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Color of vertically-suspended environmental enrichment does not effect juvenile rainbow trout growth during hatchery rearing

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Abstract
This study evaluated the effects of three different colors of physical environmental enrichment on the growth of juvenile rainbow trout *Oncorhynchus mykiss* after 54 days of hatchery rearing. Arrays of four aluminum angles painted either red, blue, or green were suspended in covered, 1.8-m diameter circular tanks. Mean (± SE) individual fish lengths (mm) (198 ± 3, 200 ± 2, and 196 ± 4 for red, green, and blue, respectively) and weights (g) (85.1 ± 3.4, 83.4 ± 1.8, and 84.6 ± 2.8 for red, green, and blue, respectively) were not significantly different among the color treatments. There were also no significant differences in total tank weight (kg) (92.8 ± 1.0, 91.7 ± 1.2, and 93.1 ± 0.6 for red, green, and blue respectively) and per cent gain (%) (106.1 ± 2.2, 103.8 ± 2.6, and 106.8 ± 1.3 for red, green, and blue respectively). These results indicate that the rearing benefits of suspended environmental enrichment are likely not influenced by the color of the structure.

Keywords: Rainbow trout, *Oncorhynchus mykiss*, color, environmental enrichment

1. Introduction

The colors experienced by fish have been shown to affect their growth, physiology, and behavior [1-8]. However, color effects appear to be species-specific. Red light produced reduced growth in gilthead seabream *Sparus aurata* [9], but increased growth in rainbow trout *Oncorhynchus mykiss* [10]. Conversely, blue light reduced stress in Nile tilapia *Oreochromis niloticus* [1] but increased stress in rainbow trout [9]. Lastly, African catfish *Heterobrachus bidorsalis* growth [11] improved in black rearing environments, but river catfish *Pangasius hypophthalmus* growth [12] was unaffected by black coloration.

Color is only one aspect of the fish hatchery rearing environment. The structural complexity of the hatchery tank may also impact fish rearing performance. Environmental enrichment by placing structure in rearing units has shown positive effects in raceways [13-14] and circular tanks [13-18]. Kientz and Barnes [17] developed a vertically-suspended array that provided beneficial environmental enrichment, while at the same time still allowing for the hydraulic self-cleaning of circular tanks. Several other studies have documented rearing benefits from similar vertically-suspended structures [18-19].

While color and environmental enrichment have been studied separately, the combination of environmental enrichment and color has received scant attention. In a series of experiments, Batzina and Karakatsouli [20-22] showed improvements to gilthead seabream *Sparus aurata* physiology using blue and red-brown substrate. No published studies have investigated the combined effects of both color and structure in salmonids, and no studies have specifically examined color and vertically-suspended environmental enrichment structures. Thus, the objective of this study was to evaluate the rearing performance of rainbow trout in different colored vertically-suspended environmental enrichment.

2. Methods

2.1 Experiment location and design

This experiment was conducted at McNenny State Fish Hatchery, Spearfish, South Dakota, USA, using degassed and aerated well water at a constant temperature of 11°C (water hardness as $CaCo_3$ = 360 mg/L, alkalinity as $CaCo_3$ = 210 mg/L, pH = 7.6, total dissolved solids = 390 mg/L). The experiment began on 19 September 2019 and ended after 54 days. Approximately 1,120 (45kg/tank) Gerrard strain rainbow trout (mean ± SE, length = 148.9 ± 4.1 mm, mean weight = 36.4 ± 3.0 g, n = 30) were stocked into twelve circular tanks (diameter = 1.8 m, height = 0.8 m, water depth = 0.6 m). Incoming water velocities were approximately 12.2 cm/s.

All experimental tanks were covered with corrugated plastic covers [23] and an array of four aluminum angles (each side 2.5 cm wide, 57.15 cm long), suspended from the overhead covers as described by Krebs *et al*. [18] (Figure 1). Experimental treatments (n = 4) consisted of each angle array being either red, blue, or green.

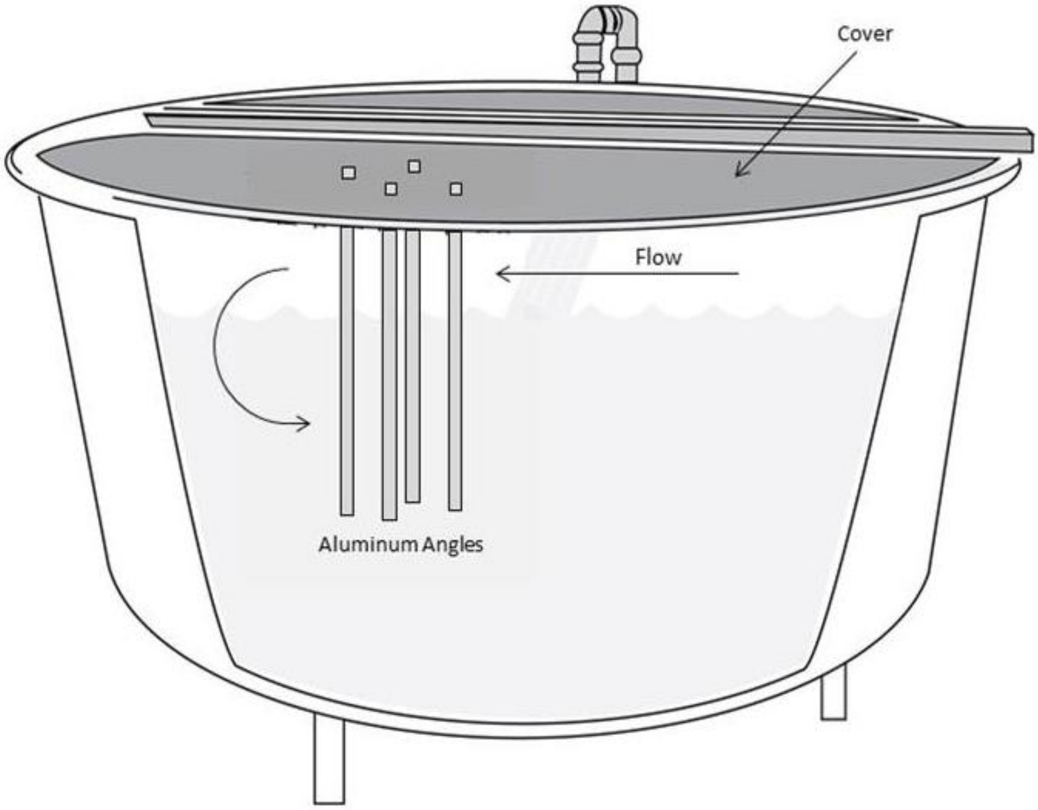

Fig 1: Circular tank with suspended array of four aluminum angles, with the peak of the angle facing in the direction of the water flow.

2.2 Experimental protocol

Fish were fed 1.5 mm floating pellets (Protec, Skretting, Tooele, Utah, USA) every 20 minutes during daylight hours using automatic feeders. Feeding rates were determined using the hatchery constant method [24], with a projected growth rate of 0.08 cm/day and a planned feed conversion ratio of 1.1, which was at or slightly above satiation. Fish were monitored daily for survival.

2.3 Data collection

At the conclusion of the experiment, total tank weights were obtained by weighing all the fish to the nearest 0.2 kg using an Intercomp CS200 hanging scale (Medina, Minnesota, USA). In addition, for individual fish metrics, ten fish were randomly selected from each tank and individually weighed to the nearest 0.1 g and measured (total length) to the nearest 1.0 mm.

The following equations were used

Total weight gain = $\frac{\text{end weight}}{\text{start weight}}$

Feed conversion ratio (FCR) = $\frac{\text{Food fed to tank}}{\text{Total weight gain}}$

Condition Factor (K) = $10^5 * \frac{\text{fish weight}}{\text{fish length}^3}$

Specific Growth Rate (SGR) =

$$100 * \frac{\ln(\text{end weight}) - \ln(\text{start weight})}{\text{number of days}}$$

Per cent Gain (%) = $\frac{\text{Total weight gain}}{\text{Start weight}}$

2.3 Data analysis

Statistical analysis was conducted using the SPSS computer program (Version 24.0; IBM, Chicago, Illinois, USA). One-way Analysis of Variance (ANOVA) was used for data analysis with significance predetermined at $p < 0.05$.

2.4 Ethics

This experiment was performed within the guidelines set out by the Aquatics Section Research Ethics Committee of the South Dakota Game, Fish and Parks and also within the guidelines for the Use of Fishes in Research set by the American Fisheries Society.

3. Results

Rainbow trout mean (± SE) total length was not significantly different among the color treatments and ranged from 196 ± 4 mm for the blue treatment to 200 ± 2 mm for the green treatments (Table 1). Likewise, mean (± SE) individual fish weight (g) was not statistically different among treatments with the red, green, and blue treatments at 85.1 ± 3.4, 83.4 ± 1.8, and 84.6 ± 2.8 g respectively. Specific growth rate and K were also not significantly different among treatments.

Table 1: Mean (± SE) individual fish total length, weight, specific growth rate (SGR[a]), and condition factor (K[b]) for rainbow trout reared with three different colors of suspended environmental enrichment (n = 4).

	Red	Green	Blue
Length (mm)	198 ± 3	200 ± 2	196 ± 4
Weight (g)	85.1 ± 3.4	83.4 ± 1.8	84.6 ± 2.8
SGR	1.18 ± 0.07	1.14 ± 0.04	1.17 ± 0.06
K	1.09 ± 0.04	1.05 ± 0.03	1.13 ± 0.04

[a]SGR = 100 * (ln (end weight) – ln (start weight)) / (number of days)
[b]K = 10^5 x [individual weight / (body length^3)]

Total tank weights were not statistically different among treatments with all treatments, and ranged from (mean ± SE) 91.7 ± 1.2 to 93.1 ± 0.6 kg/tank (Table 2). Similarly, per cent gain was not significant among each treatment with the red, green, and blue treatments averaging 106.1 ± 2.2, 103.8 ± 2.6, and 106.8 ± 1.3 respectively. Both total gain (kg), and FCR were also not significantly different among treatments.

Table 2: Mean (± SE) ending total tank weight, gain, per cent gain, and feed conversion ratio (FCR[a]) for rainbow trout reared with three different colors of suspended environmental enrichment (n = 4).

	Red	Green	Blue
Weight (kg)	92.8 ± 1.0	91.7 ± 1.2	93.1 ± 0.6
Gain (kg)	47.9 ± 1.0	46.7 ± 1.2	48.0 ± 0.6
Gain (%)	106.1 ± 2.2	103.8 ± 2.6	106.8 ± 1.3
FCR	1.13 ± 0.02	1.15 ± 0.03	1.12 ± 0.01

[a] FCR = food fed / gain

4. Discussion
The results from this study differ from other studies that have documented color preferences for rainbow trout. Karakatsouli *et al*. [9] suggested blue ambient light resulted in reduced rainbow trout growth, while Karakatsouli *et al*. [10] reported that red light resulted in the greatest growth of unstressed rainbow trout. Contrarily, Luchiari and Pirhonen [25] stated that red should be avoided during rainbow trout rearing and suggested green as their preferred ambient color for rainbow trout. Papoutsoglou *et al*. [26] concluded that black tanks reduced feeding performance of rainbow trout compared to a lighter white tank. Lastly, Üstündağ and Rad [27] reported better individual growth of rainbow trout reared in beige tanks compared to tanks colored two shades of green and grey. Unlike these prior experiments, this study could not find any influence of red, green, or blue suspended structure on rainbow trout growth.

Color vision in fish is dependent on many factors [28], and there are several possible reasons for the lack of differences in the color treatments used in the current study. First, the relatively small area of the painted aluminum angles in relation to the larger tank may have not provided enough of a color stimulus to warrant a response from the fish. Secondly, although the covered tanks have been shown to improve growth [23], the light intensity was very low which may have influenced the ability of retinal cones to detect color differences [29]. Lastly, at only 54 days, the duration of this study was relatively short, and may have been too brief for significant growth differences among the colors to be detected [30-31].

Unique to this study was the use of vertically-suspended environmental enrichment as the color medium. The possible interaction of the suspended structure color with the turquoise-green colored tanks is unknown. Other studies primarily used tank color [2, 6, 8, 26, 32] or lighting [9, 10, 33-35] as the color medium.

Vertically-suspended structures have been shown to positively impact salmonid rearing performance [17-19, 36-39]. The results of this study indicate that the rearing benefits of suspended environmental enrichment are likely not influenced by the color of the structure.

Other studies have documented species-specific effects of color. Ghavidel *et al*. [8] found that tank color had no effect on orange spotted grouper *Epinephelus coioides* growth. McLean *et al*. [40] also found no impact of tank color on weight gain in Nile tilapia *Oreochromis niloticus* or summer flounder *Paralichthys dentatus*. In contrast, Sabri *et al*. [41] found Nile tilapia preferred blue and green light and declared that red light should be avoided. Mat Nawang *et al*. [12] reported that in comparison to a black background, white or green resulted in improved juvenile river catfish *Pangasius hypophthalmus* growth. Volpato and Bareto [1] stated that blue light lowered stress in Nile tilapia *Oreochromis niloticus*, while Gaffney *et al*. [7] reported that Coho salmon *Oncorhynchus kisutch* select dark backgrounds.

6. Conclusion
In conclusion, the results of this study indicate that the color of vertically-suspended environmental enrichment does not impact rainbow trout growth over a duration of 54 days. Longer-term studies are obviously needed, as is experimentation with a wider variety of colors and different fish species and sizes.

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