Hindawi
Aquaculture Research
Volume 2024, Article ID 8897473, 10 pages
https://doi.org/10.1155/2024/8897473

Research Article

Effects of Light Spectrum on Survival, Growth, Physiological, and Biochemical Indices of Redclaw Crayfish (*Cherax quadricarinatus*) Juveniles

Cuixue Huang,[1,2] **Xiangxing Nie**,[1,2] **Jie Wei**,[2] **Yakun Wang**,[2] **Kunhao Hong**,[2] **Xidong Mu**,[3] **Chao Liu**,[3] **Zhangjie Chu**,[1] **Xinping Zhu**,[2] **and Lingyun Yu**[2]

[1]*School of Fishery, Zhejiang Ocean University, Zhoushan 316000, China*
[2]*Key Laboratory of Tropical and Subtropical Fishery Resources Application and Cultivation, Ministry of Agriculture and Rural Affairs, Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou 510380, China*
[3]*Key Laboratory of Prevention and Control for Aquatic Invasive Alien Species, Ministry of Agriculture and Rural Affairs, Guangdong Modern Recreational Fisheries Engineering Technology Center, Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou 510380, China*

Correspondence should be addressed to Lingyun Yu; lysnp@163.com

Received 16 December 2023; Accepted 13 March 2024; Published 8 April 2024

Academic Editor: Liqiao Chen

The spectrum is a key environmental factor, and light-emitting diodes (LEDs) can influence the growth and development of crustaceans by altering the composition of the spectrum. This study conducted a 30-day experiment to investigate the effects of five LED spectra (red, yellow, blue, green, and white light) on the growth, antioxidant and immune enzyme activities, stress hormone levels, and the expression of α-amylase (*α-AMY*), ecdysone receptor (*EcR*) and retinoid X receptor (*RXR*) genes in juvenile redclaw crayfish (*Cherax quadricarinatus*). The results show that the survival rate of juveniles is markedly higher in the yellow and red-light groups than in the other three groups ($P<0.05$). The green light group exhibits the lowest survival rate, yet it demonstrates the highest weight gain rate and specific growth rate. Regarding enzyme activity and hormone levels, the yellow light group shows the lowest malondialdehyde content, with higher superoxide dismutase and acid phosphatase activity than the other groups; no significant differences are observed in lysozyme activity among the groups ($P>0.05$). The melatonin content in the green and blue light groups is significantly higher than that in the other three groups ($P<0.01$). In terms of growth gene expression, the expression of *α-AMY*, *EcR*, and *RXR* in juvenile *C. quadricarinatus* is regulated by the spectrum. In conclusion, when raised under the yellow light spectrum, juvenile *C. quadricarinatus* displays elevated survival rates, rapid growth, and robust antioxidant and immune defenses. This study provides important technical parameters for optimizing and enhancing the industrial cultivation of juvenile *C. quadricarinatus*.

1. Introduction

The redclaw crayfish (*Cherax quadricarinatus*), originating from streams in Australia and Papua New Guinea, belongs to the order Decapoda and the family Parastacidae. It is a high-value freshwater shrimp species that has gradually gained popularity in aquaculture owing to its fast growth, adaptability, and profitable outcomes [1]. According to the 2021 Food and Agriculture (FAO) statistics, the total production of redclaw crayfish in countries such as Australia, Cambodia, Malaysia, and Indonesia was approximately 260 tons [2]. However, challenges regarding cultivating redclaw crayfish persist, such as low egg-carrying capacity and the immaturity of hatchery technology, which prevent the development of large-scale and industrial production [3, 4]. Currently, efforts have been made to explore factory-scale cultivation of redclaw crayfish. However, the indoor cultivation process is characterized by various instabilities due to the influence of different environmental factors. Factors such as excessively

high or low water temperatures and stocking densities can lead to slow growth and significantly reduce the survival rates of redclaw crayfish juveniles [5, 6]. Furthermore, maintaining a salinity level between 0.1% and 2% in the water environment enhances the survival and immunity of redclaw crayfish juveniles [7]. Currently, research on the influence of various environmental factors affecting factory-scale cultivation of redclaw crayfish remains limited.

In aquaculture, light exposure is closely tied to the growth and survival of aquatic animals, and different spectra play a vital role in their development [8–10]. For instance, largemouth bass (*Micropterus salmoides*) experience a significant increase in body weight and exhibit better growth performance in a blue-light environment [10]. Furthermore, the impact of spectra on aquatic animals is species-specific [11]. In lower-order crustaceans, such as the giant freshwater prawn (*Macrobrachium rosenbergii*), body length significantly increases when raised under white and green light than blue, yellow, and red light, suggesting that white and green light are beneficial for its growth [12]. For the Pacific white shrimp (*Litopenaeus vannamei*), their juvenile development period shortens under yellow light, indicating the benefit of yellow light on their growth [13]. In bivalves such as the discus abalone (*Haliotis discus hannai*), the survival and specific growth rate (SGR) of juveniles are markedly higher under red and orange light than under green and blue light, enhancing their growth performance [9]. The above research indicates that physiological activities such as growth and development in aquatic animals are regulated by changes in the spectrum. However, the mechanisms underlying physiological and biochemical regulation in response to spectrum changes are currently being explored in the scientific community.

Changes in spectra can trigger oxidative stress responses within the bodies of aquatic organisms, resulting in altered antioxidant enzyme activity and stress hormone levels [14, 15]. For instance, under red light, juvenile yellowtail clownfish (*Amphiprion clarkii*) had significantly higher serum levels of superoxide dismutase (SOD), catalase (CAT), and melatonin than those in other spectra, indicating that red light-induced oxidative stress responses in these juvenile fish [16]. In contrast, juvenile goldfish (*Carassius auratus*) reared under blue light had significantly increased cortisol levels, suggesting increased stress when cultivated under blue light [17]. Besides inducing oxidative stress responses, spectra also affect the expression levels of genes associated with growth or molting in aquatic organisms. For example, juvenile Nile tilapia (*Oreochromis niloticus*) raised under red-light spectrum conditions exhibited significant upregulation of the α-amylase (α-*AMY*) gene, promoting their growth [18]. Juvenile mud crabs (*Scylla paramamosain*) exposed to white and blue light exhibited downregulation of the molting inhibitory hormone (*MIH*) gene and increased molting frequency [19]. Currently, research on how light affects newly hatched redclaw crayfish juveniles lasts only 14 days. Given that the factory-scale cultivation period for redclaw crayfish juveniles is approximately 30 days (with the total body length ranges from 3 to 5 cm), this research requires further refinement [20]. Hence, the objective of this study was to examine that five distinct spectral conditions impact the survival, growth performance, pertinent enzyme activity, and gene expression levels associated with the growth and development of juvenile redclaw crayfish during a 30-day rearing period. The goal is to better understand the mechanisms underlying the physiological and biochemical responses of *C. quadricarinatus* juveniles to spectral regulation.

2. Materials and Methods

2.1. Experimental Operation

2.1.1. Experiment Preparation. After cleaning and disinfecting the aquariums used in the experiment, preaerated recirculating water was added. The dissolved oxygen in the water ranged from 7.25 to 8.66 mg/L, with a pH range of 7.80–8.27, while the temperature was consistently kept at 30 ± 0.5°C. We introduced nontoxic water conditioner tablets (provided by XianDe Biological Technology Co., Ltd., Guangzhou, China) into the filtration tank to improve the water quality [12]. The aeration system underwent cleaning using water containing chlorine, and residual chlorine levels were measured in each aquarium after a 24-hr period [21]. The experiment commenced when the residual chlorine level did not exceed 0.005 mg/L, at which point the experimental juveniles were introduced. Environmental conditions were kept consistent across all groups.

2.1.2. Experimental Design. The experiment utilized 510 healthy redclaw crayfish juveniles with an initial average weight of 0.03 ± 0.01 g and an initial average length of 1.06 ± 0.14 cm. These juveniles were sourced from Hengzhao Lanlong Aquaculture Co., Ltd. (Jiangmen, China) and acclimated for 1 week at the Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences (Guangzhou, China). During this acclimation period, the juveniles were housed in aquariums measuring 130 cm × 52 cm × 60 cm each. The feeding regimen during this temporary rearing period mirrored that of the formal experiment. Black plastic film was applied to cover the outside of the tanks to prevent external light sources from entering. The water depth in the tanks was maintained at 45 cm, and we employed custom-designed lighting fixtures to control the light spectrum for each aquarium. These fixtures utilized 18 W LED lights from Yicai Optoelectronics Industry Co., Ltd. (Jiangmen, China), positioned 30 cm above the water surface to ensure an illumination intensity of 1,000 Lx below the water surface. We designed five experimental groups with different visible light spectra: red (wavelength: 615–650 nm), yellow (wavelength: 580–595 nm), white (wavelength: 450–465 nm), green (wavelength: 495–530 nm), and blue (wavelength: 450–480 nm). Each group consisted of three replicates, with each replicate containing 34 juveniles. The experiment lasted for 30 days, following a natural light cycle with light fixtures set to turn on at 7:00 AM and off at 7:00 PM, providing 12 hr of illumination daily. The juveniles were given commercial crayfish feed (Tongwei Feed Co., Ltd., Chengdu, China) twice a day, at 8:00 AM and 5:00 PM, consisting of crude protein (35%), crude fat (12%), and moisture content (9%). One-third of the

Table 1: Primers used for qPCR in this study.

Gene	Sequence (5′−3′)	Product size (bp)	Amplification efficiency (%)	Sources
EcR	F: GGTTTCGGCACTCTTCAACG R: ACAGATTGCGACAAAAGCGG	208	99.45	OL963596.1
RXR	F: AGGAGATGCCGTAACCAACA R: ATGCTTCGGTGTGAGAAGGA	171	97.11	KM016907.1
α-AMY	F: CCGCTGGAGACAGATCTACG R: AACGTCACAGTAGGTGCCAG	199	98.23	OL963595.1
β-Actin	F: CCCCATGCTATCTTGCGTCT R: CGTCAGGAAGCTCGTAGGAT	220	99.66	MN396754.1

water was replaced after each feeding to clean waste, and water quality parameters were regularly monitored using an Octadem W-II water quality analyzer (Octadem Technology, Inc., Wuxi, China) to ensure water stability. Additionally, water temperature and light intensity for aquaculture were monitored using a TASI TA8121 light meter (TASI Electronics, Inc., Suzhou, China).

2.2. Sample Collection and Determination

2.2.1. Growth and Survival Indicators. After 30 days of cultivation, 30 juvenile crayfish (3 replicates, 10 individuals/replicate) were randomly selected from each group. The body length and weight of juveniles in each group were measured using the software TpsDig2 version 1.40 (F. James Rohlf, Stony Brook University, Stony Brook, NY, USA) and a Mettler Toledo AL-204 precision balance (Mettler Toledo, Inc., Shanghai, China). Calculate the weight gain rate and total length gain rate using the methods and formulas described by Nie et al. [21]. Subsequently, nine redclaw crayfish were chosen from each group for hemolymph, eyestalks, and hepatopancreatic tissue collection. These samples were flash-frozen in liquid nitrogen and stored at −80°C for further analysis, including assessments of antioxidant and immune capabilities, melatonin, and cortisol levels.

2.2.2. Analysis of Antioxidant and Immune Capacity. The collected hepatopancreatic samples were subjected to antioxidant and immune enzyme activity assays. The activities of SOD (A001-3-2), malondialdehyde (MDA) (A003-1-2), acid phosphatase (ACP) (A060-1-1), and lysozyme (LZM) (A050-1-1) were determined using the WST-1 method, thiobarbituric acid (TBA) reaction method, spectrophotometry, and turbidimetry, respectively. The procedures followed the respective instructions provided in the commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.2.3. Measurement of Melatonin and Cortisol. We collected hemolymph and eyestalk samples to measure melatonin and cortisol levels. The measurement procedure was as follows: Hemolymph and eyestalk samples were mixed with phosphate-buffered saline ($w:v = 1:9$), and the mixture was homogenized in a glass mortar containing liquid nitrogen. Afterward, the mixture was centrifuged at 5,000x *g* for 5 min at 4°C, and the supernatant was collected for further analysis. For melatonin and cortisol measurements, standard curves were prepared using ELISA assay kits (Catalog No. YJ093369; YJ085236; Ji Chun Industrial Co., Ltd., Shanghai, China). The standards were prepared at six different concentrations: 0, 5, 10, 20, 40, and 80 pg/mL for melatonin and 0, 12.5, 25, 50, 100, and 200 ng/mL for cortisol. Subsequently, microplates were incubated in a 37°C incubator for 30 min, and unbound components were washed away with a washing solution. The bound melatonin or cortisol enzyme conjugates were then determined through a reaction with the substrate tetramethylbenzidine in the presence of peroxidase. Finally, the reaction was stopped by adding 100 μL of 0.5 M H_2SO_4 at 37°C, and the absorbance was read at 450 nm using an absorbance Microplate Reader (SpectraMax 190, Molecular Devices, San Jose, CA, USA).

2.3. Total RNA Extraction, cDNA Synthesis, and Quantitative PCR (qPCR) Analysis.

Total RNA in hepatopancreatic tissues was extracted using Trizol Reagent (Invitrogen, Waltham, MA, USA). RNA integrity was evaluated via 1% agarose gel electrophoresis, and the concentration and purity were determined using a Nanodrop-2000 microspectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). To remove genomic DNA, 1 μg of total RNA underwent DNase I digestion with an enzyme from New England Biolabs (Ipswich, MA, USA) for 15 min. Subsequently, first-strand cDNA synthesis was conducted using the M-MLV reverse transcriptase reagent kit (Invitrogen, Waltham, MA, USA) following the manufacturer's instructions. Primer sequences for target and reference genes utilized in this study are provided in Table 1. Real-time qPCR was performed using the Step One Plus Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) to evaluate the expression levels of specific genes in redclaw crayfish juveniles exposed to various light spectra. Each qPCR reaction mixture contained 1 μL of cDNA (50 ng/μL), 5 μL of iTaq Universal SYBR Green Supermix, 0.5 μL of each primer (10 pmol/μL), and 3 μL of double-distilled water, making a final volume of 10 μL. Cycling conditions for qPCR were as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 40 s, annealing at 60°C for 45 s, extension at 72°C for 30 s, and a final extension step at 72°C for 10 min. Melt curve analysis involved subjecting the samples to 95°C for 5 s, 60°C for 30 s, and 95°C for 15 s to obtain dissociation curves.

2.4. Statistical Analysis.

The experimental data were analyzed using GraphPad Prism version 8.0.2 (GraphPad Software,

Table 2: Corresponding growth index under different spectra.

Light color	Survival rate (%)	Final weight (g)	Weight gain rate (%)	Final length (cm)	Total length gain rate (%)	SGR (%/day)
Red	60.33 ± 11.37^{a}	0.35 ± 0.10	$1{,}084.00 \pm 65.39^{c}$	2.16 ± 0.28	103.00 ± 16.09^{c}	0.08 ± 0.00^{b}
Yellow	62.00 ± 11.79^{a}	0.46 ± 0.06	$1{,}524.00 \pm 358.10^{ab}$	2.50 ± 0.12	137.00 ± 19.47^{ab}	0.09 ± 0.01^{ab}
Blue	48.67 ± 14.15^{bc}	0.41 ± 0.13	$1{,}295.00 \pm 155.00^{bc}$	2.41 ± 0.32	126.70 ± 10.60^{bc}	0.09 ± 0.00^{b}
Green	42.67 ± 6.43^{c}	0.68 ± 0.26	$2{,}158.00 \pm 98.78^{a}$	2.97 ± 0.35	180.00 ± 20.88^{a}	0.10 ± 0.00^{a}
White	47.67 ± 8.33^{bc}	0.47 ± 0.25	$1{,}429.00 \pm 393.50^{bc}$	2.39 ± 0.51	122.70 ± 23.29^{bc}	0.09 ± 0.01^{ab}

Note: Values are expressed as means ± SD ($N = 30$). Different superscript lowercase letters indicated significant differences between treatments ($P < 0.05$). SGR, specific growth rate.

Boston, MA, USA) with a one-way analysis of variance, followed by Tukey's post hoc test for multiple comparisons. Results are presented as mean ± SD. Differences were considered significant at $P < 0.05$ and highly significant at $P < 0.01$.

3. Results

3.1. Impact of Different Light Spectra on Juvenile Crayfish Growth and Survival Rates. After being reared under different spectral conditions for 30 days, the SGR of redclaw crayfish in the green-light group was markedly higher than that in the blue and red-light groups ($P < 0.05$), while its weight gain rate and total length gain rate increase were markedly higher than those in the white and blue-light groups ($P < 0.05$) and markedly higher than those in the red-light group ($P < 0.01$).The survival rates of juvenile redclaw crayfish in the yellow and red-light groups were significantly higher than those in the blue and white-light groups ($P < 0.05$) and markedly higher than that in the green-light group ($P < 0.01$) (Table 2), with the highest survival rate observed in the yellow-light group at 62%.

3.1.1. Effects of Different Light Spectra on the Antioxidant Capacity and Immune Parameters of Juvenile Crayfish. In the red-light group, the SOD activity in the hepatopancreas of juvenile crayfish was significantly higher than that in the blue, green, and white-light groups ($P < 0.05$), with no significant differences observed among the other groups ($P > 0.05$) (Figure 1(a)). In the green-light group, the MDA content was significantly higher than that in the red-light group ($P < 0.05$) and markedly higher than that in the yellow-light group ($P < 0.01$), while the blue and white-light groups were significantly higher than the yellow-light group ($P < 0.05$), with no significant differences observed among the other groups ($P > 0.05$) (Figure 1(b)). In the white-light group, the ACP activity of juveniles was significantly higher than that in the yellow-light group ($P < 0.05$) and markedly higher than that in the blue-light group ($P < 0.01$); the red and green-light groups were significantly higher than the blue-light group ($P < 0.05$), with no significant differences observed among the other groups ($P > 0.05$) (Figure 1(c)). Furthermore, no significant differences in LZM enzyme activity were observed between the various groups ($P > 0.05$) (Figure 1(d)).

3.1.2. Effects of Different Light Spectra on Melatonin and Cortisol Levels in Juvenile Crayfish. The melatonin levels of juveniles in the green and blue-light groups were significantly higher than those in the yellow-light group ($P < 0.01$), and the melatonin level in the yellow-light group was notably higher than that in the red-light group ($P < 0.01$). Notably, no significant differences were observed between the red and the white-light groups ($P > 0.05$) (Figure 2(a)). In the green-light group, the cortisol levels were significantly higher than those in the yellow and red-light groups ($P < 0.05$), with the red-light group significantly higher than the white-light group ($P < 0.05$), while the green, blue, and yellow-light groups were markedly higher than the white-light group ($P < 0.01$) (Figure 2(b)).

3.1.3. Effects of Different Light Spectra on Gene Expression Levels in Juvenile Crayfish. The results of the ecdysone receptor (*EcR*) expression levels in juvenile redclaw crayfish under different spectral groups showed that the white-light group had significantly higher expression levels than the other four groups ($P < 0.01$). Specifically, the green-light group had significantly higher expression levels than the yellow-light group ($P < 0.05$), while the blue-light group had markedly higher expression levels than the yellow-light group ($P < 0.01$), with no significant differences observed among the other groups ($P > 0.05$) (Figure 3(a)). Comparisons of the retinoid X receptor (*RXR*) expression levels among juvenile redclaw crayfish in different spectral groups revealed that the red-light group had markedly higher expression levels than the other four groups ($P < 0.01$), with no significant differences observed among the other four groups ($P > 0.05$) (Figure 3(b)). The results of *α-AMY* expression levels in juvenile redclaw crayfish under different spectral groups showed that the expression levels in the yellow and green-light groups were significantly higher than those in the white and blue-light groups ($P < 0.05$), and markedly higher than those in the red-light group ($P < 0.01$), with no significant differences observed among the other groups ($P > 0.05$) (Figure 3(c)).

4. Discussion

Spectra directly impact the growth, development, and survival of crustaceans [12, 22]. Different crustacean species exhibit varying degrees of photosensitivity [23, 24], which may be closely related to their environmental niches during different developmental stages [25–27]. Previously, Cheng et al. [20] found that within 14 days post-hatching, juvenile redclaw crayfish exhibited higher survival rates under red light and experienced accelerated growth rates under blue light but demonstrated decreased survival rates and slower growth under green light [20]. However, findings from this investigation reveal that under yellow light spectrum cultivation conditions, the survival rate of juvenile redclaw crayfish

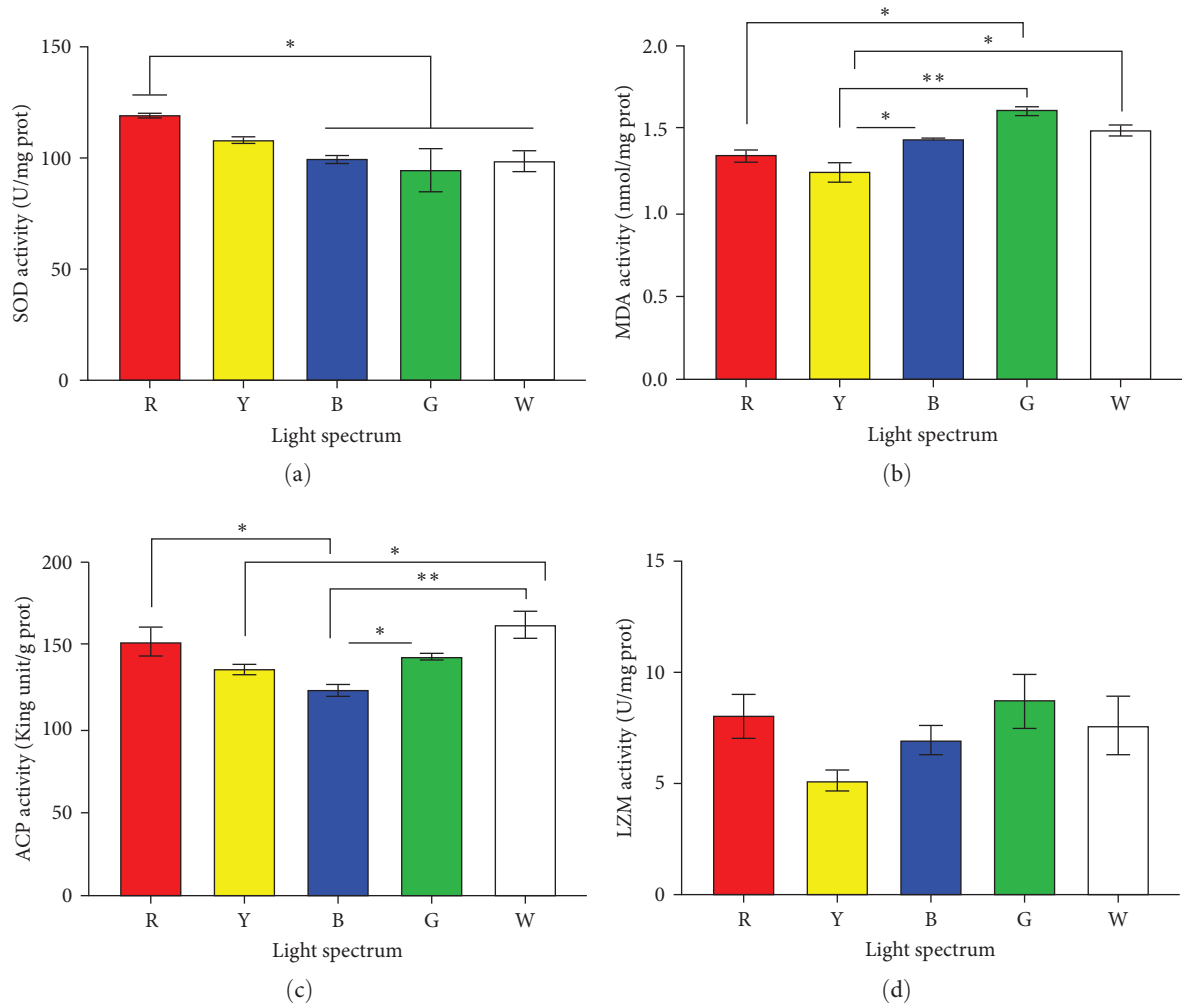

FIGURE 1: The superoxide dismutase (SOD) (a), malondialdehyde (MDA) (b), acid phosphatase (ACP) (c), and lysozyme (LZM) (d) contents of *C. quadricarinatus* reared under different light spectrums. Values are expressed as means ± SD of three replicates ($N = 9$). * For $0.01 < P < 0.05$, ** for $P < 0.01$. R: red; Y: yellow; B: blue; G: green; W: white.

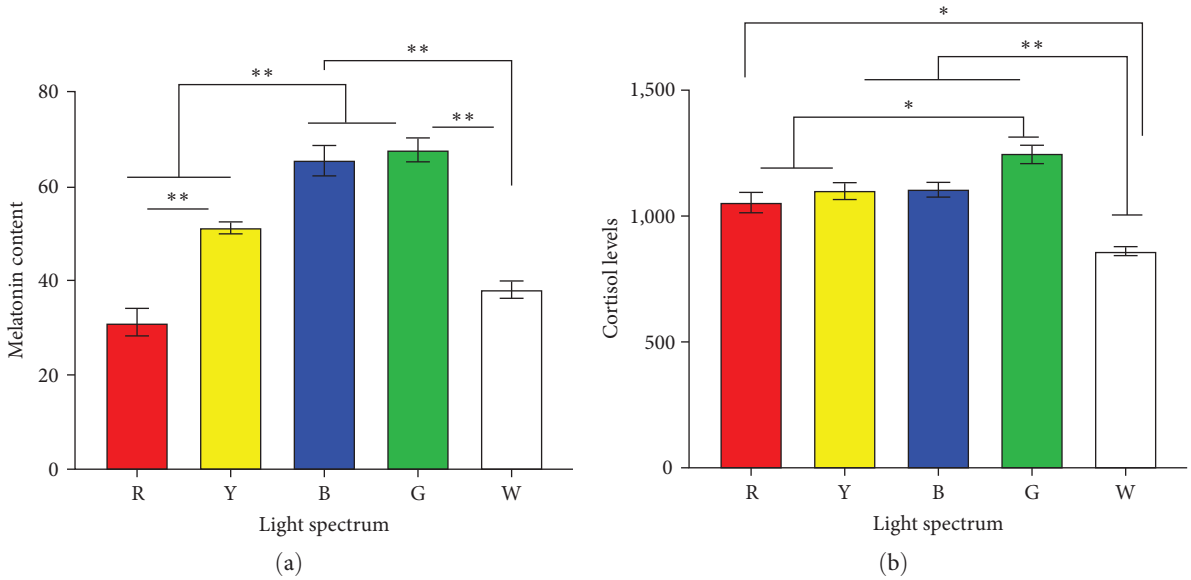

FIGURE 2: Melatonin (a) and cortisol (b) levels of juveniles *C. quadricarinatus* reared under different light spectrums. Values are expressed as mean ± SD of three replicates ($N = 9$). * For $0.01 < P < 0.05$, ** for $P < 0.01$. R: red; Y: yellow; B: blue; G: green; W: white.

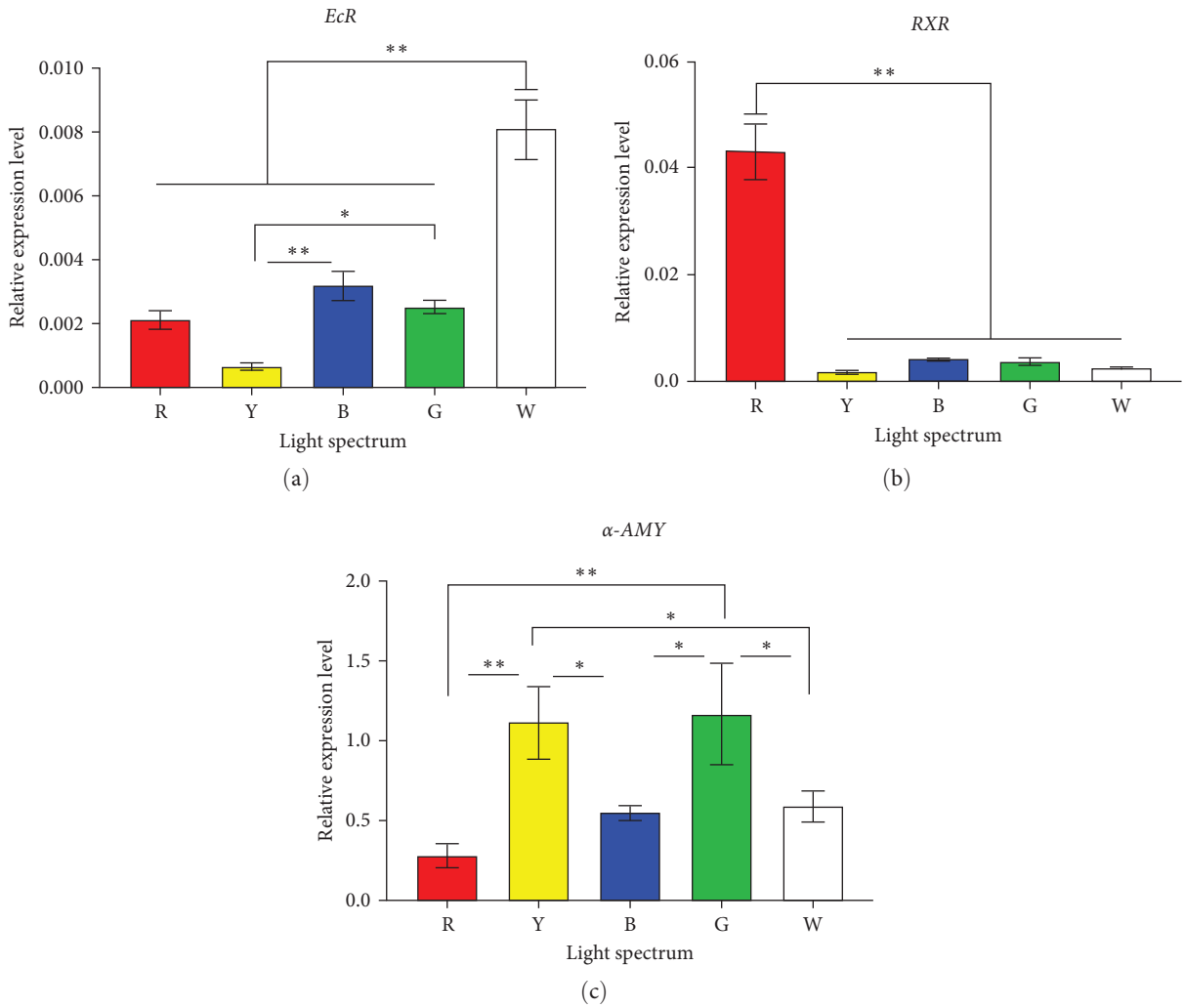

FIGURE 3: Gene expression levels of ecdysone receptor (*EcR*) (a), retinoid X receptor (*RXR*) (b), and α-amylase (α-*AMY*) (c) of *C. quadricarinatus* reared under the different light spectrum. Values are expressed as means $\pm$ SD of three replicates ($N = 9$). * For $0.01 < P < 0.05$, ** for $P < 0.01$. R: red; Y: yellow; B: blue; G: green; W: white.

was highest after 30 days posthatching, with a higher SGR. Conversely, under green light spectrum cultivation regimes, juvenile redclaw crayfish had the lowest survival rate but achieved the highest final body weight and SGR. Despite no significant difference in survival rates between the red-light and yellow-light groups, the average weight gain rate and total length gain rate within the red-light group were notably lower compared to those observed in the yellow and green-light groups. This may be due to the different durations of the two experiments; this study lasted 16 days longer than the experiment designed by Cheng et al. [20]. Consequently, the continuous development of the compound eyes of crustaceans and their corresponding changes in sensitivity to different spectra and internal response mechanisms may occur, and the optimal light conditions for growth may also change accordingly [27]. This may also be due to differences in water transparency caused by variations in the plankton and organic matter content in the aquaculture water, resulting in different survival and development effects of juvenile *C. quadricarinatus* under the same light conditions, as demonstrated in the two studies, which warrants further investigations in the future. Nevertheless, it is important to mention that in additional studies concerning crustaceans, yellow light has been demonstrated to enhance the survival rate of Pacific white shrimp (*L. vannamei*) and expedite the growth of swimming crab (*Portunus trituberculatus*) juveniles [13, 28]. Conversely, red light has been found to be detrimental to the growth, development, and survival of giant freshwater prawn (*M. rosenbergii*) and Amazon river prawn (*Macrobrachium amazonicum*) [12, 29]. To a certain extent, these studies illustrate that the yellow light spectrum is advantageous for the growth and survival of larvae in most crustacean species, while red light negatively impacts the normal life of many species.

The antioxidant capacity plays a crucial role in determining the growth performance of aquatic organisms. In aquaculture, alterations in the spectrum can trigger oxidative-reductive reactions in crustaceans, leading to fluctuations in the activity of antioxidant enzymes and the levels of oxidative products [12, 19]. Among these, SOD and MDA serve as vital markers for the antioxidant capacity of aquatic organisms [30, 31]. The findings from our experiment reveal that the SOD activity of juvenile redclaw crayfish under green, blue, and white light spectra is markedly lower

compared to that under the red light spectrum. SOD is a critical antioxidant enzyme that maintains the oxidative–reductive balance of the body by scavenging harmful superoxide anion radicals [32]. The markedly higher SOD in the red-light group than that in the other groups indicates that individuals living under red light may be constantly facing oxidative–reductive imbalances, leading to excessive energy consumption, which affects their growth [19]. MDA is the end product of lipid peroxidation in organisms, which can cause cross-linking and polymerization of macromolecules such as proteins and nucleic acids and exhibits cytotoxicity. Its content reflects the oxidative level and stress status within the organism [33]. In this experiment, the MDA levels in the yellow-light group are lower compared to those in the green, blue, and white-light groups. This suggests that juvenile redclaw crayfish living under yellow light conditions maintain a more favorable oxidative–reductive balance and experience lower stress levels. A series of reports have also indicated that the reduction in SOD activity and the elevation in MDA content is detrimental to the cellular oxidative–reductive balance in crustaceans and have negative effects on normal physiological metabolism [19, 34], which is also reflected in the enzyme activity and growth results of this study. ACP is an important nonspecific immune enzyme in crustaceans. In the immune response, it forms a hydrolytic enzyme system to degrade and eliminate foreign substances invading the body, thereby achieving immune defense [35]. It was found that the ACP activity levels in the white light spectrum cultivation environment were the highest among the groups, indicating that under different light spectrum cultivation conditions, juvenile redclaw crayfish produced a series of stress responses. Furthermore, overall, yellow light may be beneficial to the oxidative-reductive balance of cells in juvenile redclaw crayfish, positively impacting physiological metabolism by avoiding damage to the immune system and energy consumption.

Melatonin is a common and conserved biogenic amine that is synthesized and secreted in organisms ranging from unicellular organisms to mammals. In invertebrates, light exposure can transmit photic information through the visual ganglia, regulating melatonin synthesis and secretion [36, 37]. Melatonin holds considerable importance in upholding the internal environment of crustaceans, regulating diverse biological functions such as reproduction, molting, limb regeneration, antioxidative stress, and plasma glucose homeostasis [38]. For instance, the cleaner shrimp (*Lysmata amboinensis*), reared under red light, perceives the red-light environment as darkness, leading to a significant increase in melatonin levels compared to blue light conditions, thus affecting its biological rhythms [39]. On the contrary, our study findings reveal that juvenile crayfish reared under blue and green light spectra have the highest melatonin levels, while those reared under red light have the lowest melatonin levels. Similar results have been found in studies on fish melatonin, where Nile tilapia (*O. niloticus*) exhibited decreased melatonin levels under red light spectrum cultivation conditions, indicating a weaker perception of red light and, consequently, poorer growth performance [40]. Hence, different light spectra may have species-specific effects on melatonin secretion and synthesis. In juvenile crayfish reared under red light, melatonin secretion and synthesis may be inhibited, indicating a weaker sensitivity to red light, which in turn results in poor growth performance under red light.

Cortisol is a vital and conserved stress hormone, and it is considered one of the indicators reflecting the stress status of animals, including crustaceans. Elevated plasma cortisol levels, mediated by stress, can reduce the energy required for muscle growth in animals, including crustaceans, leading to growth inhibition and decreased survival rates [41–43]. Changes in light conditions represent a stressor for aquatic animals, triggering stress responses and inducing cortisol synthesis and release [19, 44–46]. Previous studies have shown that juvenile mud crabs (*S. paramamosain*) exhibit elevated cortisol levels under purple light spectrum cultivation conditions, indicating intensified stress, resulting in decreased survival rates and slower growth rates [19]. However, our study revealed that juvenile crayfish reared under green-light conditions had elevated cortisol levels, decreased survival rates, the fastest growth rate, and the largest final body weight. Thus, we hypothesized that elevated cortisol levels do not necessarily inhibit the growth of aquatic animals and may even promote growth. Villamizar et al. [45] reported that zebrafish (*Danio rerio*) raised under blue or violet light conditions exhibited increased cortisol levels and higher stress; however, they had high survival rates and significantly accelerated growth rates [45]. Zou et al. [46] reported that olive flounder larvae (*Palichthys olivaceus*) raised under green-light conditions had the highest cortisol levels, markedly surpassing those in the white-light group. Although the larvae experienced increased stress, they showed accelerated growth. However, the survival of larvae reared under white and green light did not significantly differ [46]. In summary, our results suggest that changes in light spectra can regulate cortisol levels in juvenile crayfish. Elevated cortisol levels in crayfish raised under green-light conditions accelerated growth and reduced survival rates. An appropriate increase in cortisol levels in the organism can promote the growth of certain aquatic animals; however, excessively high cortisol levels can inhibit both survival and growth.

EcR, *RXR*, and *α-AMY* are associated with the molting and digestive absorption capacity of crustaceans and serve as indicators of their growth status [47–49]. *EcR* and *RXR* form heterodimers that promote development, reproduction, and molting in organisms [50]. α-AMY is involved in energy production by hydrolyzing α-1,4-glycosidic bonds in polysaccharides such as starch, branched starch, and glycogen, subsequently affecting nutrient absorption and metabolism in the organism [51]. Reports indicate that light conditions can influence the molting frequency, digestive absorption, and gene expression related to these processes in crustaceans, thus affecting their growth performance [19, 52]. In this study, the group reared under red light exhibited the highest *RXR* expression and the lowest *α-AMY* expression but had the slowest growth rate. In contrast, the group exposed to yellow light exhibited the highest expression of the *α-AMY* and the lowest expression of the *EcR*, yet it displayed the fastest growth rate. Moreover, the expression levels of the

EcR and *α-AMY* were notably elevated in the white and green-light groups, surpassing those in the other four groups ($P < 0.01$). These findings imply that the spectrum not only induces alterations in the gene expression levels associated with molting and digestion in redclaw crayfish juveniles but also directly affects their growth performance. We speculate that changes in the spectrum may lead to variations in the expression levels of associated genes, thereby affecting the growth rate of juveniles. Specifically, the yellow light spectrum increases the expression level of the *α-AMY*, thereby promoting juvenile growth. This result suggests that the yellow light spectrum may have a positive impact on the intensive farming of redclaw crayfish juveniles.

5. Conclusions

This study examined the impacts of five distinct light spectra on the survival, growth, and pertinent physiological indicators of juvenile redclaw crayfish. The findings demonstrate that the survival rate of juvenile redclaw crayfish is highest in the yellow and red-light groups and lowest in the green-light group. Regarding antioxidant enzyme activity, juveniles in the yellow-light group exhibited elevated levels of SOD and ACP activity, along with the lowest MDA content. Furthermore, in terms of hormones, melatonin levels in juvenile redclaw crayfish were markedly higher in the green and blue-light groups compared to the other three groups (red, white, and yellow light) ($P < 0.01$), while cortisol levels in the green-light group were markedly higher than that in the other three groups (red, white, and yellow-light groups) ($P < 0.05$). Lastly, regarding gene expression levels, the expression of *RXR*, *α-AMY*, and *EcR* was highest in the red, yellow, green, and white-light groups, respectively. These results provide valuable technical support for optimizing and enhancing the factory farming of juvenile redclaw crayfish.

Data Availability

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

We greatly appreciate the support from Hengzhao Lanlong Aquaculture Co., Ltd. (Jiangmen, China) for this study, which provides juvenile redclaw crayfish and the corresponding feed. This work was supported by the Central Public-Interest Scientific Institution Basal Research Fund, CAFS (grant numbers 2021SJXK3, 2022SJ-XT1, 2020TD35, and 2020ZJTD01); China-ASEAN Maritime Cooperation Fund (grant number CAMC-2018F), National Freshwater Genetic Resource Center (grant numbers NFGR2020 and FGRC18537), and Guangdong Rural Revitalization Strategy Special Provincial Organization and Implementation Project Funds (grant number 2022-SBH-00-001).

References

[1] Y. P. Lu, P. H. Zheng, X. X. Zhang et al., "Effects of dietary trehalose on growth, trehalose content, non-specific immunity, gene expression and desiccation resistance of juvenile redclaw crayfish (*Cherax quadricarinatus*)," *Fish & Shellfish Immunology*, vol. 119, pp. 524–532, 2021.

[2] FAO, "Global aquaculture production quantity (1950–2021)," 2024, https://www.fao.org/fishery/statistics-query/en/aquaculture/aquaculture_quantity.

[3] N. C. Prymaczok, V. M. Pasqualino, V. E. Viau, E. M. Rodriguez, and D. A. Medesani, "Involvement of the crustacean hyperglycemic hormone (CHH) in the physiological compensation of the freshwater crayfish *Cherax quadricarinatus* to low temperature and high salinity stress," *Journal of Comparative Physiology B*, vol. 186, no. 2, pp. 181–191, 2016.

[4] S. Cheng, Y. Y. Jia, M. L. Chi et al., "The exploration of artificial incubation of *Cherax quadricarinatus* eggs," *Aquaculture*, vol. 529, Article ID 735576, 2020.

[5] M. García-Guerrero, P. Hernández-Sandoval, J. Orduña-Rojas, and E. Cortés-Jacinto, "Effect of temperature on weight increase, survival, and thermal preference of juvenile redclaw crayfish *Cherax quadricarinatus*," *Hidrobiologica*, vol. 23, no. 1, pp. 73–81, 2013.

[6] Y. Dong, R. Jia, Y. Hou, W. Diao, B. Li, and J. Zhu, "Effects of stocking density on the growth performance, mitophagy, endocytosis and metabolism of *Cherax quadricarinatus* in integrated rice-crayfish farming systems," *Frontiers in Physiology*, vol. 13, Article ID 1040712, 2022.

[7] G. W. Zhang, X. P. Wang, H. L. Zhou, and C. Wu, "Effects of temperature and salinity stress on survival rate and immune enzyme activity of *Cherax quadricariratus* larvae," *Acta Agriculturae Jiangxi*, vol. 34, no. 12, pp. 120–127, 2022.

[8] B. Guo, Y. C. Mu, F. Wang, and S. L. Dong, "Effect of periodic light color change on the molting frequency and growth of *Litopenaeus vannamei*," *Aquaculture*, vol. 362, pp. 67–71, 2012.

[9] X. L. Gao, X. Li, M. J. Li, C. B. Song, and Y. Liu, "Effects of light intensity on metabolism and antioxidant defense in *Haliotis discus hannai* Ino," *Aquaculture*, vol. 465, pp. 78–87, 2016.

[10] J. L. Zheng, L. Gao, H. T. Zhang, X. Chen, Q. L. Zhu, and T. Han, "LED light spectra differently affected growth, antioxidant capacity, stress response, GH/IGF and HPI axis in largemouth bass (*Micropterus salmoides*) larvae," *Aquaculture*, vol. 578, Article ID 740019, 2024.

[11] C. L. Cheng and I. N. Flamarique, "Chromatic organization of cone photoreceptors in the retina of rainbow trout: single cones irreversibly switch from UV (SWS1) to blue (SWS2) light sensitive opsin during natural development," *The Journal of Experimental Biology*, vol. 210, no. Pt 23, pp. 4123–4135, 2007.

[12] J. Wei, L. Tian, Y. K. Wang, L. Y. Yu, and X. P. Zhu, "Effects of salinity, photoperiod, and light spectrum on larval survival, growth, and related enzyme activities in the giant freshwater prawn *Macrobrachiun rosenbergii*," *Aquaculture*, vol. 530, 2021.

[13] H. S. Ou and H. F. Liang, "Effect of light on the larval metamorphosis of *Litopenaeus Vannanmei*," *Marine Sciences*, vol. 41, no. 3, pp. 55–60, 2017.

[14] D. Zhang, B. Guo, F. Wang, and L. Jia, "Effects of light color change on carbohydrate-related enzymes in *Litopenaeus vannamei*," *Aquaculture International*, vol. 25, no. 1, pp. 379–391, 2017.

[15] C. Y. Choi, J. A. Song, Y. U. Choi, T. H. Lee, and H. S. Ryu, "Effects of green wavelength light on antioxidant responses in

olive flounder *Paralichthys olivaceus* exposed to starvation and re-feeding," *Turkish Journal of Fisheries and Aquatic Sciences*, vol. 20, no. 9, pp. 659–669, 2020.

[16] H. S. Shin, J. Lee, and C. Y. Choi, "Effects of LED light spectra on oxidative stress and the protective role of melatonin in relation to the daily rhythm of the yellowtail clownfish, *Amphiprion clarkii*," *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, vol. 160, no. 2, pp. 221–228, 2011.

[17] J. A. Song and C. Y. Choi, "Effects of blue light spectra on retinal stress and damage in goldfish (*Carassius auratus*)," *Fish Physiology and Biochemistry*, vol. 45, no. 1, pp. 391–400, 2019.

[18] M. M. Yi, W. T. Zhai, M. Wang et al., "The welfare of Nile tilapia (*Oreochromis niloticus*, GIFT strain) juveniles cultured in different light spectra," *Frontiers in Marine Science*, vol. 9, Article ID 924110, 2022.

[19] S. J. Chen, C. Shi, H. Migaud et al., "Light spectrum impacts on growth, molting, and oxidative stress response of the mud crab *Scylla paramamosain*," *Frontiers in Marine Science*, vol. 9, Article ID 840353, 2022.

[20] S. Cheng, J. B. Zheng, Y. Y. Jia et al., "Effects of light color, photoperiod, and growth-related gene interference or over-expression on the survival, growth, or physiological and biochemical indices of red claw crayfish juveniles," *Aquaculture*, vol. 562, Article ID 738740, 2023.

[21] X. X. Nie, C. X. Huang, J. Wei et al., "Effects of photoperiod on survival, growth, physiological, and biochemical indices of redclaw crayfish (*Cherax quadricarinatus*) juveniles," *Animals*, vol. 14, no. 3, Article ID 411, 2024.

[22] K. Toyota, K. Usami, K. Mizusawa, and T. Ohira, "Effect of blue light on the growth of the red swamp crayfish *Procambraus clarkii* larvae-seasonal and sexual differences," *Zoological Studies*, vol. 61, 2022.

[23] Y. Xu, W. J. Yuan, Y. L. Zhao, and H. Hu, "Influence of light wavelength on the vision of the *Macrobrachium nipponense*," *Journal of Shanghai Normal University (Natural Sciences)*, vol. 32, no. 3, pp. 75–78, 2003.

[24] S. Q. Chen, A. J. Li, and C. Wang, "The structure and physiological functions of eyes in shrimp (*Penaeus chinensis*)," *Progress in Fishery Sciences*, vol. 17, no. 1, pp. 30–34, 1996.

[25] R. B. Forward Jr and T. W. Gronin, "Spectral sensitivity of larvae from intertidal crustaceans," *Journal of Comparative Physiology*, vol. 133, pp. 311–315, 1979.

[26] M. L. Fanjul-Moles and B. Fuentes-Pardo, "Spectral sensitivity in the course of the ontogeny of the crayfish *Procambarus clarckii*," *Comparative Biochemistry and Physiology*, no. 1, pp. 61–66, 1988.

[27] M. L. Fanjul-Moles, M. Miranda-Anaya, and B. Fuentes-Pardo, "Effect of monochromatic light upon the ERG circadian rhythm during ontogeny in crayfish (*Procambarus clarckii*)," *Comparative Biochemistry and Physiology*, no. 1, pp. 99–106, 1992.

[28] F. Wang, X. Wang, H. T. Liu, and S. L. Dong, "Effects of light color on growth and energy budget of swimming crab," *Periodical of Ocean University of China*, vol. 44, no. 11, pp. 25–29, 2014.

[29] A. M. Bastos, J. F. Lima, and M. Tavares-Dias, "Effects of environmental light colors on the larviculture of the Amazon river prawn *Macrobrachium amazonicum*," *Aquaculture International*, vol. 27, no. 5, pp. 1525–1534, 2019.

[30] T. C. Leite, F. Picoli, D. d. A. Lopes et al., "The effects of acai oil addition in tilapia diets on performance, hepatic energy metabolism enzymes and antioxidant responses," *Aquaculture Research*, vol. 52, no. 1, pp. 395–402, 2021.

[31] M. A. O. Dawood, M. Alkafafy, and H. Sewilam, "The antioxidant responses of gills, intestines and livers and blood immunity of common carp (*Cyprinus carpio*) exposed to salinity and temperature stressors," *Fish Physiology and Biochemistry*, vol. 48, no. 2, pp. 397–408, 2022.

[32] Q. G. Meng, J. Chen, C. C. Xu et al., "The characterization, expression and activity analysis of superoxide dismutases (SODs) from *Procambarus clarkii*," *Aquaculture*, vol. 406, pp. 131–140, 2013.

[33] K. Wei and J. Yang, "Oxidative damage induced by copper and beta-cypermethrin in gill of the freshwater crayfish *Procambarus clarkii*," *Ecotoxicology and Environmental Safety*, vol. 113, pp. 446–453, 2015.

[34] F. L. Meng, Y. Z. Zhang, J. Kong, and G. R. Ma, "The research review of prophenoloxidase activating system in crustacean," *Ocean et Limnol Sinica*, vol. 30, no. 1, pp. 110–116, 1999.

[35] J. N. Long, Y. T. Cui, R. J. Wang et al., "Combined effects of high salinity and ammonia-N exposure on the energy metabolism, immune response, oxidative resistance and ammonia metabolism of the Pacific white shrimp *Litopenaeus vannamei*," *Aquaculture Reports*, vol. 20, Article ID 100648, 2021.

[36] X. Yang, M. Xu, G. Huang et al., "The hyperglycemic effect of melatonin in the Chinese mitten crab, *Eriocheir sinensis*," *Frontiers in Physiology*, vol. 9, Article ID 270, 2018.

[37] F. E. Maciel, M. A. Geihs, M. A. Vargas et al., "Daily variation of melatonin content in the optic lobes of the crab *Neohelice granulate*," *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, vol. 149, no. 2, pp. 162–166, 2008.

[38] S. B. Sainath, C. Swetha, and P. S. Reddy, "What do we (need to) know about the melatonin in crustaceans ?" *Journal of Experimental Zoology Part a-Ecological and Integrative Physiology*, vol. 319, no. 7, pp. 365–377, 2013.

[39] J. Y. Choi, Y.-U. Choi, J. Kho, and C. Y. Choi, "Effects of various photoperiods and specific wavelengths on circadian rhythm in ornamental cleaner shrimp *Lysmata amboinensis*," *Biological Rhythm Research*, vol. 50, no. 6, pp. 897–907, 2018.

[40] E. M. Elkadom, M. F. A. El-Kader, B. A. Bakr, A. M. Abozeid, and R. A. Mohamed, "Impacts of various single and mixed colors of monochromatic LED light on growth, behavior, immune-physiological parameters, and liver and brain histology of Nile tilapia fingerlings," *Aquaculture*, vol. 577, Article ID 740007, 2023.

[41] A. S. K. Yong, W. Y. Mok, M. L. M. Tamrin, R. Shapawi, and Y. S. Kim, "Effects of dietary nucleotides on growth, survival and metabolic response in whiteleg shrimp, *Litopenaeus vannamei* against ammonia stress condition," *Aquaculture Research*, vol. 51, no. 6, pp. 2252–2260, 2020.

[42] B. Sadoul and M. M. Vijayan, "5—stress and growth," *Fish Physiology*, vol. 35, pp. 167–205, 2016.

[43] C. Lebigre, M. Woillez, H. Barone et al., "Temporal variations in scale cortisol indicate consistent local-and broad-scale constraints in a wild marine teleost fish," *Marine Environmental Research*, vol. 182, Article ID 105783, 2022.

[44] S. M. Noureldin, A. M. Diab, A. S. Salah, and R. A. Mohamed, "Effect of different monochromatic LED light colors on growth performance, behavior, immune-physiological responses of gold fish, *Carassius auratus*," *Aquaculture*, vol. 538, Article ID 736532, 2021.

[45] N. Villamizar, L. M. Vera, N. S. Foulkes, and F. J. Sánchez-Vázquez, "Effect of lighting conditions on zebrafish growth and development," *Zebrafish*, vol. 11, no. 2, pp. 173–181, 2014.

[46] Y. X. Zou, Z. Z. Peng, W. X. Wang et al., "The stimulation effects of green light on the growth, testicular development and stress of olive flounder *Paralichthys olivaceus*," *Aquaculture*, vol. 546, Article ID 737275, 2022.
[47] K. Xie, Y. Chen, Z. H. Zhang, and G. F. Qiu, "A new alternative splice variant of ecdysteroid receptor (*EcR*) transcript differentially expressed during embryogenesis in the oriental river prawn *Macrobrachium nipponense*," *Aquaculture Research*, vol. 53, no. 17, pp. 6020–6028, 2022.
[48] Z. Li, W. Q. Wang, E. F. Zhang, and G. F. Qiu, "Identification of spliced mRNA isoforms of retinoid X receptor (*RXR*) in the oriental freshwater prawn *Macrobrachium nipponense*," *Genetics and Molecular Research*, vol. 13, no. 2, pp. 3914–3926, 2014.
[49] L. D. García-Rodríguez, J. C. S. Hernández, J. A. Aguiñaga-Cruz, C. G. J. Acosta, A. Santamaría-Miranda, and J. P. Apun-Molina, "Enzymatic activity in the shrimp *Penaeus vannamei* fed at different feeding frequencies," *Latin American Journal of Aquatic Research*, vol. 49, no. 2, pp. 280–288, 2021.
[50] M. Horigane, K. Ogihara, Y. Nakajima, and D. M. Taylor, "Isolation and expression of the retinoid X receptor from last instar nymphs and adult females of the soft tick *Ornithodoros moubata* (Acari: Argasidae)," *General and Comparative Endocrinology*, vol. 156, no. 2, pp. 298–311, 2008.
[51] L. Rodriguez-Viera, E. Perera, J. A. Martos-Sitcha et al., "Molecular, biochemical, and dietary regulation features of alpha-amylase in a carnivorous crustacean, the spiny lobster *Panulirus argus*," *Plos One*, vol. 11, no. 7, Article ID e0158919, 2016.
[52] Y. Z. Liu, H. H. Yao, T. T. Zhou, Z. H. Lin, and Y. H. Dong, "The discovery of circadian rhythm of feeding time on digestive enzymes activity and their gene expression in *Sinonovacula constricta* within a light/dark cycle," *Frontiers in Marine Science*, vol. 8, Article ID 744212, 2021.