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Research Article

Effects of Background Color on Growth, Stress, Biochemical, Hematological, and Immunological Responses, and Expression of Growth-Related Genes in Oscar Fish (*Astronotus ocellatus*)

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The aim of the current study was to assess the impacts of tank color on the growth, stress, biochemical, hematological, and immunological responses, and expression of growth-related genes in juvenile Oscar (*Astronotus ocellatus*). Therefore, a total of 120 fish (9.14 ± 0.34 g) were distributed into 12 aquariums ($60 \times 50 \times 35$ cm) and divided into four treatments (aquariums with blue, white, yellow, and red colors) in three replicates (10 fish per aquarium). The fish were daily hand-fed ad libitum in three meals at 7:00, 12:00, and 17:00 hr for 56 days. Results showed that Oscar fish cultured in the red aquariums had higher final weight and weight gain and lower feed conversion ratio. Plasma cortisol, glucose, and lactate values of fish were significantly higher, and the amount of white blood cells was lower in the blue aquariums compared to other tank colors. Plasma triglyceride values were significantly higher in the white and yellow groups than the blue aquariums. Moreover, fish cultured in yellow and red aquariums had significantly higher melatonin levels than the blue aquariums. Plasma cholesterol, total protein, albumin, complement 3, and total immunoglobulin values were highest in Oscar fish cultured in the red aquariums. Also, lysozyme and alternative complement (ACH50) activities and complement 4 values of fish cultured in yellow and red aquariums were meaningfully higher compared to blue and white groups. Growth hormone relative gene expression levels were meaningfully higher in Oscar fish cultured in white, yellow, and red aquariums than the blue aquariums. Furthermore, insulin-like growth factor-1 (IGF-1) relative gene expression levels were significantly higher in fish cultured in yellow and red aquariums compared to those cultured in blue and white tank colors. Thus, the most suitable tank color for rearing juvenile *A. ocellatus* is red, while blue tank color is unsuitable.

1. Introduction

Fish culture in artificial environments may affect physiological responses and behavior of farmed fish [1–4]. The tank color is an important parameter for fish welfare and it must be considered for enhancing performance of cultured fish [5, 6]. Rearing tanks can be produced in a wide range of colors. Many studies exhibit that different fish species have better growth and feed conversion ratio (FCR) when cultured in certain tank colors [7]. Tank color can influence the ability of fish to identify food and consequently their feed intake, growth, and survival [8–10]. Different tank colors also influence fish health, stress responses, and even level of aggression [7]. Inappropriate tank colors stress fish, which is verified by the elevation in cortisol values and accordingly affect fish welfare, growth, and survival [11, 12]. Stress has a serious impact on immune modulation in fish [13, 14]. As the tank color affects stress parameters in fish, the immune system may be indirectly influenced by different tank colors [1, 15]. Tank color also affects the secretion of somatotropin and thyroxine hormones, physiological condition, and expression of growth-related genes and regulates the somatic growth of farmed fish [12, 16, 17]. Moreover, tank color impacts fish skin color and consequently affects fish price and consumer confirmation [11].

Suitable tank colors can change depending on different parameters, such as the fish species, the color of feed pellet,

the fish life stage, light intensity, and visual adjustment [1, 9, 18, 19]. For instance, white is an optimal tank color for goldfish (*Carassius auratus*) in terms of growth performance [1] and haddock (*Melanogrammus aeglefinus*) [20], while red is preferred for Caspian Kutum (*Rutilus frisii kutum*) [21], and yellow is optimal color for milkfish (*Chanos chanos*) [22]. Snakeskin gourami (*Trichogaster pectoralis*) cultivates better in blue-colored tank [23], river catfish (*Pangasius hypophthalamus*) prefers white and green tank colors [24], and Asian catfish (*Clarias magur*) cultivates better in black tank colors [19]. Also, red was the most beneficial tank color for hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) and fish cultured in the red tanks had a comparable skin color to catfish sold in market [11]. These results must be taken into attention during preparing the physical environment of tanks or aquariums for rearing fish in order to improve their production [16]. However, there is no information as to which tank color is the best for rearing Oscar fish (*Astronotus ocellatus*).

Some studies on the effects of tank color on the physiological and growth parameters of different fish species have been performed previously. According to Ninwichian et al. [11], fish cultured in the red tanks had higher growth and survival, and less stress as shown by lower values of plasma cortisol as compared to fish cultured in other tank colors (white, green, blue, and black). Similarly, the higher specific growth rates were recorded in Asian seabass (*Lates calcarifer*) reared in the red tanks followed by white and black tank colors [16]. Also, lower values of cortisol were observed in fish cultured in the red tanks as compared to the other colors [16]. Moreover, the snakeskin gourami cultured in blue-colored tanks had a meaningfully higher final weight and less FCR than the fish cultured in black-colored tanks [23]. Also, fish cultured in black-colored tanks showed greater glucose values than the other tanks [23]. The contradictory results were also reported that red and blue backgrounds cause chronic stress and suppress immune response in goldfish [1]. However, no data are available on the impacts of tank colors on stress, physiological and immunological responses, and growth performance in Oscar fish.

The Oscar fish (*Astronotus ocellatus* Agassiz, 1831) belongs to the Cichlidae family and it is one of the most precious species of the ornamental fish. Furthermore, this species is one of the most popular aquarium fish and has a high market price. Because of a worldwide increasing interest in ornamental fishes, this field is growing throughout the world [1]. Therefore, this study was done to assess the impacts of different aquarium colors (blue, white, yellow, and red) on the growth, feed utilization, stress, biochemical, hematological, and immune parameters as well as growth-related genes expression in *A. ocellatus*. Our results are applied as a procedure for enhancing the efficiency of Oscar fish production.

2. Materials and Methods

2.1. Fish and Rearing Conditions. The experiment was done in agreement with the Animal Ethics of Guilan University, Iran. The Oscar (*Astronotus ocellatus* Agassiz, 1831) juveniles were obtained from Guil Berkeh Company located in Rasht, Guilan, Iran. Before the start of the experiment, the fish were stocked into two 300 L glass aquariums and adapted to laboratory conditions for 15 days. During the adaptation period, fish were fed ad libitum in three meals at 7:00, 12:00, and 17:00 hr with a commercial feed (Faradaneh Co., Shahrekord, Iran; 5%–10% moisture, 46%–50% protein, 11%–15% fat, 9%–13% ash, 1.5%–3% fiber, and 1%–1.5% phosphorus). Approximately, 30% of water in the glass aquariums was exchanged daily. After the adaptation phase, a total of 120 Oscar fish with an average weight of 9.14 ± 0.34 g that were approximately the same size in terms of weight and length were randomly distributed into 12 aquariums ($60 \times 50 \times 35$ cm) and divided into four treatments (aquariums with blue, white, yellow, and red background colors) and each treatment in three replicates (10 fish per aquarium). The aquariums walls were covered with blue, white, yellow, or red sheeting, and all these aquariums were placed in the same light intensity. In this research, the fish were reared for 56 days. During the rearing period, fish were also fed ad libitum [25] in three meals at 7:00, 12:00, and 17:00 hr with the aforementioned commercial feed (Faradaneh Co., Shahrekord, Iran). Uneaten foods were gathered 1 hr after each meal, dried to constant at 70°C, and subsequently weighed to estimate food conversion ratio [26]. Feces and other residual materials were siphoned from the aquariums daily. The photoperiod was 12 hr light and 12 hr dark. The water quality factors were measured weekly throughout the experimental period and the ranges were for temperature = 23−24°C, oxygen = 7–7.5 mg/L, total dissolved solids = 250–280 mg/L, pH = 7–7.6, and NH_3 lower than 0.02 mg/L.

2.2. Fish Growth Performance and Sampling. At the end of the experimental period, feeding was stopped for 24 hr and then all fish of different treatments were weighed [25] and growth parameters including weight gain (WG), FCR, condition factor (CF), and survival calculated by the following equations:

$$\text{Weight gain (WG, \%)} = 100 \times \frac{(\text{final weights} - \text{initial weights})}{\text{initial weights}}. \quad (1)$$

$$\text{Feed conversion ratio(FCR)} = \frac{\text{dry feed intake}}{(\text{final weights} - \text{initial weights})}. \quad (2)$$

$$\text{Condition factor(CF)} = 100 \times \frac{\text{final weight(g)}}{(\text{final length(cm)})^3}. \quad (3)$$

$$\text{Survival (\%)} = 100 \times \frac{\text{final number of fishes}}{\text{initial number of fishes}}. \quad (4)$$

Clove powder (200 mg/L) was used to anesthetize the fish before sampling. In order to evaluate hematological factors, six fish from each group (two from each aquarium) were randomly captured and then blood was drawn from the

Table 1: Nucleotide sequences of primers applied to determine growth hormone (GH) and insulin-like growth factor-1 (IGF-1) mRNA expression by real-time PCR.

Sequences of primers			Amplification efficiency (%)
Gene name	Forward	Reverse	
GH	GCAACGTCAGCTCAACAAAA	AAACTCCCAGGACTCAACCA	96
IGF-1	CACTACTGCTGTGCGTCCTC	AATAAAAGCCTCGCTCTCCA	98
Ef1-α	GGCTCGTTTTGAGGAAATCA	ATCTTCCATCCCTTGAACCA	97

Abbreviations. Ef1-α, elongation factor 1-α; GH, growth hormone; and IGF-1, insulin-like growth factor-1.

caudal vein using 2 mL heparinized syringe. Moreover, nine fish from each group (three from each aquarium) were randomly captured and then blood was drawn from the caudal vein using 2 mL heparinized syringes. The blood sample was centrifuged at 5,000 *g* for 10 min at 4°C. The plasma samples were used for stress, biochemical, and immunological analysis. Additionally, nine other fish from each treatment (three from each aquarium) were captured and brain and liver tissues were collected for gene expression studies [25]. Plasma and tissue samples were then immediately frozen in liquid nitrogen and kept at −80°C.

2.3. Hematological Parameters. In order to measure hematocrit, heparinized microhematocrit capillary tubes were filled with whole blood samples and then their ends were closed with a special paste, centrifuged at 650 *g* for 10 min, and finally the amount of the packed cell volume was measured. Hemoglobin content was also determined using cyanmethemoglobin procedure [27]. Red and white blood cells (RBCs and WBCs) counts were measured by the Neubauer counting chamber of the hemocytometer according to the method of [28].

2.4. Plasma Stress and Biochemical Factors. The values of plasma cortisol were measured by radioimmunoassay (RIA), as detailed in Ghaedi et al.'s [29] study. Plasma glucose and lactate values were measured by commercial kits (Pars Azmoon, Karaj, Iran and Biorex Diagnostics, Antrim, UK, respectively) following the manufacturer's procedures.

Plasma cholesterol, triglyceride, total protein, and albumin values were determined by commercial kits (Pars Azmoon, Karaj, Iran) based on the manufacturer's instructions with an automated biochemical analyzer (Technicon RA-1000, USA). Melatonin levels in plasma were measured by commercial enzyme-linked immunosorbent assay (ELISA) kits (IBL-Hamburg, Germany), as described by company.

2.5. Plasma Immune Parameters. Lysozyme activity in plasma was measured turbidimetrically following the procedure of Demers and Bayne's [30] study, according to the lysis of the lysozyme-sensitive bacterium, *Micrococcus luteus* (Sigma). Alternative complement (ACH50) activities were measured using the protocol of Sunyer and Tort [31]. The volumes of plasma complement presenting 50% hemolysis (ACH50) were determined, and the number of ACH50 U/mL was determined. In order to measure the values of complement 3 (C3) and complement 4 (C4), the available commercial kits (Pars Azmoon, Karaj, Iran) were used as explained by company. Plasma total immunoglobulin (Ig) values were measured by precipitation of Ig with polyethylene glycol solution [32]. Total protein values of the sample before and after precipitation were used to calculate total Ig values [32].

2.6. Expression of Growth-Related Genes. The brain and liver tissues of fish were sampled for total RNA extraction by a high pure RNA extraction kit (Roche) based on the manufacturer's procedures. Quantity and purity of RNA were evaluated by absorbance at 260 and 280 nm and sample with the RNA ratio (A260 : A280) greater than 1.8 was selected following electrophoresis on the 1% agarose gels. Elongation factor 1-α (Ef1-α) was applied as the reference gene. Reverse transcription (RT) was performed with 1 μg of total RNA with the random Hexamers and M-MuLV Reverse Transcriptase enzyme kits (Vivantis) based on the manufacturer's instruction.

Real-time quantitative PCR was conducted in triplicates to detect the impacts of the tank colors on the mRNA transcription levels of growth hormone (GH) and insulin-like growth factor-1 (IGF-1) in the brain and liver tissues of Oscar fish, respectively. The sequences of primers (Table 1) used in real-time PCR were designed using Primer 3 software on the basis of the sequences deposited in Gene Bank (NCBI). Real-time quantitative RT-PCR was conducted by a real-time PCR (Rotor Gene-2000), containing 6.25 μL of SYBR Green qPCR Master Mix (×2; Cinnagen), 0.5 μL of cDNA, 1 μL of each primer (Table 1), 4.65 μL of double-distilled and DNase free water, which added a total volume of 13.4 μL. Real-time quantitative RT-PCR program consisted of a denaturation stage at 94°C for 2 min, followed by 40 amplification cycles in 15 s, and then 30 s annealing at 60°C, 30 s extensions at 72°C, and final extension at 72°C for 5 min. The specificity of PCR was determined using the melt curve analysis. After PCR amplification, melt curve analysis was done to verify that there was only one amplified product. Data analysis of the real-time quantitative PCR (relative gene expression) was done in triplicates with Rotor Gene, RG-2000 (Australia) software. The relative CT procedure ($2^{-\Delta\Delta CT}$) was applied [33].

2.7. Statistical Analysis. Data analysis was done using SPSS 25 software. One-way ANOVA was applied to analyze the data (means ± SE) followed by Tukey test to compare the means among treatments. The agreed significance value was $p < 0.05$. Before analysis, data were evaluated for normality and homogeneity of variance with Shapiro–Wilk and Levene tests, respectively.

Table 2: Growth performance and feed utilization of Oscar fish cultured in blue, white, yellow, and red color aquariums for 8 weeks (mean ± SE, $n = 3$).

	Tank color			
	Blue	White	Yellow	Red
Initial weight (g)	9.15 ± 0.12	8.89 ± 0.25	9.33 ± 0.27	9.19 ± 0.11
Final weight (g)	51.90 ± 2.12^{a}	51.06 ± 3.08^{a}	50.69 ± 0.37^{a}	60.38 ± 1.13^{b}
WG (%)	466.27 ± 27.63^{a}	475.21 ± 24.17^{a}	443.59 ± 22.50^{a}	556.61 ± 26.93^{b}
FCR	2.90 ± 0.05^{b}	2.92 ± 0.03^{b}	2.96 ± 0.08^{b}	2.60 ± 0.05^{a}
CF	2.51 ± 0.09	2.33 ± 0.03	2.53 ± 0.11	2.43 ± 0.09
Survival (%)	87.00 ± 5.77	83.33 ± 4.73	90.00 ± 11.54	85.00 ± 7.32

Note. A different superscript in the same row indicates statistically significant differences ($p < 0.05$). *Abbreviations.* CF, condition factor; FCR, feed conversion ratio; WG, weight gain.

Table 3: Plasma stress and biochemical parameters of Oscar fish cultured in blue, white, yellow, and red color aquariums for 8 weeks (mean ± SE, $n = 3$).

	Tank color			
	Blue	White	Yellow	Red
Cortisol (ng/mL)	52.22 ± 0.82^{c}	38.33 ± 0.44^{a}	42.68 ± 1.81^{ab}	38.21 ± 0.59^{a}
Glucose (mg/dL)	63.68 ± 0.50^{c}	54.02 ± 0.68^{b}	56.94 ± 0.89^{b}	50.18 ± 0.71^{a}
Lactate (mg/dL)	29.90 ± 0.57^{b}	23.65 ± 1.45^{a}	26.00 ± 0.57^{a}	22.65 ± 1.52^{a}
Triglyceride (mg/dL)	82.68 ± 0.83^{a}	96.24 ± 2.11^{b}	141.74 ± 1.88^{c}	87.03 ± 1.96^{ab}
Cholesterol (mg/dl)	151.26 ± 1.13^{ab}	150.47 ± 0.94^{a}	155.57 ± 1.43^{b}	161.39 ± 0.85^{c}
Total protein (g/dL)	3.30 ± 0.04^{ab}	3.22 ± 0.02^{a}	3.40 ± 0.01^{b}	3.58 ± 0.08^{c}
Albumin (g/dL)	1.45 ± 0.02^{b}	1.23 ± 0.08^{a}	1.33 ± 0.06^{ab}	1.68 ± 0.01^{c}
Melatonin (ng/L)	9.80 ± 0.35^{a}	10.68 ± 0.40^{ab}	11.33 ± 0.12^{bc}	12.51 ± 0.33^{c}

Note. A different superscript in the same row indicates statistically significant differences ($p < 0.05$).

3. Results

3.1. Growth Performance and Food Efficiency. Growth performance and feed efficiency of Oscar fish cultured in different color aquariums for 8 weeks are presented in Table 2. Fish cultured in the red aquariums had significantly higher final weight and WG compared to those cultured under the other tank colors. Also, Oscar fish cultured in the red aquariums had significantly lower FCR than the fish cultured in the blue, white, and yellow color aquariums. CF and survival of fish were not significantly different among different tank color groups.

3.2. Plasma Stress and Biochemical Parameters. Plasma stress and biochemical parameters of Oscar fish cultured in different color aquariums for 8 weeks are presented in Table 3. Plasma cortisol, glucose, and lactate values of Oscar fish were significantly higher in the blue aquariums compared to other tank colors. Our results also showed that red color aquariums presented the lowest values of plasma glucose.

In the current study, plasma cholesterol level was highest in Oscar fish cultured in the red aquariums. Plasma triglyceride values were meaningfully higher in the white and yellow treatments compared to the blue aquariums (Table 3). Also, plasma total protein and albumin levels were highest in Oscar fish cultured in the red aquariums. Moreover, Oscar fish cultured in yellow and red aquariums had meaningfully higher melatonin values than those cultured in the blue aquariums (Table 3).

3.3. Hematological Parameters. Hematological parameters of Oscar fish cultured in different color aquariums for 8 weeks are presented in Table 4. The significant increase in the amount of WBCs was recorded in Oscar fish cultured in white, yellow, and red aquariums compared to the blue aquariums. Hematocrit (Hct), hemoglobin (Hb), and RBCs of fish were not significantly different among different tank color groups.

3.4. Plasma Immune Responses. Plasma immune parameters of Oscar fish cultured in different color aquariums for 8 weeks are presented in Table 5. Lysozyme and alternative complement (ACH50) activities and complement 4 (C4) values of fish cultured in yellow and red aquariums were meaningfully higher compared to blue and white groups. Also, complement 3 (C3) and total immunoglobulin (total Ig) values were highest in Oscar fish cultured in the red aquariums.

3.5. Gene Expression. Expression of growth-related genes in Oscar fish cultured in different color aquariums for 8 weeks is presented in Table 6. GH relative gene expression levels were meaningfully higher in fish cultured in white, yellow, and red aquariums than the blue aquariums. Moreover, insulin-like growth factor-1 (IGF-1) relative gene expression levels were significantly higher in Oscar fish cultured in

Table 4: Hematological parameters of Oscar fish cultured in blue, white, yellow, and red color aquariums for 8 weeks (mean ± SE, $n = 3$).

	Tank color			
	Blue	White	Yellow	Red
WBC ($\times 10^3$ cell/mm^3)	4.29 ± 0.06^{a}	5.17 ± 0.07^{b}	5.66 ± 0.09^{c}	5.30 ± 0.13^{bc}
RBC ($\times 10^6$ cell/mm^3)	1.68 ± 0.04	1.61 ± 0.01	1.66 ± 0.03	1.60 ± 0.03
Hct (%)	31.07 ± 0.47	30.66 ± 0.43	31.00 ± 0.35	30.16 ± 0.57
Hb (g/dL)	5.70 ± 0.05	5.73 ± 0.04	5.80 ± 0.08	5.55 ± 0.07

Note. A different superscript in the same row indicates statistically significant differences ($p < 0.05$). *Abbreviations.* Hb, hemoglobin; Hct, hematocrit; RBC, red blood cells; and WBC, white blood cells.

Table 5: Plasma immune responses of Oscar fish cultured in blue, white, yellow, and red color aquariums for 8 weeks (mean ± SE, $n = 3$).

	Tank color			
	Blue	White	Yellow	Red
Lysozyme (U/mL)	34.66 ± 1.20^{a}	33.33 ± 0.88^{a}	42.33 ± 3.17^{b}	43.66 ± 2.40^{b}
ACH50 (U/mL)	130.01 ± 0.57^{a}	131.01 ± 0.57^{a}	136.66 ± 1.45^{b}	140.01 ± 2.51^{b}
C4 (mg/dL)	9.23 ± 0.53^{a}	9.93 ± 0.69^{a}	13.73 ± 0.14^{b}	15.53 ± 1.05^{b}
C3 (mg/dL)	43.66 ± 0.88^{a}	46.33 ± 1.20^{a}	49.33 ± 1.45^{a}	56.66 ± 2.72^{b}
Total Ig (mg/mL)	18.16 ± 0.24^{a}	18.66 ± 0.23^{a}	18.96 ± 0.26^{a}	21.36 ± 1.09^{b}

Note. A different superscript in the same row indicates statistically significant differences ($p < 0.05$). *Abbreviations.* ACH50, alternative complement; C4, complement 4; C3, complement 3; and Total Ig, total immunoglobulin.

Table 6: Expression of growth-related genes in Oscar fish cultured in blue, white, yellow, and red color aquariums for 8 weeks (mean ± SE, $n = 3$).

	Tank color			
	Blue	White	Yellow	Red
GH (relative expression)	0.29 ± 0.03^{a}	0.59 ± 0.05^{b}	0.85 ± 0.09^{b}	0.59 ± 0.04^{b}
IGF-1 (relative expression)	2.57 ± 0.27^{a}	3.18 ± 0.14^{a}	7.45 ± 0.65^{b}	7.26 ± 0.97^{b}

Note. A different superscript in the same row indicates statistically significant differences ($p < 0.05$). *Abbreviations.* GH, growth hormone; IGF-1, insulin-like growth factor-1.

yellow and red aquariums compared to fish cultured in blue and white tank colors.

4. Discussion

The impacts of tank colors on the growth performance and feed efficiency in terms of the FCR of fish may be described by the contrasting color between the food pellet and the tanks [1, 5, 10]. In the current study, Oscar fish cultured in the red aquariums had meaningfully higher final weight and WG and significantly lower FCR than the fish cultured in the other tank colors. Similarly, WG, SGR, and protein efficiency ratio (PER) were meaningfully higher in Asian seabass juveniles cultured in the red tanks compared to the blue tanks [16]. Furthermore, juvenile hybrid catfish cultured in the red tanks showed higher final weight, WG, and SGR and the lower FCR compared to fish cultured in other tanks [11]. Moreover, the FCR of juvenile common carp cultured in red background meaningfully decreased compared to other colors [34]. The use of red culture tank can improve the color contrast between the food pellet and the tank, and consequently, the visibility of food, supporting feed efficiency, and growth performance. If the color of the food pellet obviously contrasts with the tank color, fishes are capable to see the food obviously. Also, background lighting can influence the visual adjustment of fish, and consequently the fish capability to see food. Several fish species presented better visual adaptation to low-light culture environment, while some fish species act better in relatively clear culture environment [35, 36]. The capability to see food obviously allows fishes to use more food and reduces the energy demands of searching for food, which contributes to higher growth rate and feed utilization efficiency [11, 18, 23].

Our findings reveal that red is the most suitable aquarium color for rearing Oscar fish juveniles in terms of growth and feed utilization. In contrast, Ruchin [37] indicated the higher growth rate in guppies (*Poecilia reticulata*) when reared in blue tanks. Also, Eslamloo et al. [1] exhibited that growth performance factors of goldfish, except for the CF, obviously improved in white backgrounds compared to the other colors. Moreover, daily feed intake, FCR, and plasma glucose levels were higher in fingerling grouper (*Epinephelus Coioides*) cultured in the blue tanks compared to the black and white tank colors [38]. Furthermore, some researches have shown that tank color does not meaningfully influence the growth performance of several fish species, for

instance, scaled carp (*Cyprinus carpio*) [39], juvenile beluga (*Huso huso*) [40], convict cichlid (*Cichlasoma nigrofasciatum*) [41], and juvenile Caspian Kutum [5]. The previous reports and our findings indicate that tank color can be a key parameter that affects the growth performance and feed efficiency in cultured fish, and the level of this effect depends on the fish species [1, 18, 42]. Besides, the fishes of different sizes may react differently to tank color [9, 16].

Inappropriate tank colors intensified stress response in farmed fish [6, 16]. It is shown that chronic stress leads to lower growth in fish which is because of the energy consumption to compensate stress-related elevated energy demands and for setting of body homeostasis [43]. Ruchin [37] also indicated that optimal light color for each fish species plays the role of antistress factor. Our results showed that white, yellow, and red tank colors presented lower values of plasma cortisol and therefore seem to be less stressful to Oscar fish. Similarly, plasma cortisol values of juvenile hybrid catfish cultured in the red tanks were lower than the other (white, green, blue, and black) tanks [11]. Furthermore, juvenile Asian seabass cultured in the red tanks showed a lower values of cortisol as compared to the other tank colors [16], indicating that red tank color is less stressful for rearing this species. This can be one of the reasons for the enhanced growth of fish cultured in the red tanks in their study; because it occurred with the higher WG, SGR, and PER [16].

Plasma cortisol values of juvenile beluga sturgeon were lowest in the black tanks as compared to white and blue tank colors [44]. Also, plasma glucose levels of beluga were meaningfully lower in the black tanks as compared to white and blue tanks [40]. The values of plasma cortisol considerably increased in postsmolt Atlantic salmon (*Salmo salar*) cultured under high blue light intensity [45]. Moreover, various tank colors caused significant changes in plasma cortisol values in scaled carp and goldfish [1, 39]. Thus, the tank colors may influence differently the stress indices of fishes in various experiments.

Glucose values have been associated with cortisol as secondary stress responses. Plasma cortisol enhances plasma glucose because of the elevating requirements for energy in stressful conditions [46]. Plasma glucose and lactate values of Oscar fish were meaningfully higher in the blue aquariums which were in line with higher cortisol values as compared to other tank colors in our study. Thus, the current study indicates that blue aquariums can cause stress in Oscar fish and must be avoided in the culture of this species. Blue aquariums may have contributed to the decline in growth caused by the elevated plasma cortisol, glucose, and lactate values. The decline in growth as an indirect result of the response to inappropriate tank color has been reported in some fish species, for instance, scaled carp [39] and African catfish (*Clarias gariepinus*) fingerlings [47]. The significantly higher plasma glucose values of Oscar fish in the blue, white, and yellow aquariums may describe the meaningfully high FCR in these aquariums than the red aquariums. When fishes are cultured in inappropriate colored tanks, the energy stores that might have contributed to growth can be consumed to cope with stressful conditions, leading to lower fish growth performance [48].

Monitoring physiological condition and fish health is usually done by evaluating blood biochemical and hematological parameters [1, 16]. In fingerling grouper, plasma triglyceride values were meaningfully higher in the white tanks [38], while no significant differences had been reported in cholesterol and triglyceride levels among scaled carp adapted to black, green, and white backgrounds [39]. In the current study, plasma cholesterol level was highest in Oscar fish cultured in the red aquariums. Also, plasma triglyceride values were meaningfully higher in fish cultured in white and yellow aquariums and lower in the blue aquariums, suggesting that blood lipid stores were decreased as a result of stressful conditions to cope with the elevated energy demands, as lipids are the important energy store in most fish [49, 50]. The results coincided with the higher plasma cortisol values in the blue color aquariums, exhibiting the reduction of the blood lipid stores in these fish.

Blood proteins have important functions in fish physiology and immune responses [38]. Plasma total protein and albumin were greater in fingerling grouper cultured in the black tanks compared to the blue and white tanks [38], while plasma total protein, albumin, and globulin in goldfish did not markedly alteration in different background colors [1]. Moreover, plasma albumin levels in beluga juveniles were not influenced by tank color [40]. In the current study, plasma total protein and albumin were highest in Oscar fish cultured in the red aquariums, exhibiting fish immune status was improved by rearing in the red color aquariums.

Melatonin is known to be the important light perception hormone secreted by the pineal organ in fish with a daily light/dark pattern [51]. Melatonin can adjust the physiology of growth, feed intake, stress and immune responses, and reproduction by special behavioral patterns [52]. According to Migaud et al. [45], no significant difference was recorded in plasma melatonin values among postsmolt Atlantic salmon subjected to blue low, blue medium, blue high, and white high constant light regimes. Amiya et al. [53] also showed that melanin-concentrating hormone (MCH) values in the brain and plasma were higher in barfin flounder (*Verasper moseri*) cultured in the white tank compared to the black tank. Their results indicate that production and release of MCH are increased with the white tank color, and MCH is associated with somatic growth and skin pigmentation. In our study, Oscar fish cultured in the red aquariums had higher melatonin levels and growth rates and lower values of plasma cortisol, glucose, and lactate than those cultured in the blue aquariums. It is also reported that intraperitoneal injection of melatonin increased WG and growth in goldfish [54]. Moreover, our study has argued that the value of melatonin in Oscar fish is associated with the stress level. This was also revealed in tilapia (*Oreochromis mossambiques*) that plasma cortisol reduces melatonin secretion in the pineal gland [55].

Hematological parameters are measured as reliable indices of fish health and physiological condition [1, 56]. The elevation in Hct and Hb is a method to meet the elevated

requirement for oxygen by enhancing the oxygen-carrying capacity of blood in stressful conditions [57]. In the current study, Hct, Hb, and RBC of fish were not significantly different among different tank color groups. Some previous researches have reported similar findings. Hematological parameters of juvenile beluga, including RBC, WBC, Hct, and Hb, did not significantly differ among tank color groups [40]. Similarly, neither Hct nor Hb were influenced by tank color in rearing of juvenile beluga sturgeon [44]. Tank colors did not influence Hct, Hb, RBC, lymphocytes, and WBC in juvenile Asian seabass at the end of the experimental period [16]. Also, no significant impacts of background color were reported on the Hct and Hb in Asian seabass [58]. Furthermore, WBC, RBC, Hct, and Hb values of goldfish did not significantly alteration in the various background colors [1].

In the present study, the significant increase in the amount of WBC in Oscar fish cultured in white, yellow, and red aquariums apparently stemmed from lower plasma cortisol values in these groups [1]. Few studies on the impacts of tank colors on fish immune response are available. Eslamloo et al. [1] exhibited that red and blue background colors cause chronic stress and suppress immune system in goldfish. In their study, plasma ACH50 and bactericidal activities of fish among different background colors were not statistically significant, while plasma total antiprotease and lysozyme activities in white and black background colors were meaningfully higher compared to those cultured in blue or red background colors [1].

In the current study, lysozyme and ACH50 activities and C4 values of Oscar fish cultured in yellow and red aquariums were meaningfully higher compared to blue and white groups. Also, C3 and total Ig values were higher in fish cultured in the red aquariums. Thus, the majority of immune factors evaluated in our study were suppressed in blue and white groups, and red tank color was the most appropriate color with regard to the fish immunity. Such immunomodulation by the tank color might be described by the impact of tank color on the α-melanocyte-stimulating hormone (αMSH) and MCH, which in turn cause the changes in immunological parameters [59]. It is reported that αMSH and MCH may activate immune factors of fish such as respiratory burst activities and leucocytes proliferation [60, 61, 62]. Stress-related hormones like cortisol are also the immunomodulators in fish [14, 59, 63]. On the other hand, continued release of cortisol is known to be a robust immunosuppressive factor [14]. In our research, the lower values of C4, C3, and total Ig and lower activities of lysozyme and ACH50 in blue color aquariums were accompanied by the greater cortisol values, indicating the immunosuppressive impacts of the long-term release of this hormone in Oscar fish.

Compared to the other vertebrates, the GH/IGF-1 axis seemed to be strongly related to the growth performance in fish [64]. In the current study, GH relative gene expression levels were meaningfully higher in Oscar fish cultured in white, yellow, and red aquariums than the blue aquariums. Moreover, IGF-1 relative gene expression levels were meaningfully higher in fish cultured in yellow and red aquariums compared to those cultured in blue and white aquariums. Our results also showed that fish cultured in the red color aquariums presented the greatest growth. In addition, Oscar fish cultured in the red aquariums had higher melatonin levels than the blue aquariums in our study. According to Cánepa et al. [65], higher transcript values of somatolactin α, which belongs to the GH superfamily, in the pituitary gland were reported in the cichlid (*Cichlasoma dimerus*) cultured in the black background color compared to fish cultured in the white backgrounds. A high number of melanophores was also reported in fish reared in the black background compared to the white backgrounds [65]. Tsalafouta et al. [17] showed that expression levels of genes related to the GH/IGF growth axis in greater amberjack (*Seriola dumerili*) larvae cultured in the tanks with white background were increased compared to the fish cultured in black and green background colors. These variations coincide with the higher survival rates in fish cultured in the tanks with white background [17]. Tian et al. [66] also showed that long photoperiod can increase the growth of blunt snout bream (*Megalobrama amblycephala*) juveniles and relative mRNA expression levels of GH and IGF-1 enhanced as light time raised from 8 to 16 hr daily. Furthermore, plasma IGF-1 values correlate positively with growth rate changes in olive rock fish (*Sebastes serranoides*) [67].

5. Conclusions

The Oscar fish cultured in the red aquariums had higher final weight and WG, lower FCR, and higher immune competence when compared to those cultured in aquariums of other colors. Also, GH and IGF-1 relative gene expression levels were meaningfully higher in fish cultured in yellow and red aquariums. The fish cultured in the blue aquariums had the highest stress levels as exhibited by high values of plasma cortisol, glucose, and lactate compared to other tank colors. These findings reveal that the most suitable tank color for the efficient production of juvenile *A. ocellatus* is red, while blue tank color is unsuitable. It is suggested that this research be performed in different life stages of the Oscar fish. Also, the effects of other colors on the performance of this species can be evaluated.

Data Availability

The data are valid from the corresponding author upon credible requests.

Conflicts of Interest

The authors state that they have no known competing financial interests or personal relationships that could have appeared to affect the experiment written in the current paper.

Authors' Contributions

Hakimeh Dopeikar contributed to fish maintenance, sample collection, and writing the manuscript. Majidreza Khoshkholgh

contributed to data interpretation, design of the experiment, and funding acquisition. Seyed Ahmad Ghasemi contributed to gene expression analysis and methodology. Vahid Morshedi contributed to analysis of the data, design of the experiment, and methodology.

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