Effects of different color artificial lights on growth, survival, and sex ratio on an experimental population of freshwater ornamental emperor tetra fish *Nematobrycon palmeri*

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Abstract. This study evaluated the effects of white, green, blue, and red colors of LED light had on survival, growth, and sex ratio of the ornamental emperor tetra fish *Nematobrycon palmeri*. Fish used in the study were approximately two months old at trial start and throughout the study duration individual fish appeared healthy and fed normally. Fish deaths occurred but were attributed to common injuries that happen with initial capture and handling; overall survival was 79%. External sex differentiation occurred during the 12-week experimental period. All males became more colorful than females and developed the characteristic extended ray in the middle of the caudal fin, giving it the appearance of a trident. The males also became significantly ($p < 0.05$) heavier (468±125 mg) and longer (3.4±0.2 in total length, TL) than females (305±5 mg; 2.8±0.4 TL). Fish in all treatment groups gained significant ($p < 0.05$) length and body weight; 1.8±0.1 to 2.4±0.1 cm standard length, and 169±24 mg to 359±53 mg. However, different colors of light did not have a significant effect ($p > 0.05$) on survival or growth performance on the emperor tetra. More important was the observation that different colors of artificial LED light affected the sex ratio of the experimental population, with significant skew ($p < 0.05$) showing approximately 3 females to 1 male when the population was raised under blue and red lights. Under white and green lights the sex ratio did not deviate significantly ($p > 0.05$) from parity, 1:1. After extensive literature review we found no other information regarding altering the sex ratio in fishes raised under color light of different wavelengths. This finding may be applicable to other ornamental fish species, and become a very practical tool in their commercial production. Our finding also further supports the idea that the effect of color lights on fish biological parameters appears to be species specific.

Key Words: aquarium, husbandry, LED colored light bulbs, photoperiod, reproduction.

Introduction. Although the impact of photoperiod and tank color manipulation to improve welfare parameters in fishes is well studied (Chemineau et al 2007; McLean et al 2008; Villamizar et al 2011), the photobiological effects of different light colors on fishes has only recently received the attention it deserves. Investigating light color should be a given since the properties of light (such as wave length and color) change rapidly in the water, especially with water clarity and increased depth where fish live (Luria & Kinney 1970; Warrant & Johnsen 2013; Sliney 2016). In water, colors disappear in the same order as they appear in the continuous visible color spectrum: red, with the longest wavelengths, is the first to be absorbed, followed by orange, yellow, green, and cyan. The blue and violet have the shortest, highest-energy wavelengths and remain visible at the deepest depth.

A literature review indicated that different species of fish can have different responses to different colors of light, which means light responses are highly dependent on the species (Villamizar et al 2011). For example, red light reduced growth in the crucian carp *Carassius carassius*, zebrafish *Danio rerio*, European sea bass *Dicentrarchus labrax*, guppy *Poecilia reticulata*, the Chinese sleeper *Perccottus glenii*, and the marine clownfish *Amphiprion clarkii* (Ruchin 2004; Villamizar et al 2009; Shin et al 2012; Adatto et al 2016). It was further suggested that red light induced oxidative stress in *A. clarkii* (Shin et al 2011). Red light also resulted in a lower fecundity in zebrafish (Adatto et al 2016). Differently, the common carp *Cyprinus carpio*, and tilapia (*Oreochromis niloticus* and the hybrid *O. mossambicus* x *O. hornorum*) exhibited highest growth values under red light (Aly et al 2017; Nasir & Farmer 2017; Lopez-Betancur et al 2020); red light also stimulated reproductive performance and feeding behavior in the tilapia (Volpato et al 2004; Volpato et al 2013); red light also stimulated ovarian development in the tropical damselfish *Chrysiptera cyanea* (Bapary et al 2011). On the other side of the spectrum, blue light prevented stress in tilapia and enhanced growth in koi carp *Cyprinus carpio*, Texas cichlid *Herichthys cyanoguttatus*, and two species of marine clownfish *A. clarkii* and *A. melanopus* (Volpato & Barreto 2001; Choi et al 2012; Montajami et al 2012; Shin et al 2012; Bairwa et al 2017). Yellow light promoted growth in embryonic and < 2 g juvenile rainbow trout *Oncorhynchus mykiss* (Dadfar et al 2017), while the green light was also suitable for growth in koi carp, and best for juvenile rainbow trout, flounder *Paralichthys olivaceus*, and tiger puffer *Takifugu rubripes* (Luchiari & Pirhonen 2008; Kim et al 2016; Bairwa et al 2017; Benedict et al 2019). On the other hand, green light negatively affected growth in the Texas cichlid (Montajami et al 2012). Additionally, green light irradiation induced female-to-male sex reversal in the medaka *Oryzias latipes* (Hayasaka et al 2019). However, a high proportion of malformations and low survival rates were observed in zebrafish larvae when exposed to green light (Villamizar et al 2014). Except for the negative effect red light had on zebrafish larvae, the different colors of visible light seem to have little or no effect on fish survival rates, as reported in the cited literature.

Given the vivid coloration of many ornamental fish, the background color of the tank, the color of the lights, the physical distortions of light underwater, and the perception of color by the fish eye and brain, color can greatly influence their structural, physiological and behavioral adaptations that ensure their welfare in the wild and in captivity. In this study, we compared the effects of different color artificial light on growth, survival, and the sex ratio on an experimental population of the freshwater ornamental emperor tetra fish *Nematobrycon palmeri*. To our knowledge, this is the first study of its kind for the species and one that can lead to practical developments using color light-driven practices to increase fish farm efficiency, especially those involved in ornamental fish production. The emperor tetra is among the most valuable of ornamental fish collected from the wild and exported from Colombia (Ajiaco-Martínez et al 2012). Colombia has traditionally been a major exporter of freshwater ornamental fish to the world, primarily to Europe and the United States (Chapman 2000). The culture of ornamental fish is among the most valuable fishery commodities worldwide (Chapman 2000). The commercial aquaculture production of ornamental fishes can lead to socio-economic development and wild resource conservation.

Material and Method. Newly transformed juvenile emperor tetras (~ 1 month old) were obtained from a culture facility near the city of Buenaventura, on the Pacific coast of Colombia; the emperor tetra is native to the rivers and streams of the region. The fish were placed in small plastic bags filled with water, and carried inside an insulated ice chest for transport to the laboratory. In the laboratory, fish were poured into a plastic water storage tank (1.5 m diameter x 0.5 cm height), and acclimated for three weeks until the start of the study. The tank was covered with shade cloth and filled with well water (~500 L) maintained at ambient air temperature (25-28°C). Water was continuously filtered and aerated as it passed through a sponge attached to a submersible powerhead pump. The fish were fed a commercially prepared sinking granules (TetraColor, Tetra Spectrum Brands Pet), 3 times daily to satiation.

For the experiment, the fish were transferred to a temperature-controlled room (24-26°C), and randomly selected and assigned to aquariums and treatment groups then acclimated again for one week to the test conditions (aquariums, feed, light). Three replicates of fish groups (10 fish tank^{-1}) were placed in plastic aquariums (28 L, 34 x 32 x 30 cm), and exposed to four different colors of 1 W LED light bulbs: white, red, green, and blue (Figure 1). The lights were turned on and off automatically by a timer set to 12 h light-dark cycle. Barriers were placed between aquaria to prevent light interference from other sources. Good water quality (dissolved oxygen > 6.5 mg L^{-1}, NH_3 < 0.02, pH 7.0) was maintained using a sponge filter supplied with air from a linear piston air pump, and a weekly replacement of water (15%). General observations were made daily, with special attention to the behavior of the fish and their health condition, such as loss of apetite, difficulty in swimming, rubbing against objects in the tank, and presence of skin lesions. Mortalities were removed immediately and recorded.

Figure 1. Photo of experimental setup (aquariums and filters) exposed to four colors LED lights (white, red, green and blue) during the experimental period. Tank in green space in middle row was removed for photo to show light barriers more clearly.

The experiment was conducted for 12 weeks (October 2019 to January 2020), and survival and growth measurements were recorded at the beginning and end of the trial. Percent survival was estimated by dividing total number of individuals from each treatment group at the end with that at the initial, and multiplying that number by one hundred. Individual fish were weighed (0.01 g) and measured for total and standard lengths (0.1 cm). Male fish were easily identified by their prominent, trident-shaped caudal fin (Weitzman & Fink 1971); an external sex phenotype characteristic of the species which they start exhibiting when they are approximately three months old (pers. observation). At the end of the trial period, sex ratios were calculated as the proportion of males relative to females [M/(F + M)] for each population under each different color light treatment. Data obtained from the trial were analyzed by a one way analysis of variance and a chi-square test to determine if the sex ratios deviated from the predicted 1:1 norm at a 90% and 95% confidence level. Calculations were performed using the computer software Excel (2016, Microsoft).

Results and Discussion. During the initial acclimation period of three weeks (prior to initiation of the trial), the young emperor tetras appeared healthy and fed normally. Fish deaths occurred but were attributed to common injuries that happen with initial capture and handling of fish at a young age. Throughout the trial the fish behaved and fed normally and appeared healthy. Growth performance and survival of emperor tetra at the end of the experiment are presented in Figure 2. The overall survival among fish in all treatment groups was 79%, which was similar to that of other ornamental fish species like angelfish *Pterophyllum scalare*, molly *Poecilia latipinna*, gourami *Trichogaster leeri*, goldfish *Carassius auratus*, zebrafish *Danio rerio*, and the marine ornamental clownfish

Amphiprion percula (Siccardi et al 2009; Chambel et al 2015; Ali et al 2016; Kumari et al 2017). Also, fish in all treatment groups, gained significant ($p < 0.05$) length and weight indicative that rearing conditions were appropriate during the study period. During the experimental period, fish exhibited first signs of morphological sex differntiation, which by the end of the period, all individual fish could be correctly assigned to a female or male category. The males were significantly ($p < 0.05$) heavier (468±125 mg) and longer (3.4±0.2 in total length, TL) than females (305±5 mg; 2.8±0.4 TL) but there was no difference in their standard lengths (2.3±0.3 and 2.6±0.1, females and males respectively). Males were more colorful than females, and all had the characteristic extended ray in the middle of the caudal fin, giving it the appearance of a trident. In a separate study we confirmed with histology that only males have the trident-shape caudal fin, as has been correctly reported in the aquarium hobby literature and Weitzman & Fink (1971). Also as repeatedly mentioned by hobbyists, external sexual phenotypes of emperor tetras were readily distinguished within the first three months of age.

Color of light	Initial weight, mg	Final weight, mg	Survival
White	178 ± 24	⇧ 396 ± 46	83 %
Green	160 ± 20	⇧ 329 ± 63	67 %
Blue	163 ± 23	⇧ 336 ± 49	87 %
Red	175 ± 29	⇧ 374 ± 54	77 %

Figure 2. Growth performance and survival, of emperor tetra *Nematobrycon palmeri* reared under illumination of LED lights of four different colors (white, green, blue, and red) for a period of 12 weeks. Fish gained significant weight ($p < 0.05$, ⇧) compared to baseline, however no significant differences ($p > 0.05$) were observed in weight or survival under the different color illuminations.

Results from this study indicated that different color LED lights did not have a significant effect ($p > 0.05$) on growth and survival of emperor tetra (Figure 2). These results were different from what was expected since light is one of the important environmental factors that influence survival and growth in many different species of fish. Light intensity influences and mediates photoperiodic adaptations through the functional relationship between the sensory stimulation (receptors) of the brain-hypothalamic-pituitary axis and the various peripheral tissues, organs, and regions of the body (de Vlaming 1972; Munro et al 1990). Such influences were demonstraded in the numerous studies cited in the introduction.

Emperor tetras raised under white and green lights exhibited a sex ratio of 1:1 (chi-square, $p > 0.01$). However, under blue and red lights the sex ratio was skewed to favor females aproximately 3:1 (blue, $p < 0.05$; red, $p < 0.10$) (Figure 3).

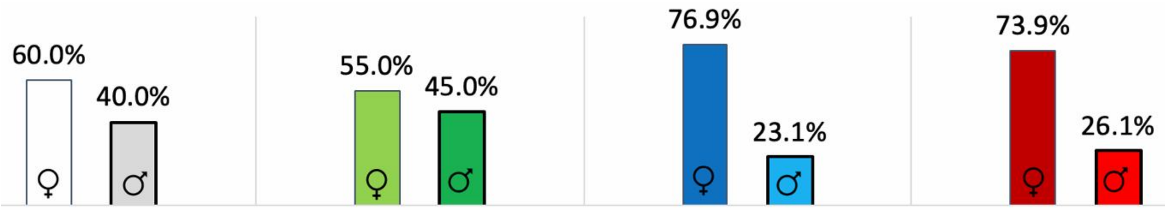

Figure 3. Sex ratios (♀•female and ♂• male) of emperor tetra *Nematobrycon palmeri* reared under illumination of LED lights of four different colors (white, green, blue, and red) for a period of 12 weeks.

Sex determination in fishes is typically determined genotypically (e.g., sex chromosomes or by multiple regions in the genome), as a function of temperature (temperature-dependent sex determination), response to social cues, and anthropogenic pollutants, such as estrogenic compounds (Ospina-Álvarez & Piferrer 2008; Godwin 2010; Bachtrog et al 2014; McCormick & Romero 2017). The first documented case of photoperiod-dependent sex determination in fish (California grunion *Leuresthes tenuis*) was reported by Brown et al (2014), where a long-photoperiod (15 L:9 D) resulted in a higher proportion of females and a standard photoperiod (12 L:12 D) produced more males than females. Also, it has been documented that green light induced female-to-male sex reversal in the medaka *Oryzias latipes* (Hayasaka et al 2019). After extensive literature review we found no other information regarding altering the sex ratio in fishes raised under color light of different wavelengths. Controlling the sex ratio in fishes has great practical applications in aquaculture (Martínez et al 2014). In ornamental fish farming, given the small-scale nature of typical facilities and microlivestock, obtaining a higher proportion of females will be very useful to reduce the number of brood fish needed and increase production of larvae in the farm. The manipulation of photoperiod and light colors will be easy to implement and manage, especially in ornamental fish production.

Conclusions. Different colors of artificial LED light affected the sex ratio but not the growth or survival of an experimental population of the freshwater ornamental emperor tetra fish, *Nematobrycon palmeri*. The sex ratio of emperor tetras was skewed 3 females to 1 male when the population was raised under blue and red LED lights. If this finding is applicable to other ornamental fish species, it may become a very practical tool to reduce the number of brood fish needed and increase production of larvae in the farm. Our finding and that of others, further support the idea that the effect of color lights on fish biological parameters (behavior, survival, growth, reproduction) appears to be species specific.

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