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Use of different colours of vertically-suspended structure during the hatchery rearing of juvenile landlocked fall Chinook salmon (*Oncorhynchus tshawytscha,* Walbaum)

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This study investigated the effects of coloured structures on the growth of juvenile landlocked fall Chinook salmon (*Oncorhynchus tshawytscha*, Walbaum) during hatchery rearing. Structures consisted of an array of four aluminum angles painted one of four colours: Silver, red, black and green, which were vertically-suspended in circular tanks. After 25 days, mean total lengths and weights of individual salmon reared in tanks with the green arrays were significantly ($P < 0.05$) larger than those reared with silver, red or black arrays. Condition factor was not significantly different ($P > 0.05$) among the colour treatments. Final total tank biomass and gain were also not significantly different ($P > 0.05$) among the colour treatments. The results of this study indicate that structural colours could be considered to maximize juvenile Chinook salmon growth during hatchery rearing.

Key words: Chinook salmon, colour, *Oncorhynchus tshawytscha*, hatchery rearing.

INTRODUCTION

Relatively little is known about the impacts of colour on cultured fish. The colours used during hatchery rearing may affect feed intake, growth, aggression, stress response and body colouration (Volpato and Bareto, 2001; Strand et al., 2007; Qin et al., 2012; Eslamloo et al., 2015; Gaffney et al., 2016; Ghavidel et al., 2019). However, the effects of specific colours are not universal among species. For example, blue light was found to reduce stress in Nile tilapia (*Oreochromis niloticus*) (Volpato and Bareto, 2001) but increase stress in rainbow trout (*Oncorhynchus mykiss*) (Karakatsouli et al., 2007). African catfish (*Heterobrachus bidorsalis*) growth improved in black tanks (Solomon and Ezigbo, 2018), but black had no impact on the growth of river catfish (*Pangasius hypophthalmus*) (Mat et al., 2019). Color preferences in fish may also change over time (Ullmann et al., 2011).

Colour and substrate interact to influence the hatchery rearing performance of gilthead seabream (*Sparus aurata*). In particular, blue and red-brown substrate

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Table 1. CIELAB color space digital colour values for the tank and painted arrays. L* is the black to white component, a* is the green-red component, and b* is the blue-yellow component.

Colour	L	a*	b*
Tank: Turquoise-Green	50.41	-46.19	1.35
Array: Silver	71.48	-1.92	2.01
Red	31.89	49.84	26.27
Black	9.14	-0.99	1.18
Green	26.54	-23.39	12.57

improved fish growth and reduced aggression compared to green substrate or no substrate at all (Batzina and Karakatsouli, 2012, 2014a, b). In a study comparing blue substrate, a photo of blue substrate, and a plain control, Batzina and Karakatsouli (2014b) observed positive effects on growth from only the blue substrate; the blue colour (photo of blue substrate) was not enough to produce the improvements caused by the substrate itself. Thus, interaction between colour and substrate determines effectiveness.

Vertically-suspended structure is a relatively new form of environmental enrichment. Most studies evaluating vertically-suspended structure have used unpainted aluminum angles, unpainted aluminum rods, or light grey polyvinyl chloride electrical conduit (Kientz and Barnes, 2016; Krebs et al., 2018; White et al., 2018, 2019; Jones et al., 2019). However, strings of randomly coloured spheres were used by Kientz et al. (2018) and Crank et al. (2019), which produced dramatic improvements in weight gain and feed conversion ratio in rainbow trout in comparison to those trout reared in barren tanks. While the focus of these studies was the structures themselves, both studies suggest that colour may have helped to maximize the benefits of vertically-suspended structures. The effects of colour in combination with vertically-suspended structures on fish rearing performance are unknown. In addition, the effects of colour in general during the rearing of landlocked fall Chinook salmon (*Oncorhynchus tshawytscha*, Walbaum) are also unknown. Thus, the objective of this study is to evaluate the effects of different coloured vertically-suspended arrays on landlocked juvenile Chinook salmon growth and feed conversion.

MATERIALS AND METHODS

Experimental design

A 25-day experiment was conducted at McNenny State Fish Hatchery, Spearfish, South Dakota, USA, using 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 using standard methods described by Federation et al. (2005)]. Water temperature did not vary because it originated from 100-m deep wells. On May 3, 2019, approximately 25,000 juvenile landlocked Chinook salmon originating from feral stock from Lake Oahe, South Dakota, USA (mean (± SE) length = 105 ± 3 mm, mean (± SE) weight = 12.3 ± 0.6 g, N = 30) were divided

evenly into 12 turquoise-green circular tanks (diameter = 1.8 m, height = 0.8 m, water depth = 0.6 m). Each tank initially received 25.7 kg of fish (approximately 2,084 fish). The tanks were near-fully covered by corrugated black plastic overhead covers, with only 0.1 lux of light entering the tank (Walker et al., 2016). Each tank also had an array of four aluminum angles (each angle side 2.5 cm wide, 57.15 cm long), suspended from the overhead covers as described by Krebs et al. (2018). Water flows were set to maintain dissolved oxygen levels above 7 mg/L to eliminate any potential effects of relatively low oxygen levels on fish growth (Mallya, 2007) and also to maintain circular tank hydraulic self-cleaning. Substantial turbulence was only observed near the spray bar where water entered the tank.

Colours

The four angles of the array were all of the same colour in each tank. In addition to the unpainted aluminum (silver colour), the other colour treatments were safety red, semi-gloss black, or hunter green. Treatments were randomly assigned with each coloured-array replicated in three tanks (12 total tanks with three tanks per colour). The red array was created by painting the angles with Occupational Safety and Health Administration standard coloured spray paint (Krylon, Krylon Products Group, Cleveland, Ohio, USA). The black and green arrays were created by spray painting the angles with gloss enamel (Rust-oleum, Rust-oleum Corporation, Illinois, USA). Digital colour values were obtained using a MiniScan XE Plus spectrophotometer (HunterLab, Reston, Virginia, USA) and are listed in Table 1.

Feeding

All fish were fed 1.0 mm BioVita (BioOregon, Longview, Washington, USA) daily using automatic feeders. Aliquots of feed were dispensed for one minute at 20-minute intervals over eight hours each day. The hatchery constant method (Buterbaugh and Willoughby, 1967) was used to determine feeding rates with a projected growth rate of 0.07 cm/day and a planned feed conversion ratio of 1.1. Any mortality were removed and counted daily.

Data collection

At the end of the experiment, five randomly sampled individual fish from each tank were both weighed to the nearest 0.1 g and total length measured to the nearest 1.0 mm. In addition, total tank weights were obtained to the nearest 0.2 kg using an Intercomp CS200 hanging scale (Medina, Minnesota, USA). The following equations were used (Piper et al., 1982):

Total weight gain = final tank weight – initial tank weight.
Feed conversion ratio (FCR) = total feed fed to tank / total tank

Table 2. Mean ± SE individual fish total lengths, weights and condition factors (K[a]) for Chinook salmon reared with different coloured enrichment structures. Means in a row with different letters are significantly different (P < 0.05, N = 3).

Colour	Silver	Red	Black	Green	P
Length (mm)	118 ± 1 z	117 ± 3 z	115 ± 1 z	126 ± 2 y	0.034
Weight (g)	16.4 ± 0.7 z	15.7 ± 1.1 z	15.1 ± 0.6 z	20.7 ± 0.7 y	0.004
K	0.99 ± 0.02	0.98 ± 0.02	0.98 ± 0.03	1.05 ± 0.03	0.201

[a]$K = 10^5$ x [individual weight / (body length^3)]

Table 3. Mean ± SE tank total weights, gain, food fed, and feed conversion ratios (FCR[a]) for Chinook salmon reared with different coloured enrichment structures (N = 3).

Variable	Silver	Red	Black	Green	P
Initial weight (kg)	25.7	25.7	25.7	25.7	
Food Fed (kg)	14.7	14.7	14.7	14.7	
Final weight (kg)	37.0 ± 0.6	35.8 ± 0.4	38.2 ± 0.2	37.8 ± 1.7	0.343
Gain (kg)	11.3 ± 1.7	10.1 ± 0.4	12.5 ± 0.2	12.1 ± 0.5	0.343
FCR	1.30 +/- 0.06	1.45 +/- 0.05	1.18 +/- 0.01	1.21 +/- 0.15	0.240
Mortality (%)	0.08 ± 0.03	0.85 ± 0.45	0.06 ± 0.04	0.08 ± 0.02	0.100

[a] FCR = total food fed / tank gain.

weight gain.
Condition factor (K) = 10^5 × [weight / (body length)^3].
Mortality (%) = 100 × (number of fish that died in a tank / initial number of fish in a tank).

Statistical analysis

One-way Analysis of Variance (ANOVA) was used to analyze the data with the SPSS (version 24.0) statistical analysis program (IBM Corporation, Chicago, Illinois, USA). Because the tanks were the experimental units, not individual fish, nested ANOVA was conducted on the individual fish data. If the ANOVA indicated significant differences, Fisher's Protected Least Significant Difference procedure was used for pair-wise comparisons. Significance was pre-determined at P < 0.05.

RESULTS

Individual fish length and weight were significantly different among the colour treatments (Table 2). Individual salmon reared with the green angles were significantly heavier and longer than those reared in any of the other colour treatments. There was no significant difference in condition factor among the treatments. Total tank ending biomass, gain, and feed conversion ratio were not significantly different among the coloured angle treatments (Table 3). Percent mortality was low and not significantly different among the treatments. However, the mortality of 1.7% in one of the tanks containing the red angles was an outlier.

DISCUSSION

The positive impact of green vertically-suspended structures on individual salmon growth after the relatively short time period of only 25 days was somewhat surprising. However, these results are supported by Luchiari and Pirhonen (2008), who observed that rainbow trout preferred green environments over those that were white, blue, yellow, or red. They also noted that the trout reared in green environments were significantly larger after 42 days. This preference could be due to the predominance of blue visual pigment cones in juvenile Chinook salmon (Flamarique, 2005), which would align most closely with the green colour used in this study. Unfortunately, blue-coloured angles were not included in this study for comparison. Ullmann et al. (2011) also described an inherent green colour preference in barramundi (*Lates calcarifer*).

Interestingly, Luchiari and Pirhonen (2008) noted that trout preference for green was temperature dependent, with rainbow trout preferring green at water temperatures of 12°C, but shifting their preference to blue at 1°C. The green colour preference for Chinook salmon in this study was obtained using 11°C water; lower temperatures were not investigated. Colour preferences in fish may not only be influenced by temperature, but also by long term exposure to different colours (Ullmann et al., 2011). It is also possible that salmon color preferences may change seasonally and with developmental stage (Beatty, 1966; Flamarique, 2005).

In contrast to the results of this study with Chinook salmon, Karakatsouli et al. (2007, 2008) reported that red light improved rainbow trout length. The red angles in the present study were associated with significantly shorter salmon in comparison to green angles. These differing results may be due to how the colour was applied (light

versus structure), or the saturation of colour in the rearing environment. The red arrays presented a relatively small area of colour, whereas a red-light source would produce a much more intense colouration.

Papoutsoglou et al., (2005) observed that rainbow trout growth was reduced during rearing in black tanks. Lighter coloured tanks have been associated with increased growth, likely because feed detection is improved due to feed-background contrast (Tamazouzt et al., 2000; Papoutsoglou et al., 2005; Karakatsouli et al., 2007, 2008; Strand et al., 2007; McLean et al., 2008; Üstündağ and Rad, 2015). Strand et al. (2007) reported that at low light intensities, feed intake was higher in lighter tanks than dark tanks, but there was no significant difference at higher light intensities. Light intensity likely does not explain the results of this study, even though the tanks were nearly completely covered. The painted surfaces of the suspended angles occupied a small area in the tank, and the high L values of the silver and red colours indicate that they were lighter than the green and black angles.

While tanks containing the green angles produced significantly larger individual salmon, the possible influence of the turquoise-green rearing tank colour on these results is unknown. Other studies examining colour during rearing have focused on tank colours, rather than suspending novel colours within an already-coloured tank (Browman and Marcotte, 1987; Papoutsoglou et al., 2005; Strand et al., 2007; Eslamloo et al., 2015; Ghavidel et al., 2019). Similarly, studies examining the colour of lighting typically use tanks that are colourless, opaque, or match the lighting colouration (Karakatsouli et al., 2007, 2008; Banan et al., 2011; Elnwishy et al., 2012; Kawamura et al., 2017). The lack of significant differences in total tank gain or feed conversion ratio in this study may be due to the relatively short duration (Weathercup and McCraken, 1999), which occurred because the fish needed to be moved to make room for additional production; the tanks of salmon were only available for 25 days. The duration of this study was shorter than the minimum study duration of 56-84 days recommended by the National Research Council (2011) for fish feeding trials. In a study by de Francesco et al. (2004) differences in rearing performance from trout fed different diets did not appear until after 84 days.

The feed conversion ratios observed in this study are typical for landlocked fall Chinook salmon from Lake Oahe (Barnes et al., 2013). The relatively high rearing densities likely affected feed conversion ratio (Piper et al., 1982; Mazur and Iwama 1993; Mazur et al., 1993; Procarione et al., 1999). Feed conversion ratios of under 1.00 with this strain of Chinook salmon fed this particular diet have only been observed using very low rearing densities (Barnes et al., 2013).

In conclusion, despite the short duration of this study, significant improvements in Chinook salmon growth were observed during rearing with green-coloured vertically-suspended structures as environmental enrichment. Longer duration trials may indicate improvements in gain or feed conversion ratio and should be conducted. Additionally, experiments involving colour and Chinook salmon rearing at different temperatures, different light intensities, or different rearing tank colours would be beneficial.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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