* 2008). These results showed that there is a species-specific response in terms of environmental color, and that, information as to the most appropriate background color is of high importance.

CONFLICT OF INTEREST

The author declares that there is no human or any institutional conflict of interests in this research work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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