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The effects of blue and red light color combinations on the growth and immune performance of juvenile steelhead trout, *Oncorhynchus mykiss*

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ABSTRACT

To examine the growth and immune response of steelhead trout (*Oncorhynchus mykiss*) under conditions of differing light color, we investigated the effects of different red and blue light color combinations on plasma non-specific immunity, hepatic antioxidant capacity, and GH/IGF-1 axis gene expression in juvenile steelhead trout (initial weight: 34.67 ± 2.69 g). The 16-week trial was set up with six treatments in which light color varied at a constant light intensity of 150 lx: 12 h white light + 12 h dark (12 W), 12 h blue light + 12 h dark (12B), 12 h red light + 12 h dark (12 R), 1.5 h blue light + 9 h red light + 1.5 h blue light + 12 h dark (3B9R), 3 h blue light + 6 h red light + 3 h blue light + 12 h dark (6B6R), total 12 h of blue and red light + 12 h dark (T12BR). The results revealed that the 12B and 3B9R light environments significantly enhanced plasma immunoglobulin M levels in steelhead trout, and that the 3B9R light environment also promoted significant increases in the gene expression of hepatic growth hormone receptor, insulin-like growth factor 1, and insulin-like growth factor-binding protein. The 3B9R light environment was also found enhance the antioxidant capacity of trout by increasing hepatic superoxide dismutase, catalase, and glutathione peroxidase activities. In contrast, trout exposed to the 6B6R light environment showed significant reductions in growth and immune performance, and there were similar reductions in hepatic antioxidant capacity. Furthermore, fish exposed to the 12R light environment were found to be characterized by elevated levels of lipid peroxidation and malondialdehyde, which are indicative of hepatic stress. In addition, we observed that in response to light variation, there were elevations in the activities of glutamic oxaloacetic transaminase and lactic dehydrogenase in the plasma of steelhead trout, thereby indicating an inhibition of cardiomyocyte function. In summary, our findings indicate that a 3B9R light environment simulates the blue-red-blue daily rhythm of light color variation in natural underwater environments, which is beneficial with respect to enhancing the growth and immunity of steelhead trout, and could accordingly be used to provide suitable light conditions in commercial trout production. However, sudden changes in light color should be avoided in the aquaculture of trout.

1. Introduction

In aquaculture, cultivated animals experience natural rhythmic changes in water temperature, light, and other environmental factors, the appropriate variation of which can accelerate their growth (Dong et al., 2006; Guo et al., 2012). Light, as an important exogenous

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environmental factor, affects all developmental stages of aquatic animals (Villamizar et al., 2011). Not only can it stimulate the visual organs but also influences growth and immunity by regulating the synthesis and secretion of endocrine hormones. Consequently, a suitable light environment is conducive to improving fish welfare (Ruchin, 2021).

The light environment comprises light intensity (quantity), photoperiod (duration), and light color (spectrum) (Zhang et al., 2020), and given the diverse ecological habits of fish and their inherent visual functions, different light colors have varying influences on specific physiological characteristics, thereby having differing effects on growth and immune performance (Ruchin, 2018; Wu et al., 2020). A red light environment can, for example, strengthen the growth performance of pikeperch (*Sander lucioperca*) (Baekelandt et al., 2019) and yellow perch (*Perca flavescens*) (Head and Malison, 2000), as well as enhancing the antioxidant capacity of Atlantic salmon (*Salmo salar*) (Vera et al., 2010), whereas blue light has been found to improve the growth performance of goldfish (*Carassius auratus*) (Noureldin et al., 2021) and turbot (*Scophthalmus maximus*) (Wu et al., 2021), and reduce oxidative stress and enhance immunity in cinnamon clownfish (*Amphiprion melanopus*) (Choi et al., 2012). Interestingly, Karakatsouli et al. (2008, 2007) have reported that whereas a red light environment is beneficial in terms of rainbow trout (*Oncorhynchus mykiss*) growth, a blue light environment was found to significantly inhibit its growth. Conversely, Guller et al. (2020) found that a blue light environment can enhance the antioxidant capacity of rainbow trout, thereby improving immune performance. However, most of the previous studies examining these effects of light have tended to focus of the influence of single light colors or white light (full spectrum) on fish growth and immunity, whereas there is currently limited information available regarding the effects of light color variation on the physiological ecology of fish.

In natural water bodies, the spectral composition (light color) of sunlight that penetrates to a given water layer changes corresponding to daily changes in the sun's altitude (i.e., the angle at which incident light strikes the water) (Ruchin, 2018). Rainbow trout, a species that is extensively cultivated worldwide, has two recognized ecotypes, namely, the landlocked and anadromous forms, the latter of which is also referred to as the steelhead trout (Pearse et al., 2019). In the present study, we investigated the light color requirements of steelhead trout from a physiological perspective. Specifically, we investigated the effects of six combinations of blue and red light on plasma immunobiochemical indices, hepatic antioxidant capacity, and hepatic GH/IGF-1 axis gene expression to identify suitable light color conditions.

2. Materials and methods

2.1. Sampling

Juvenile triploid steelhead trout eggs were purchased from Troutlodge Inc (Washington, USA) and hatched at the Wanzefeng Fishery Company (Rizhao, Shandong, China). The trial was conducted in the laboratory of the Wanzefeng Fishery company using the flow through rearing system.

Prior to commencing the trial, the juvenile trout were acclimatized to a brackish saltwater environment (salinity: 14.2 ± 0.7) for two weeks, during which time, the fish were fed to apparent satiation twice daily at 08:00 and 18:30 with a commercial trout feed and maintained on a 24-h oxygen supply and 12L:12D photoperiod.

2.2. Trial design

For the purposes of light evaluation, we utilized the mean value of light intensity measured in the surface, middle, and bottom water layers as a baseline, and established the following six light color treatments: 12 h white light + 12 h dark (12 W, Full spectrum, 150 lx); 12 h blue light + 12 h dark (12B, peak at 454.9 nm, 150 lx); 12 h red light + 12 h dark (12 R, peak at 614.8 nm, 150 lx); 1.5 h blue light + 9 h red light + 1.5 h blue light + 12 h dark (3B9R, peaks at 454.9 nm and 614.8 nm, respectively, 150 lx); 3 h blue light + 6 h red light + 3 h blue light + 12 h dark (6B6R, peaks at 454.9 nm and 614.8 nm, respectively, 150 lx); 12 h blue light and red light + 12 h dark (T12BR, peaks at 454.9 nm + 614.8 nm, respectively, 75 lx). For all treatments, the photoperiod was a 12L:12D cycle (light period: 07:30–19:30). Light intensity and spectra were determined using a hand-held illuminometer (PLA-300; Everfine Inc., China). The 16-week trial was based on a completely randomized block design with four replicates per treatment, and with each replicate group comprising 20 fish per tank (380-L volume, 0.72 m height × 0.95 m diameter) with the total 480 fish. Each fish was weighed individually using an electronic balance (Mettler-Toledo International, Inc., Greifensee, Switzerland).

At the beginning and end of the trial, feeding was suspended for 36 h to ensure that the digestive tracts of fish were completely empty. Prior to commencing the trial, the fish were anesthetized by immersing in a 30 mg/L solution of tricaine methanesulfonate (MS-222; Sigma Chemicals Inc., USA) solution, after which they were gently blotted dry with a tissue, and weighed. During the period of culture, all trout (initial weight 34.67 ± 2.69 g) were fed twice daily (at 08:00 and 18:30) with commercial trout feed. Residual unconsumed feed was collected after feeding for 30 min, and the daily feed intake was calculated by measuring the feed moisture content and with correction of feed intake for leaching. Water was renewed via a single-flow system at a water flow rate of 1.15 L/min. Water temperature, salinity, dissolved oxygen content, and pH were monitored three times daily using a YSI ProPlus handheld multiparameter meter (YSI Inc., USA). Water samples were collected at 3-day intervals, and total ammonia nitrogen (TAN), phosphate, nitrite nitrogen, and nitrate nitrogen concentrations were analyzed using a Cleverchem 380 automatic chemical analyzer (DeChem-Tech Inc., Hamburg, Germany).

During the culture period, values for the water quality parameters (mean ± standard deviation) temperature, salinity, dissolved oxygen, pH, TAN, phosphate, nitrite nitrogen, and nitrate nitrogen were maintained at 16.5 ± 0.2 °C, 14.2 ± 0.7, 8.7 ± 0.3 mg/L, 7.3 ± 0.1, at 0.03 ± 0.03 mg N/L, 0.11 ± 0.08 mg P/L, 0.09 ± 0.05 mg N/L, and 3.43 ± 1.9 mg N/L, respectively.

At 4-week intervals, the fish in each tank were treated with 30 mg/L tricaine MS-222, counted, and all the fish were group weighed for each tank. At the conclusion of the trial, he survival rate was 93.75 ± 6.95, and no significant difference was observed among all groups. At the time of sampling, three fish were randomly selected from each tank and immediately anesthetized with 100 mg/L MS-222. Hepatic tissues were immediately collected and frozen in liquid nitrogen, whereas blood samples were collected in heparin sodium anticoagulant EP tubes, centrifuged at 10,000 rpm for 10 min at 4 °C (Sorvall RC 3C Plus centrifuge; Thermo Fisher Scientific, USA) to separate plasma, and immediately thereafter placed in liquid nitrogen. All the samples were then transferred to a − 80 °C ultra-low temperature freezer for provisional preservation.

2.3. Plasma immunobiochemical analyses

The plasma levels of immunoglobulin M (IgM), alkaline phosphatase (ALP), alanine transaminase (ALT), total bilirubin (T-Bil), aspartate transaminase (AST), and lactate dehydrogenase (LDH) were determined using a Cobas C-311 automatic biochemical analyzer (Roche/Hitachi Inc., Basel, Switzerland). All commercial kits were obtained from Roche Inc. (Basel, Switzerland).

2.4. Hepatic antioxidant enzyme and oxidative damage marker analyses

Having been accurately weighed, hepatic tissues were placed in prechilled saline according to a weight (g):volume (mL) ratio of 1:9, and thereafter the tissues were homogenized using an automatic freezer mill

grinder (Jingxin Industrial Development Inc., Shanghai, China). The tissue homogenate solutions thus obtained were centrifuged at 3500 rpm and 4 °C for 10 min, and the resulting supernatants were collected for analyzes. Commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China; A001–3–2, A007–1–1, A005–1–2, A045–4–2, A106–1–3, and A003–1–2) were used to determine the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), and the contents of total protein (TP), lipid peroxide (LPO), and malondialdehyde (MDA) in the liver homogenates. SOD activity was determined by the WST-1 method, CAT activity by a visible light method, GPx activity by a colorimetric method, TP content by the bicinchoninic acid (BCA) method, and LPO and MDA content by the thiobarbituric acid (TBA) method. The activities of SOD, CAT, and GPx were determined as previously described by Li et al. (2020)., and the TP, LPO, and MDA levels were similarly determined by Guo et al. (2020). All assays were conducted strictly in accordance with the manufacturer's instructions. The assays generated colored products, the absorbance values of which were measured at 450, 405, 405, 562, 586, and 532 nm, respectively, using a microplate reader (Synergy2; BioTek Inc., USA). The activities of SOD, CAT, GPx, and MDA were presented as units per milligram of protein, and the activity of LPO was presented as units per gram of protein.

2.5. Total RNA extraction and gene expression analyses

Total RNA was extracted from hepatic tissues using a UNIQ-10 column Trizol total RNA extraction kit (Sangon Biotech Inc., Shanghai, China), with the concentration and purity of the extracted RNA being determined at OD_{260} and OD_{280}, respectively, using a Nano-300 microspectrophotometer (Allsheng Inc., Zhejiang, China). RNA quality was evaluated by the electrophoresis of samples on 1.5% agarose gels.

cDNA was amplified from the extracted RNA by reverse transcription using a Goldenstar RT6 cDNA Synthesis Kit (Ver 2) according to the manufacturer's instructions. The cDNA products were diluted to appropriate concentrations and used as qPCR templates. The quantity of cDNA in each sample was normalized using a 2 × T5 Fast qPCR Mix (SYBR Green I) with the β-actin housekeeping gene being used as an endogenous control. All data were analyzed using the $2^{(-\triangle\triangle Ct)}$ method (Abraham et al., 2020). The PCR reaction conditions were as follows: pre-denaturation at 95 °C for 1 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 15 s, and extension at 72 °C for 30 s. The sequences of the primers used for amplification are listed in Table 1.

Table 1
Sequences of the primers used for quantitative real-time PCR.

Target gene	Primer sequence (from 5′ to 3′)	GenBank accession no.	Product size (bp)
GH-1	F: CCCCAAACAAACGACAACATACT R: GCGATGTTGAAGAGCCGTTG	XM_020486328.1	174
GH-2	F: CTCCAGGGGTTTTCAGGCATT R: CAGTAGCACCGCCATGAAC	XM_020486328.1	157
GH-3	F:GCCAACTGCACTCTGTAGAC R: AGAATCTCAATTGAAAATGCACCTC	XM_020486328.1	124
GHRH	F: GCTCAGTTCATACACTCTGGC R: TGCATTTGTTTCAGTTGGTAGGA	XM_021609419.2	124
GHR	F: TGCAGATGGAATGTCGGAGT R: CGTAGCGAGGACATTCACCC	NM_001124535.1	118
IGF-1	F: TGGGGATGTCTAGCGGTCAT R: AAGTCAGGGTTAGGACGCAC	XM_021577176.2	120
IGFBP	F: GGGATCCTAGACCCCTCCACT R: CAAAGGGGGTGTTGAGTCCC	NM_001124561.1	132
β-actin	F: GGAACGGTGAAACAGCGGA R: CAGCCTTCACAGAGGCAAATAC	NM_001124235.1	182

Note: GH: growth hormone; GHRH: growth hormone-releasing hormone; GHR: growth hormone receptor; IGF-1: insulin-like growth factor 1; IGFBP: insulin-like growth factor binding protein; β-actin: housekeeping gene

2.6. Parameter calculations

The specific growth rate (SGR) of trout was calculated as follows:

$$SGR\ (\%/day) = 100 \times (\ln W_t - \ln W_0)/t,$$

where W_t and W_0 are the final and initial wet body mass (g) of fish, respectively, and t is the feeding duration (day).

Standardized and normalized hepatic immune indices were calculated as follows:

Zero-mean Standardization:

$$x_i^{'} = (x_i - \bar{x})/s$$

Min-max Normalization:

$$x_i^{'} = [x_i - min(x)]/[max(x) - min(x)],$$

where x_i, $\bar{x}$, and s are the sample data, sample mean, and standard deviation, respectively, and $max(x)$ and $min(x)$ are the maximum and minimum values for the sample data, respectively.

Comprehensive hepatic immunity was evaluated using the following equation:

$$T = X_{CAT} + X_{GPx} + X_{SOD} - X_{LPO} - X_{MDA}$$

where T is the comprehensive immune index for the hepatic tissue, and X_{CAT}, X_{GPx}, X_{SOD}, X_{LPO}, and X_{MDA} are the standardized or normalized values for the respective selected hepatic immune indices.

2.7. Statistical analyses

All data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, North Carolina, USA). The normal distribution and homogeneity of variance of the data were initially assessed using Shapiro–Wilk and Levene tests, respectively. Normally distributed homogeneous data were analyzed using a one-way analysis of variance (ANOVA), with mean values being compared using Tukey's multiple comparison tests. For analysis of the non-normally distributed data, we applied the non-parametric Mann–Whitney rank sum test and a Kruskal–Wallis ANOVA of ranks. The results are expressed as means ± standard deviation (means ± SD), with differences being considered significant at $P < 0.05$.

3. Results

3.1. Plasma immunobiochemistry

Data obtained of steelhead trout for the SGR in Supplementary Fig. 1. The plasma levels of IgM, ALP, ALT, T-Bil, AST, and LDH are presented in Table 2. We found that the SGR of trout in the 3B9R and 12 R groups was significantly higher than that of 6B6R group fish ($P < 0.05$). Levels of immunoglobulin IgM, were observed to be significantly higher in the 12B and 3B9R group trout than in those of the 12 W group, whereas ALP content in the 12 R and 6B6R groups were significantly higher than those in the 12B and 12 W groups ($P < 0.05$), and highest ALT contents were detected in the 6B6R group, which were significantly higher than those recorded in the 12B and 12 W groups ($P < 0.05$). Furthermore, 12 R group trout were found to have the highest T-Bil contents, which were significantly higher than those in the 3B9R, 12B, and 12 W groups ($P < 0.05$), and the 12B and 12 W groups had AST levels that were significantly lower than those in the 3B9R group ($P < 0.05$). Lowest LDH levels were detected in the 12 W group trout, which were significantly lower than those recorded in the 3B9R, 6B6R, and 12B group fish ($P < 0.05$).

Table 2
Plasma levels of IgM, ALP, ALT, T-BIL, AST, and LDH of steelhead trout exposed to different light colors.

Variable	12W	12B	12R	3B9R	6B6R	T12BR	*P-value*
IgM (mg/mL)	10.83 ± 1.67^{c}	19.17 ± 3.19^{a}	13.33 ± 2.72^{bc}	16.67 ± 2.72^{ab}	11.67 ± 1.92^{bc}	14.17 ± 1.67^{abc}	0.0009
ALP (U/L)	33.83 ± 7.61^{d}	54.50 ± 4.44^{c}	86.17 ± 6.14^{a}	59.17 ± 6.31^{bc}	68.25 ± 7.45^{b}	68.09 ± 3.52^{bc}	< 0.0001
ALT (U/L)	5.90 ± 0.50^{b}	6.42 ± 0.31^{b}	7.76 ± 0.33^{ab}	6.81 ± 0.34^{ab}	8.57 ± 0.48^{a}	7.03 ± 1.96^{ab}	0.0062
T-BIL (umol/L)	0.16 ± 0.02^{d}	0.20 ± 0.02^{cd}	0.37 ± 0.03^{a}	0.27 ± 0.05^{bc}	0.33 ± 0.08^{ab}	0.28 ± 0.02^{abc}	< 0.0001
AST (U/L)	251.61 ± 15.38^{d}	308.05 ± 19.93^{c}	367.23 ± 13.67^{ab}	375.92 ± 22.39^{a}	326.43 ± 13.41^{bc}	330.83 ± 29.43^{bc}	< 0.0001
LDH (U/L)	457.42 ± 28.47^{c}	480.83 ± 20.35^{bc}	511.59 ± 26.92^{bc}	613.50 ± 41.05^{a}	530.00 ± 19.81^{b}	521.17 ± 15.89^{b}	< 0.0001

Note: IgM: immunoglobulin M; ALP: alkaline phosphatase; ALT: alanine transaminase; T-BIL: total bilirubin; AST: aspartate aminotransferase; LDH: lactate dehydrogenase. Values (mean ± SD) are the means of four replicate tanks, with three fish per replicate (n = 12). Values in the same line denoted by different superscript letters are significantly different ($P < 0.05$) based on a one-way analysis of variance (ANOVA) with Tukey's test.

3.2. Hepatic antioxidant enzymes and markers of oxidative damage

Results obtained for the activities of antioxidant enzymes (SOD, CAT, and GPx) and levels of oxidative damage markers (LPO and MDA) in hepatic tissues are presented in Table 3. Highest levels of SOD activity were detected in 12 R group trout, which were significantly higher than the levels recorded for 12W, T12BR, and 6B6R group trout ($P < 0.05$). CAT activity in the 12R and 3B9R groups was found to be significantly higher than that in the 6B6R and 12 W groups ($P < 0.05$), whereas activities of GPx in the 3B9R and 12R groups were significantly higher than those of the 6B6R group ($P < 0.05$). The highest contents of LPO were detected in 6B6R group trout, which were significantly higher than those in all other groups ($P < 0.05$). MDA contents in 3B9R group fish were found to be significantly lower than those in the 6B6R, 12W, and 12R group fish ($P < 0.05$). Despite there are no significant difference for LPO and MDA contents in the 12R group ($P > 0.05$), but it is still relatively high.

In order to evaluate the comprehensive hepatic immune capacity of trout in the different light exposure groups, we standardized and normalized the data obtained for hepatic antioxidant enzyme activity and oxidative damage markers. We accordingly found that by using both the standardized and normalized values, we were able to obtain good evaluations of the comprehensive hepatic immune capacities of each group (Fig. 1), which indicated that the responses of trout in the 3B9R and 12R groups were significantly higher than those in the 12W and 6B6R groups ($P < 0.05$).

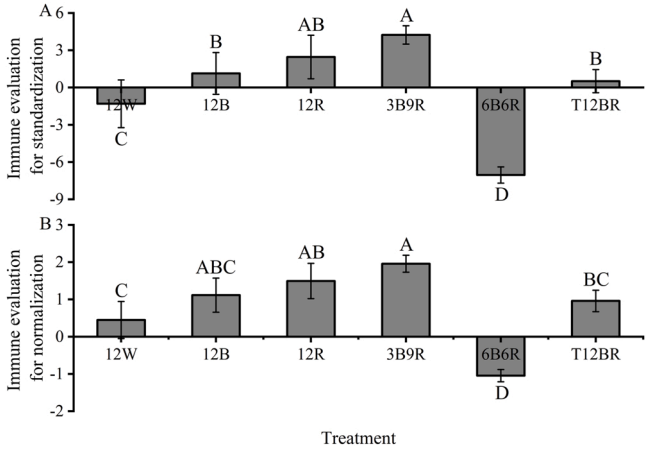

Fig. 1. Standardized (A) and normalized (B) data were used to evaluate the comprehensive hepatic immune response of steelhead trout exposed to different light colors. Different capital letters on the bar indicate the significant difference ($P < 0.05$) based on a one-way analysis of variance (ANOVA) using Tukey's test. Values (mean ± SD) are means of four replicates, with three fish per replicate (n = 12).

3.3. Growth-related gene expression

Data relating to the expression of GH/IGF-1 axis-associated genes for juvenile steelhead trout are presented in Fig. 2. We detected no significant differences were observed in the gene expression of growth hormone-releasing hormone (GHRH) among the assessed six groups ($P > 0.05$). However, in the case of growth hormone receptor (GHR), insulin-like growth factor-1 (IGF-1), and insulin-like growth factor-binding protein (IGFBP), which showed similar trends, we detected significantly higher gene expression in 3B9R and 12R group trout than in the 6B6R group trout ($P < 0.05$).

qPCR amplification of the aforementioned GH/IGF-1 axis genes, performed using three pairs of GH-specific primers designed based on GenBank sequences, failed to reveal any pronounced peaks in expression. Nevertheless, by plotting the normalized gene expression data as a heat map (Fig. 3), we found that the comprehensive expression levels of the GH/IGF-1 axis-associated genes in 3B9R and 12R group trout were significantly higher than those in the 6B6R group fish ($P < 0.05$), whereas expression of the GHR, IGF-1, and IGFBP genes in the 3B9R and 12R groups were significantly higher than those in the other groups ($P < 0.05$).

4. Discussion

In this study, we found that the expression of GH/IGF-1 axis-associated genes was higher in those steelhead trout exposed to a red light environment, whereas exposure to blue light was observed to promote a significant enhancement in their immune performance. These findings are consistent with those of previous studies that have shown that a red light environment is beneficial with to respect to growth, whereas blue light is more conducive to strengthening the immune capacity of these

Table 3
The levels of SOD, CAT, GPx, LPO, and MDA in the livers of steelhead trout exposed to different light colors.

Variable	12W	12B	12R	3B9R	6B6R	T12BR	*P-value*
SOD (U/mgprot)	116.19 ± 8.04^{bcd}	124.63 ± 3.53^{abc}	132.72 ± 8.97^{a}	129.48 ± 5.31^{ab}	106.42 ± 4.79^{d}	113.14 ± 10.09^{cd}	0.0004
CAT (U/mgprot)	6.58 ± 0.31^{b}	7.22 ± 0.55^{ab}	7.80 ± 0.41^{a}	7.77 ± 0.55^{a}	6.60 ± 0.32^{b}	7.08 ± 0.61^{ab}	0.0041
GPx (U/mgprot)	492.57 ± 102.98^{ab}	427.48 ± 51.20^{ab}	541.38 ± 13.59^{a}	545.29 ± 25.63^{a}	352.04 ± 47.58^{b}	487.78 ± 88.34^{ab}	0.0033
LPO (umol/gprot)	0.71 ± 0.38^{b}	0.52 ± 0.11^{b}	0.91 ± 0.27^{b}	0.34 ± 0.06^{b}	1.68 ± 0.43^{a}	0.51 ± 0.04^{b}	< 0.0001
MDA (umol/mgprot)	0.45 ± 0.08^{b}	0.28 ± 0.07^{cd}	0.41 ± 0.03^{bc}	0.25 ± 0.06^{d}	0.69 ± 0.09^{a}	0.30 ± 0.05^{cd}	< 0.0001

Note: SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; LPO: lipid peroxidation; MDA: malondialdehyde. Values (mean ± SD) are the means of four replicate tanks, with three fish per replicate (n = 12). Values in the same line denoted by different superscript letters are significantly different ($P < 0.05$) based on a one-way analysis of variance (ANOVA) with Tukey's test.

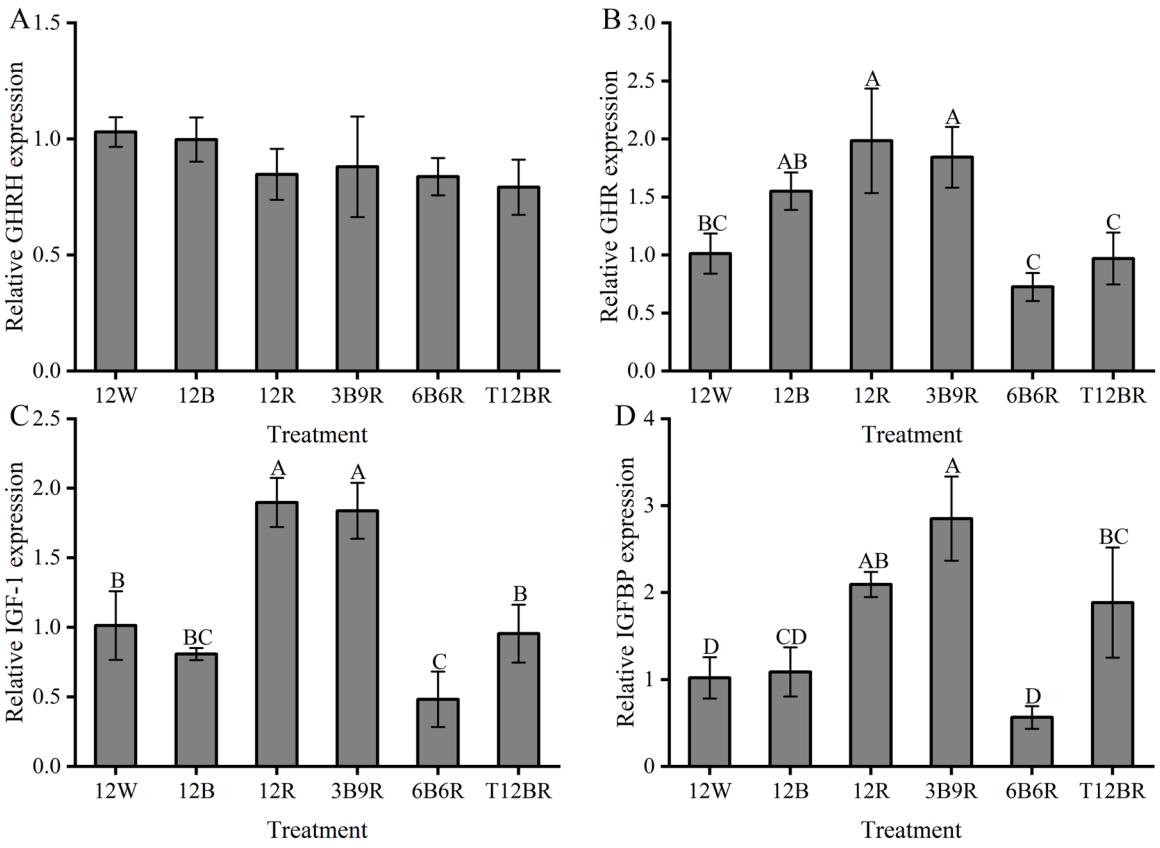

Fig. 2. GH/IGF-1 somatotropic axis gene expression in the liver of steelhead trout. GHRH: growth hormone-releasing hormone, A; GHR: growth hormone receptor, B; IGF-1: insulin-like growth factor 1, C; IGFBP: insulin-like growth factor-binding protein, D. Different capital letters on the bar indicate the significant difference ($P < 0.05$) based on a one-way analysis of variance (ANOVA) with Tukey's test. Values (mean ± SD) are the means of four replicates, with three fish per replicate (n = 12).

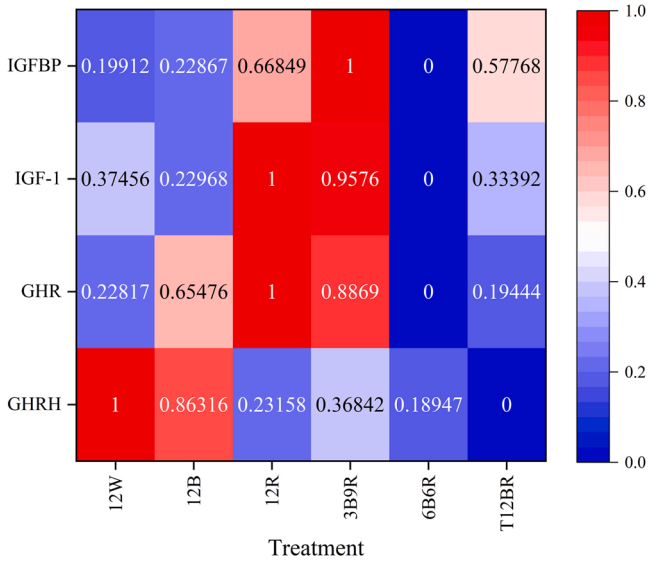

Fig. 3. A heat map of GH/IGF-1 axis gene expression in the liver of steelhead trout. The heat map shows normalized expression values, with the red and blue colors representing higher and lower somatotropic axis gene expression, respectively.

fish (Guller et al., 2020; Karakatsouli et al., 2008, 2007). Correspondingly, trout exposed to the 3B9R light environment were found to be characterized by simultaneous enhancement of both growth and immune performance, thereby tending to indicate that these conditions, which simulate the blue-red-blue daily rhythm of light color variation in natural underwater environments, are more conducive to the healthy growth and development of these fish. Conversely, however, if the proportion of blue light in the blue-red combination exceed a threshold of 6 h, this has a significant inhibitory effect on the immune and growth performance.

4.1. *Plasma immunobiochemistry*

As in all vertebrates, blood functions as a mediator of metabolism, immune regulation, and nutrient transport in fish, and as such, is widely used to evaluate physiological health and environmental adaptability (Chen et al., 2021). Indeed, it has been demonstrated that the immunobiochemical characteristics of fish blood can show marked variations in response to environmental change (Wang et al., 2021).

IgM is the most abundant and widely distributed immunoglobulin in teleost fishes, in which it plays a vital role in specific humoral immune responses (Salinas et al., 2021). Consequently, plasma IgM levels can serve as a useful index for assessing the immune capacity of fish (Tian et al., 2015). In the current study, we found that levels of IgM in the blood of group 12B and 3B9R steelhead trout were significantly higher than those in the blood of trout in other groups (Table 2). We speculate that this difference could be associated with a promotion of IgM synthesis in response to blue light stimulation, whereas a red light environment may have the effect of inhibiting IgM synthesis. Similarly, Choi et al. (2016) have demonstrated that exposure to green light can stimulate IgM synthesis in rock bream (*Oplegnathus fasciatus*), whereas synthesis is inhibited by a red light environment. Moreover, the effects of different light colors on IgM synthesis in fish have been shown to vary according to species.

Our results regarding the light-induced responses of three markers (ALP, ALT, and T-Bil) of hepatic health (Table 2) tended to indicate normal liver function in steelhead trout exposed to the 12B and 3B9R environments, whereas the livers of 12R and 6B6R trout showed evidence of a high metabolic load or oxidative stress. Moreover, fish in the 3B9R group were characterized by a higher SGR. Consequently, in terms of hepatic performance, a 3B9R light environment may be the more suitable for steelhead trout cultivation. Although in the liver, the enzymes ALP and ALT are non-functional and T-Bil is a normal hemoglobin degradation product (Syu et al., 2005; Yousefi et al., 2020), under conditions of high metabolic load or oxidative stress, an increase in the permeability hepatocyte cell membranes can result in a pronounced release of ALP and ALT into the bloodstream, whereas damaged red blood cells can release bilirubin, thereby potentially leading to elevated levels of T-Bil (Ghelichpour et al., 2020; Xiao et al., 2017). Similarly, Nepal et al. (2018) have observed elevated levels of ALP, ALT, and T-Bil in the plasma of carp (*Cyprinus carpio*) with hepatic injury.

We also found that plasma levels of AST and LDH were significantly increased in those trout exposed to the 3B9R light environment (Table 2), which we suspect could represent a stress response associated with a sudden change in the color of the light environment, to which these fish had yet to become fully adapted. This stress is initially expressed in the myocardial system, in which damage to cardiomyocytes leads to pronounced increases in the levels of plasma AST and LDH, which accordingly serve as vital markers that can be used to assess the degree of myocardial tissue damage (Kuerban et al., 2020). In this regard, Mork and Gulbrandsen (1994) found that four salmonid species showed a considerable elevation in stress levels in response to a sudden transition between light and darkness, whereas Ryu et al. (2020) reported that by exposing goldfish (*C. auratus*) to slowly dimming light changes (gradient light), they could mitigate the stress response induced by a changing light environment. In the light of these observations, it would therefore seem prudent that when applying different combination of light color in the rearing steelhead trout, a “buffer interval” should be incorporated during light color transition to prevent cardiomyocyte damaged, and thus reduce the associated stress.

4.2. Hepatic antioxidant capacity

SOD, CAT, and GPx are the main components of the fish antioxidant defense system, the activities of which can contribute to maintaining the dynamic equilibrium of free radical concentrations and prevent, or at least minimize, oxidative cell damage (Abdel-Tawwab and El-Araby, 2021). This defense system can also accurately reflect the antioxidant capacity of fish (Jia et al., 2021). The data obtained in the present study indicated that the steelhead trout in all light exposure groups showed similar trends with respect to the activities of hepatic SOD, CAT, and GPx (Table 3), although activities in the 6B6R group trout were found to be significantly lower than those in the 3B9R and 12R group trout. This difference in response would thus tend to indicate that a 6B6R light environment may have the effect of reducing the hepatic antioxidant capacity of these fish. One plausible explanation that might account for these findings is that a 6B6R light environment can cause a stress response in steelhead trout, and that an excess of reactive oxygen species (ROS), produced as a consequence accelerated metabolism, exceeds the decomposition capacity of hepatic antioxidant enzymes, thereby leading to the oxidative damage of cells and/or tissues. Our findings also revealed that the hepatic contents of LPO and MDA in 6B6R group trout were significantly higher than those in the fish of other groups. Together with the significantly lower activities of SOD, CAT, and GPx in 6B6R group trout, these observations indicate that exposure to the 6B6R environment had the effects of inducing severe oxidative stress and reducing the antioxidant capacity of the liver. Moreover, the elevated levels of liver LPO and MDA in 12R group trout indicates the requirement for higher metabolic activity to eliminate the ROS produced by oxidative reactions, and thereby reduce the associated stress. Consequently, exposing steelhead trout to a 12-h red light period would probably be unsuitable for the long-term cultivation of these fish. Similarly, Hoseini et al. (2020) found that under stress conditions, carp (*C. carpio*) enhances the activities of SOD, CAT, and GPx activity to balance MDA, thereby against oxidative damage. It should be noted in this regard that although oxidative stress contributed to promoting the activities of SOD, CAT, and GPx in the 12R group of steelhead trout, this does not necessarily imply a corresponding enhancement of antioxidant capacity. Muthulakshmi et al. (2018) showed that exposure of zebrafish (*Danio rerio*) embryos to toxin stress stimulated increases in the activities of SOD, CAT, and GPx in response to elevated levels of LPO and MDA, thereby countering the adverse effects of oxidative stress. Consequently, when attempting to assess the antioxidant capacity of fish, it is advisable to collectively examine all five of the aforementioned stress-related indicators, which would facilitate a more accurate and comprehensive evaluation of immune performance (Cui et al., 2020; Duan et al., 2016; Liu et al., 2014, 2020).

Accordingly, to comprehensively evaluate the hepatic antioxidant capacity of steelhead trout in response to variations in light color, we transformed the data obtained for the five immunological indicators SOD, CAT, GPx, LPO, and MD (Fig. 1) based on standardization and normalization to obtain dimensionless values, with both procedures revealing similar trends in comprehensive hepatic immune performance. Moreover, we established that the comprehensive hepatic immune performance of trout in the 3B9R group was significantly higher than that of fish in group 6B6R, which indicates that those fish exposed to the 3B9R environment showed a higher level of resistance to environmental stress, whereas fish in the 6B6R group would be more susceptible to the adverse effects of the light environment.

4.3. GH/IGF-1 axis gene expression

Our analysis of the expression of the GH/IGF-1 axis-associated genes GHR, IGF-1, and IGFBP in response to the six assessed light environments revealed similar trends in the livers of steelhead trout (Figs. 2 and 3), with the expression of these genes in the 6B6R group fish being significantly lower than those in the 3B9R group fish. The GH/IGF-1 axis is a vital regulator of somatic growth and development (Ayres, 2020), and our observations indicate that the growth performance of steelhead trout may be correlated with the hepatic expression of the aforementioned three genes, and that the patterns of expression are to a certain extext influenced by the color of light perceived by these fish. Thus, regulating the endocrine system of fish by manipulating the expression of GH/IGF-1 axis genes would predictably have an effect on growth performance (Guo et al., 2021). Indeed, in a recent study, Yang et al. (2021) reported that a high expression of GH/IGF-1 axis genes in sturgeon (*Acipenser baeri*♀ × *Acipenser schrenckii*♂) can significantly improve growth performance. Moreover, not only light but also other environmental factors, including temperature, salinity, and culture density, have been found to influence the expression of GH/IGF-1 axis-associated genes in teleost fish, as reviewed by Triantaphyllopoulos et al. (2020). IGF-1 is a vital mediator of somatic growth, the biological effects of which are regulated by the binding protein IGFBP. In response to a change in the light environment, activation of the GH/IGF-1 axis stimulates the synthesis of IGF-1 in the liver and other target tissues via the JAK2/STAT5 signal transduction pathway. Thereafter, this growth factor, binds to IGFBP, and is subsequently transported to target tissues via blood circulation to promote cell proliferation and differentiation (Frank, 2020). GHR functions as a central link in the GH/IGF-1 axis by promoting growth through the synergistic action of hepatic GHR, IGF-1, and IGFBP (Yu et al., 2021). Consequently, any marked changes in the hepatic expression of GHR, IGF-1, or IGFBP would be predicted to have a pronounced effect on the growth performance of steelhead trout. Analogously, in a study of olive flounder (*Paralichthys olivaceus*) (Zou et al., 2022), exposure of fish green light was found to promote a significant increase in the expression of GH/IGF-1 axis genes. Nevertheless,

it is conceivable that the expression of GH/IGF-1 axis genes in response to variations in environmental light color may differ depending on the species of fish and/or tissue type.

5. Conclusions

In this study, in which we examined the responses of juvenile steelhead trout to changes in the color of the light environment to which they were exposed, we established that a 3B9R light environment simulated the blue-red-blue daily rhythm of light color variation in natural underwater environments, which can simultaneously improve the growth and immune performance of these trout. Our results indicate that trout can adapt to variations in the daily rhythm of light conditions during long-term natural evolution. Furthermore, we found that a 12B light environment can strengthen the immune performance of trout by increasing the levels plasma IgM, although had no significant effect on fish growth. Despite being favorable for growth, exposure to a 12R light environment was observed to be associated with relatively high liver contents of LPO and MDA, which is taken to be indicative of the occurrence of oxidative stress. Consequently, a 12R light environment is considered unsuitable for the long-term cultivation of steelhead trout. Moreover, we detected significant reductions in the growth and immune performance of trout exposed to a 6B6R light environment, which again indicates that the application of these light conditions would be unsuitable for trout aquaculture. Collectively, our findings indicate that among the six experimental light conditions assessed, a 3B9R light environment would be the most conducive in meeting the growth requirements of steelhead trout, although we additionally propose the incorporation of a gradient transition in light conditions in the light cycle to minimize the likelihood of stress induction in response to sudden changes in light color.

Animals and ethics approval

All methods used in this study were conducted according to the guiding principles of the Chinese Legislation on the Use and Care of Laboratory Animals. The Academic Council approved the animal protocol of the Ocean University of China.

CRediT authorship contribution statement

Xueweijie Chen: Investigation, Methodology, Writing – original draft, Formal analysis, Data curation, Visualization. **Yangen Zhou:** Writing – review & editing, Supervision, Project administration. **Jinze Huang:** Software, Formal analysis, Data curation. **Dong An:** Writing – review & editing. **Yunwei Dong:** Writing – review & editing. **Qinfeng Gao:** Writing – review & editing. **Shuanglin Dong:** Conceptualization, Methodology, Writing – review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2022.101156.

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