frontiers in Marine Science

ORIGINAL RESEARCH
published: 04 March 2022
doi: 10.3389/fmars.2022.840353

Light Spectrum Impacts on Growth, Molting, and Oxidative Stress Response of the Mud Crab *Scylla paramamosain*

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An 8 weeks trial was performed to test the effects of light spectra [full-spectrum, violet (405 nm), blue (470 nm), cyan (500 nm), green (525 nm), yellow (570 nm), and red (625 nm)] on growth performance, molting, antioxidant capacity, stress response and expression of molting, and apoptosis-related genes in *Scylla paramamosain*. Results showed that spectrum had a significant effect on *S. paramamosain* physiology. Compared to blue light, crabs exposed to violet light had a significantly lower survival rate (79.5 ± 3.6% vs. 94.9 ± 3.6%), weight gain (49.2 ± 5.4 vs. 67.6 ± 6.7), molt frequency (4.2 ± 0.2 vs. 4.5 ± 0.1), and extended intermolt intervals between instar 1 and 2 stages (C1–C2) (6.3 ± 0.3 vs. 5.0 ± 0.1 days). Expression of the molt-inhibiting hormone (*mih*) gene was upregulated in crabs reared under violet light. According to the regression analysis, maximum SGR would be at 449.97 nm. Crabs exposed to blue light also had lower melatonin levels than under full-spectrum and lower cortisol levels than violet and yellow groups. Regarding oxidative stress, crabs in full-spectrum had lower H_2O_2 and MDA contents, however, no significant difference was found in total antioxidant capacity (T-AOC), superoxide dismutase (SOD), and catalase (CAT) in hepatopancreas from crabs under different spectra. Gene expression of *hsp40*, *hsp70*, *hsp90* were down-regulated in crabs exposed to the full-spectrum light group. Regarding apoptosis-related genes, *bcl-2* gene expression in crabs under cyan and the *cox IV* and *caspase 3* in green were upregulated, suggesting cyan light may inhibit, while green light may promote apoptosis. Taken together, these results suggest that blue or cyan light would promote growth performance, while full-spectrum light could reduce stress response in *S. paramamosain*.

Keywords: light spectrum, molting, oxidative stress, apoptosis, mud crab

OPEN ACCESS

Edited by:
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Hanafiah Fazhan, Shantou University, China
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Specialty section:
This article was submitted to Aquatic Physiology, a section of the journal Frontiers in Marine Science

Received: *21 December 2021*
Accepted: *31 January 2022*
Published: *04 March 2022*

Citation:
Chen S, Shi C, Migaud H, Song C, Mu C, Ye Y, Wang C and Ren Z (2022) Light Spectrum Impacts on Growth, Molting, and Oxidative Stress Response of the Mud Crab Scylla paramamosain. Front. Mar. Sci. 9:840353. doi: 10.3389/fmars.2022.840353

INTRODUCTION

The mud crab (*Scylla paramamosain*) is a marine decapod crustacean species which plays a significant commercial and ecological role in marine aquaculture. *Scylla paramamosain* is widely distributed and farmed in Indo-West-Pacific, including south and southeastern China. *S. paramamosain* has been the most productive marine crab species cultured in China since 2009,

and its output reached 159, 433 t in 2020 (China FBOAMO, 2021). Nevertheless, due to the unreliability of the hatchery phase in aquaculture settings, the mud crab industry still highly relies on wild harvested mud crab juveniles, raising concerns over the sector's sustainability. Unreliable hatchery supply is mainly attributed to the lack of optimized and standardized husbandry protocols during early development leading to high mortality, and variable growth performances (Chen et al., 2021a).

Light, as an important environmental cue that influence growth performance, behavior, and physiology of aquatic animals (Gao et al., 2016a; Takahashi et al., 2018; Nasr et al., 2019; Yang et al., 2020), has three core elements, i.e., intensity, spectrum, and photoperiod. The visible spectrum is composed of short-wavelength (violet, 380–440 nm and blue, 440–485 nm), middle wavelengths (green 500–565 nm and yellow, 565–590 nm), and long-wavelengths (orange, 590–625 nm and red, 625–740 nm) (Wu et al., 2021). Unlike terrestrial animals, photic conditions experienced by aquatic animals can be highly variable and depend on water properties including plankton, suspended particles, dissolved organic substances, and water depth acting as a chromatic filter (Mukai, 2011; Peng et al., 2019). In aquatic animals, understanding the impact of light and specifically spectrum on key physiological functions is complex and poorly characterized when compared to other abiotic factors. Studies performed in fish showed that shorter wavelengths (blue and green) can promote larvae growth performance in Atlantic cod (*Gadus morhua*), turbot (*Scophthalmus maximus*), and European sea bass (*Dicentrarchus labrax*) (Villamizar et al., 2009; Sierra-Flores et al., 2016). In crustaceans, enhanced growth was reported in the giant freshwater prawn (*Macrobrachium rosenbergii*) when exposed to green as opposed to red light (Wei et al., 2021). However, specific growth rate (SGR) was enhanced in the Chinese shrimp *Fenneropenaeus chinensis* reared under natural light while SGR was suppressed under blue light (Wang et al., 2003). In shellfish, previous studies on abalone *Haliotis discus* have shown improved hatching and larvae growth under blue and green light (Gao et al., 2015) contrasting with positive effect of orange and red light reported on growth performance of juvenile *H. discus* (Gao et al., 2016a). Such contrasting results reported in the literature clearly illustrate the species and stage of development specific effects of spectrum on aquatic animals and most studies so far have been performed in fish.

The effect of light on aquatic vertebrate animals is thought to be mainly mediated through melatonin (N-acetyl-5-methoxy-tryptamine), the light perception hormone which is remarkably conserved across vertebrate phyla (Falcón et al., 2010). In fish, studies have suggested that melatonin is involved in the circadian and seasonal entrainment of many essential physiological functions (Falcón et al., 1992; Oliveira et al., 2007). However, light regulation of pineal melatonin production diverged in teleost species as a result of evolution and striking differences in spectral sensitivities were reported between species (Migaud et al., 2007a; Vera et al., 2010). In crustaceans, light perception and transduction has not been studied much and while melatonin is mainly secreted by the eyestalks, it has also been detected in the hemolymph and nervous systems (Pape et al., 2008; Zhang et al., 2018). Melatonin was reported to impact on a range of biological processes in crustaceans including glucose metabolism, oxidative stress, limb regeneration, and molting (Sainath and Reddy, 2010a,b; Sainath et al., 2013; Girish et al., 2015). A recent study from our laboratory showed melatonin levels in the eyestalks of *S. paramamosain* were significantly affected by light intensity (Chen et al., 2021a), however, light spectral effects remain unknown in the species.

Many biological functions (e.g., reproduction, metabolism, growth, behavior) and life transition events (e.g., spawning, hatching, metamorphosis, smoltification) in fish are regulated by environmental signals including light with strong relevant applications to the sector. In crustaceans, molting is particularly interesting as it directly controls the growth of the animals (Kobayashi, 2012; Yang et al., 2018). Crustaceans molting is mainly controlled by ecdysteroids and molt-inhibiting hormone (MIH) secreted by the Y-organs and the complex X-organ–sinus gland (XO-SG) (Imayavaramban et al., 2007). The fundamental function of MIH is to suppress ecdysteroid biosynthesis to regulate molting (Qiao et al., 2018). Molting, and subsequently growth, in crab species are also regulated by many abiotic factors including temperature (Yuan et al., 2017), salinity (Gong et al., 2015), and light intensity (Li et al., 2011; Chen et al., 2021a). Previous studies indicated that light fluctuations from blue to green or yellow spectra could promote growth and molting frequency of *L. vannamei* (Guo et al., 2011). In contrast, the effect of light spectrum on mud crab molting remains little studied.

Oxidative stress is another critical function in animals which is impacted by external factors. The reactive oxygen species (ROS) such as superoxide anion radical (O^{2-}), hydroxyl radical (OH^{-1}), and hydrogen peroxide (H_2O_2) are produced as a result of metabolism and immune defense response in cells (Bogdan et al., 2000; Kohen and Nyska, 2002). Excessive accumulation of ROS may result in oxidative stress, cellular damage, and ultimately compromise cell functions (Guo H. et al., 2013; Guo Z.-X. et al., 2013; Cheng et al., 2020). In response to ROS, animals have evolved various antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxiredoxins (Prx) to counteract oxidation (Chen et al., 2021b). Light spectrum have been suggested to influence the antioxidant capacity of aquatic organisms as reported in abalone (*Haliotis discus hannai*) (Gao et al., 2016b), whiteleg shrimp (*Penaeus vannamei)* (Fei et al., 2020a), and turbot (*Scophthalmus maximus*) (Wu et al., 2021). Furthermore, as an end-product of lipid peroxidation, malondialdehyde (MDA) is used as an indicator of oxidative damage in organisms (Liu et al., 2011). Recent studies have demonstrated that ROS and the resulting oxidative stress play a pivotal role in activating apoptosis (Kannan and Jain, 2000). Apoptosis plays an essential role in removing the excess, damaged, necrotic, and potentially dangerous cells (Wyllie et al., 1980). The expression levels of apoptosis-related genes such as *bcl-2*, *p53*, and *caspase 3* can be used as biomarkers of tissue apoptosis. A recent study showed that exposure to dark condition suppressed apoptosis-related gene expression in *Litopenaeus vannamei* (Fei et al., 2020a). In addition, gene expression of *bcl-2*, *p53,* and *cytochrome c* in the hepatopancreas of *L. vannamei* was significantly reduced in shrimp reared under a full spectrum + UVA and full spectrum + UVB (Fei et al., 2020b).

In the present study, the Light Emitting Diodes (LED) were used to create seven different light spectra to investigate the effects of light spectrum on growth, molting, antioxidant capacity, and apoptosis-related gene expression in the mud crab *S. paramamosain*. The overall goal of this work is to identify optimal environmental conditions for *S. paramamosain* produced in land-based hatcheries and nurseries to boost juvenile outputs, performance and reduce the reliance of the sector on wild-harvested stocks.

MATERIALS AND METHODS

Experimental Animal, Rearing Conditions, and Experimental Design

A total of 273 juvenile mud crab (Initial weight: 12.05 ± 2.15 mg) were obtained from Choupijiang farm (Ningbo City, Zhejiang province, China) and transferred to the experimental tanks on the Meishan campus of Ningbo University. Crabs were randomly distributed into seven treatments in triplicates (13 crabs per replicate, 39 per treatment). Crabs were individually stocked in polypropylene containers (14.1 cm × 8.4 cm × 5.0 cm) with 750 mL of seawater. During the experiment, formulated diet with crude protein ≥ 40.0%, crude lipid ≥ 6.0%, and crude fiber ≤ 5.0% (Ningbo Tech-Bank Feed Co., Ltd., Ningbo, China) was given once daily at 17:00, and 100% rearing water was changed daily at 08:00 am. During the experiment, the water temperature was maintained at 26 ± 1°C, salinity 24 ± 1 ppt, ammonia and nitrite < 0.5 mg L^{-1} (HACH, 2604545 and 2608345, respectively), and dissolved oxygen > 6.0 mg L^{-1} (Proplus, YSI, Yellow Springs, Ohio, United States).

Seven LED lamps (Institute of Semiconductors, Chinese Academy of Sciences, Semiconductor Lighting R&D Center) with specific narrow bandwidths were used in the experiment. The light intensity and light spectrum (**Figure 1**) in each treatment were measured by a spectroradiometer (PLA-20 Plant Lighting Analyzer, Hangzhou, China). Peak and full width at half maximum (FWHM) wavelengths for the seven treatments were 634.4/233.4 nm (full-spectrum), 400.7/12.4 nm (Violet), 460.0/20.2 nm (Blue), 510.4/33.7 nm (Cyan), 518.5/34.1 nm (Green), 576.5/15.8 nm (Yellow), and 619.7/23.8 nm (Red), respectively. Systems were light proofed using black clothes to prevent light pollution between treatments. The light intensity was set at 1 W m^{-2} by adjusting dimmers and the distance between lamps and the water surface. The photoperiod was set as 12L:12D (photophase between 6:00 and 18:00).

Sampling and Data Calculations

The final weight (W_f), carapace width (CW), carapace length (CL), and body height (BH) of all living crabs were measured after 24 h starvation at the end of the experiment (8 weeks). The hepatopancreas and eyestalks were collected and snap frozen in liquid nitrogen. Samples were stored at −80°C for subsequent analysis.

The number of days between two consecutive molts was monitored as the molting interval. Survival rate, weight gain, SGR, molting interval, and molting frequency were calculated using the following equations:

$$\text{Survival rate (SR, \%)} = (N_t - N_i)/N_i \times 100$$

$$\text{Weight gain (WG, \%)} = (W_f - W_i)/W_i$$

$$\text{Specific growth rate (SGR, \% day}^{-1}) = 100 \times (\text{Ln}\, W_f - \text{Ln}\, W_i)/t$$

$$\text{Condition factor (CF)} = 100^* \text{ (body weight/CL}^3)$$

$$\text{Molting frequency (MF)} = \Sigma\, ((C_n - 1) \times N_n)/N_t$$

$$\text{Molting interval (MI, days)} = \text{Date } (C_n) - \text{Date } (C_{n-1})$$

Where N_t is the number of crabs at the beginning of the experiment; N_i is the number of crabs at the end of the experiment, W_f is final body weight in gram; W_i is initial body weight in gram, and the "t" is the experimental duration (days); N_n, the number of molting stages; N_t, the total number of survival crabs; C_n, the developmental stage of crab.

Analysis of Antioxidant Capacity

Samples (n = 3, 6 hepatopancreas per treatment) were homogenized in ice-cold normal saline and centrifuged at 825 g min^{-1} at 4°C for 15 min. The activity of superoxide dismutase (SOD, A001-3-2, Jiancheng, Nanjing, China) was measured by WST-1 method (Peskin and Winterbourn, 2000). Catalase (CAT, A007-1-1, Jiancheng, Nanjing, China) was tested using the hydrogen peroxide decomposition method (Góth, 1991). Total antioxidant capacity (T-AOC, A015-2-1, Jiancheng, Nanjing, China) was assessed via the ABTS method (Re et al., 1999). Malondialdehyde concentration (MDA, A003-1-2, Jiancheng, Nanjing, China) was measured by thiobarbituric acid (TBA) reaction (Ohkawa et al., 1979). Finally, hydrogen peroxide (H_2O_2) was tested using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). All the above analyses have been validated previously for *S. paramamosain* (Xu et al., 2019).

Measurement of Melatonin and Cortisol

The eyestalks from each treatment (n = 3, 6 individuals per treatment) were homogeneized and dissolved in PBS. After centrifuging at 825 g at 4°C for 15 min, the supernatant was collected to analyze melatonin and cortisol contents. Sample analyses were performed using crab's specific melatonin and cortisol ELISA kits supplied by Enzyme-linked Biotechnology (Qiaodu-Bio, Shanghai, China). Kits have been validated previsouly in *S. paramamosain* (Chen et al., 2021a). Detection limit was 1 pg mL^{-1}, and both intra-assay and inter-assay coefficients of variation for both kits were less than 15%.

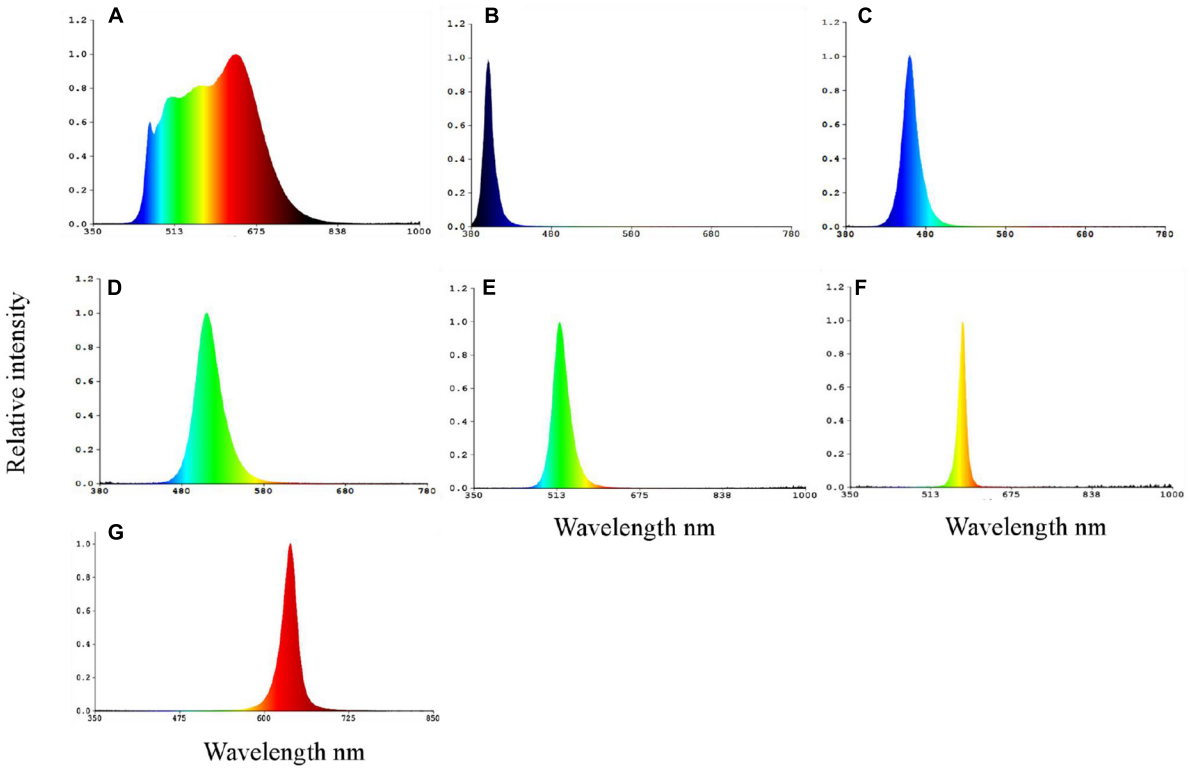

FIGURE 1 | The spectral composition of full-spectrum **(A)**, violet **(B)**, blue **(C)**, cyan **(D)**, green **(E)**, yellow **(F)**, and red **(G)** spectra LEDs on the water surface.

RNA Extraction, cDNA Synthesis, and Quantitative PCR Analysis

Trizol Reagent (Invitrogen, United States) was used to extract the total RNA from hepatopancreas samples according to manufacturer instructions. The quality and quantity of the RNA was assessed by electrophoresis and was measured with a small volume spectrophotometer (Eppendorf NanoDrop 2000). The RNA pellet was eluted in 20 μL of nuclease-free water prior to cDNA synthesis using HiFiScript cDNA Synthesis Kit (CW Biotech. Co., Lid., Shanghai, China) with 2 μg RNA. The synthesized cDNA stored at −80°C until use.

Real-time PCR assays were carried out in a Lightcycler 96 (Roche) using SYBR green as a fluorescent dye. The primers used for Quantitative PCR (qPCR) are listed in **Table 1**. The qPCR amplifications were carried out in total reaction volumes of 20 μL, which included 10 μL of SYBR green mixed reagent [Magic SYBR Mixture (CW3008H), CW Biotech. Co., Lid., Shanghai, China], 1 μL of each of the forward and reverse primers, 1 μL of cDNA template and 7 μL of ddH_2O. All detection for each sample was performed twice. The procedure of quantitative PCR contained an initial activation step at 95°C for 2 min, followed by 45 cycles of 95°C for 10 s and 55°C for 10 s, and 72°C for 20 s. To verify that the primer pair produced a single product, the dissociation curve of the product was also tested by heating from 55 to 95°C at the end of the reaction. The molting inhibiting hormone (*mih*), heat shock protein 40, 70, and 90 (*hsp40*, *hsp70,* and *hsp90*), tumor suppressor protein p53 (*p53*), *bcl-2*, *caspase-3,* and cytochrome *c* oxidase IV (*cox IV*) were normalized with β-*actin* used as the housekeeping gene. Target genes' critical threshold (Ct) quantities were standardized with quantities of housekeeping gene using the optimized comparative $2^{-\Delta\ \Delta\ CT}$ method (Livak and Schmittgen, 2001), and the results were presented as n-fold changes relative to the housekeeping gene.

Data Calculations and Statistical Analysis

All data are presented as the mean ± standard deviation (mean ± SD). Data were analyzed using SPSS 25.0 statistical software. The normality and homogeneity were checked by Kolmogorov–Smirnov test and Levene's test, respectively. The normal distributed and homogenous data were compared with one-way ANOVA and Tukey's *post hoc* multiple comparisons. Non-parametric tests, including the Kruskal-Wallis test, Mann-Whitney test, and Bonferroni correction, were performed to compare SR (after being subjected to arcsine square-root transformation), molting frequency, and molting interval. Bonferroni correction was used for multiple comparisons. To determine the relationship between wavelength, growth, and molting, the Pearson correlation analysis was performed and *t*-test were used. A significance of $P < 0.05$ was applied to all statistical tests.

TABLE 1 | Primers used for qPCR in this study.

Gene	Sequence (5′–3′)	Tm (°C)	References or accession number
β-*actin*	F: GAGCGAGAAATCGTTCGTGAC	56	Xu et al., 2019
	R: GGAAGGAAGGCTGGAAGAGAG		
mih	F: CCGCGCTAACTCCAGATTTT	57	JQ855710.2
	R: TTGCCAGTATCGGTGTGAGA		
hsp40	F: CATTGACTGAAAGTGCGAAAG	55	JQ864186.1
	R: AAACGGATGTCCACCCAAG		
hsp70	F: CAACAGAACTACGCCCTCC	57	EU754021.1
	R: AATCAGCCTCTTGGCATCA		
hsp90	F: AAGCGTATGACTTTGTGGA	54	JX987068.1
	R: CTTCTTCGTCTTGGGTTTG		
p53	F: AAGCAAGTCAATGAACGCTATGTG	55	Cheng et al., 2020
	R: AATGGGCTGCGAAGGACG		
caspase 3	F: ACGAAGTGAGGGGATTATGCC	55	
	R: CAGCCCATCCAGCGAGC		
bcl-2	F: GAAGTGGACCTGGAAAGTAA	55	MK426684.1
	R: GCTCACAGGGAGAAGCATAG		
Cytochrome *c* oxidase IV (*cox IV*)	F: GGCGAGGAAGGGATAC	55	FJ774694.1
	R: GGAAGTCAACACGGTCATA		

RESULTS

Survival, Growth, and Molting

Survival rates in the blue and cyan groups were significantly higher than in the yellow and violet groups but was not significantly different than in the full-spectrum, green and red groups (**Figure 2A**). Crabs reared under violet had significantly lower W_f and WG than the blue group (**Figures 2B,C**). No significant differences in W_f and WG were detected between full-spectrum, blue, red, yellow, green, and cyan groups. The coefficient of variation for weight gain (CV_{WG}) was higher in the full-spectrum group (41.1 ± 10.6%) than violet group (18.7 ± 8.1%), but not significantly different than any other groups (**Figure 2D**). The relationship between light wavelength and SGR based on a 4-parameter saturation kinetic models (4-SKM) showed maximum SGR for spectrum of 449.97 nm ($R^2 = 0.5961$) (**Figure 3**). No significant differences were detected in CW and BH between treatments (**Table 2**). However, CL was significantly influenced by light spectrum, with crabs reared under a blue light showing a higher CL than under violet. In addition, there was no significant differences in CF between treatments (**Table 2**).

Crabs reared under full-spectrum and cyan light had a higher molting frequency than yellow and violet (**Figure 4A**). No significant differences were detected in the molting interval (C2–C5). However, significant differences in molting interval (C1–C2) and molting interval (C5–C6) were detected between treatments (**Figures 4B–F**). The molting interval from C1 to C2 in the violet group increased significantly compared to full-spectrum and blue groups, but was not significantly different to any other groups (**Figure 4B**). For crabs of, A higher molting interval from C5 to C6 was detected in blue and red groups, which was significantly higher than for violet and yellow groups.

Melatonin and Cortisol

Melatonin levels in the eyestalks of crabs exposed to full-spectrum were significantly higher than for blue, cyan, and yellow groups but not significantly different from violet, green, and red

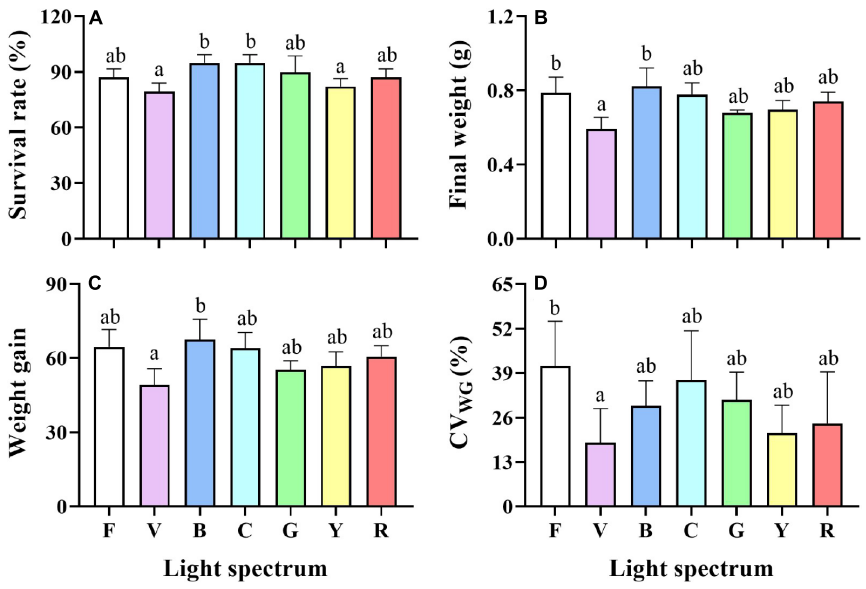

FIGURE 2 | Survival rate **(A)**, Final weight **(B)**, Weight gain **(C)**, coefficient of variation of weight gain **(D)** of *S. paramamosain* reared under the different spectra. Values are expressed as means ± SD ($n = 3$). F, Full spectrum; V, Violet; B, Blue; C, Cyan; G, Green; Y, Yellow; R, Red. Different superscripts denote significant differences between treatments ($P < 0.05$).

TABLE 2 | Morphology indexes of *S. paramamosain* reared under different light spectra during 8 weeks, including final carapace length (CL) and width (CW), body height (BH), and condition factor (CF).

Treatments	CL (cm)	CW (cm)	BH (cm)	CF
Full spectrum	1.61 ± 0.08	1.14 ± 0.05^{ab}	0.61 ± 0.03	19.01 ± 2.32
Violet (405 nm)	1.46 ± 0.11	1.03 ± 0.06^{a}	0.56 ± 0.02	19.27 ± 2.77
Blue (470 nm)	1.62 ± 0.09	1.16 ± 0.07^{b}	0.63 ± 0.05	19.16 ± 0.96
Cyan (500 nm)	1.49 ± 0.03	1.08 ± 0.02^{ab}	0.57 ± 0.01	23.63 ± 2.92
Green (525 nm)	1.51 ± 0.04	1.07 ± 0.02^{ab}	0.56 ± 0.02	19.89 ± 1.16
Yellow (570 nm)	1.51 ± 0.08	1.08 ± 0.04^{ab}	0.58 ± 0.03	20.52 ± 1.78
Red (625 nm)	1.56 ± 0.04	1.11 ± 0.01^{ab}	0.60 ± 0.01	19.64 ± 0.67

Values are expressed as the means ± SD (n = 3 replicate, and 34, 31, 37, 37, 35, 32, and 34 per treatment). Different superscripts denote significant differences between treatments (P < 0.05).

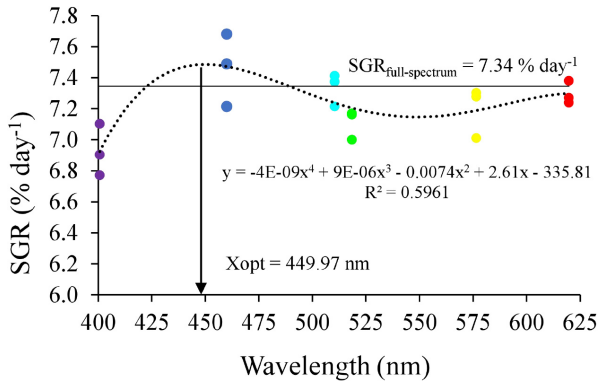

FIGURE 3 | Relationship between light wavelength and specific growth rate (SGR) in mud crab based on 4-SKM. The horizontal line represents as SGR of F group.

groups (**Figure 5A**). Cortisol levels increased in violet and yellow groups compared to blue light treatment, while no significant difference was detected between full-spectrum, cyan, green, and red groups (**Figure 5B**).

Antioxidant Capacity

Antioxidant capacity was significantly impacted by light spectrum (**Figure 6**). No significant differences were detected in T-AOC levels, SOD, and CAT activities in the hepatopancreas of crabs under different spectra. However, significant differences in H_2O_2 and MDA contents were detected between treatments (**Figures 6D,E**). The H_2O_2 content in crabs exposed to violet light (239.6 ± 23.1 U mgprot^{-1}) was significantly higher than in the green light (153.2 ± 24.8 U mgprot^{-1}). Crabs from red (11.1 ± 0.6 mmol mgprot^{-1}), violet (10.5 ± 1.3 mmol mgprot^{-1}), and blue (9.1 ± 0.6 mmol mgprot^{-1}) groups had higher MDA content than full-spectrum group (5.0 ± 0.8 mmol mgprot^{-1}).

Gene Expression

Molt-Inhibiting Hormone in Eyestalks

The relative expression of *mih* gene in eyestalks of crabs reared under violet and yellow groups were significantly higher than in all other treatments. No significant difference was detected between full-spectrum, blue, cyan, green, and red groups (**Figure 7**).

Heat Shock Protein Genes in Hepatopancreas

Light spectrum affected the relative mRNA gene expression of *hsp40*, *hsp70*, and *hsp90* measured in *S. paramamosain* hepatopancreas (**Figure 8**). The relative expression levels of *hsp40* in the violet group were significantly higher than in the other groups (**Figure 8A**). The relative expression levels of *hsp70* in the blue and yellow groups were significantly higher than in the other groups except for cyan p (**Figure 8B**). The expression of *hsp90* was significantly higher in blue compared to full spectrum and violet groups (**Figure 8C**).

Apoptosis Related Genes in Hepatopancreas

Significant differences were found in the relative expression levels of *bcl-2* between treatments. Compare to the full-spectrum and violet groups, *bcl-2* was significantly upregulated in crabs exposed to cyan light (**Figure 9A**). The relative expression levels of *p53* were not significantly different between treatments (**Figure 9B**). Relative expression levels of *cox IV* were also significantly influenced by light spectrum with the highest expression levels detected in the green group, which was significantly higher than all other groups except cyan group (**Figure 9C**). Expression levels of *caspase 3* were significantly higher in crabs reared under green light than in all other groups (**Figure 9D**).

Correlation Analysis

Correlations between wavelength and growth (WG), molting (MF), relative expression of *mih* and oxidative stress (H_2O_2 and MDA) parameters were analyzed by Pearson correlation coefficient (**Figure 10**). H_2O_2 content was negatively correlated with wavelength ($R^2 = -0.647$, $p = 0.004$), while WG was significantly positively correlated with molting frequency ($R^2 = 0.703$, $p = 0.001$) and negatively correlated with the relative expression of *mih* ($R^2 = -0.592$, $p = 0.01$) (**Figure 10**). In addition, the relative expression of *mih* was negatively correlated with molting frequency ($R^2 = -0.495$, $p = 0.037$).

DISCUSSION

In aquaculture, most research on the effects of light have been performed in fish with less studies done in invertebrates and especially crustaceans. Results from the current study showed that crabs reared under blue (460 nm) and cyan (510 nm) narrow bandwidth lights grew significantly better than for any other spectra tested, especially violet and yellow. Additionally, higher molting performance and lower oxidative stress and apoptosis were observed in the blue groups. The 4-SKM showed that the optimal light wavelength for maximum SGR in mud crab was 449.97 nm.

Published studies suggested that light spectrum can affect the feeding behavior of crabs impacting on feeding efficiency and growth (Cohen and Forward, 2002; Luchiari et al., 2009). However, contrasting results have been reported in the literature

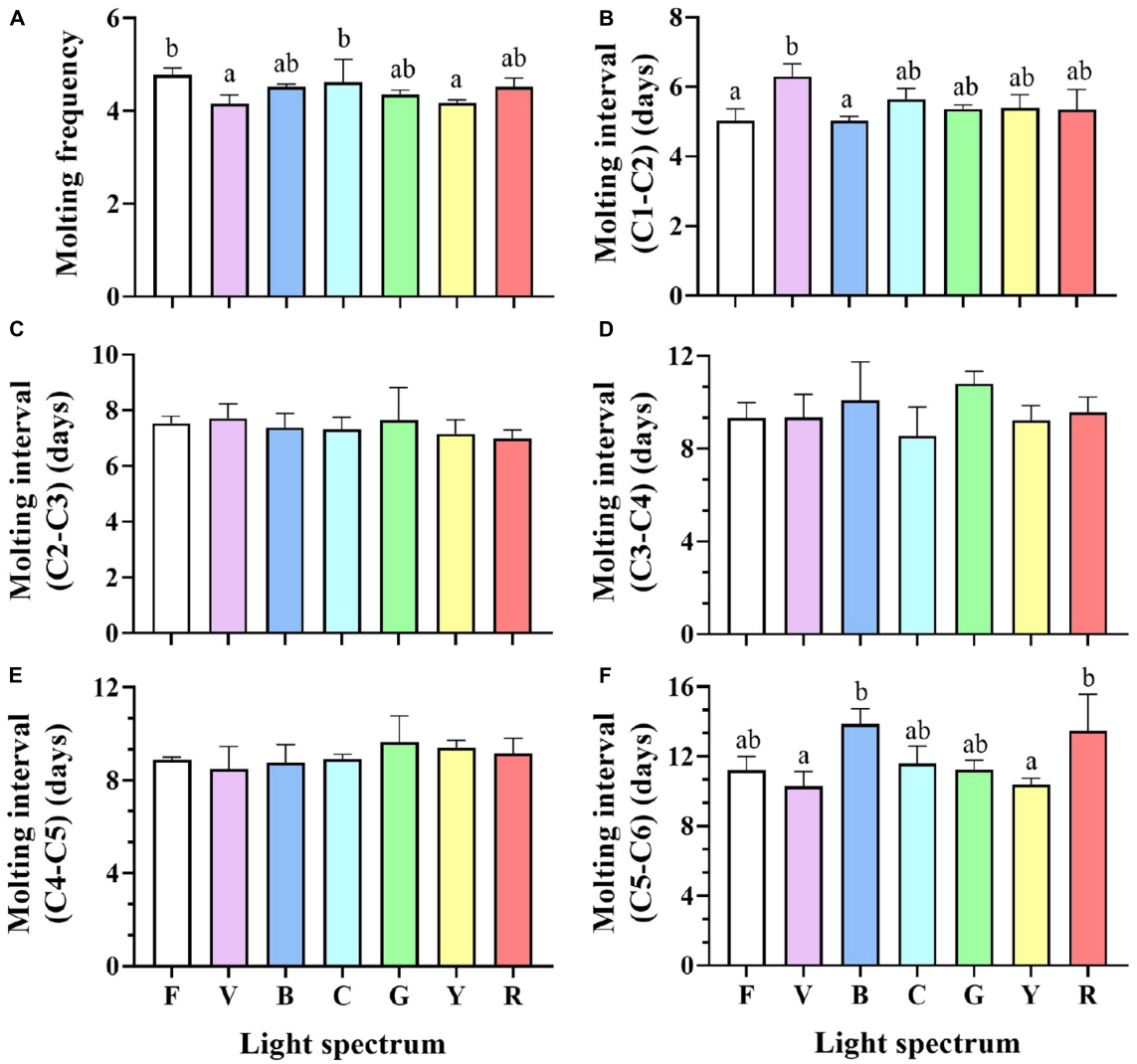

FIGURE 4 | Molting frequency **(A)**, Molting interval (C1-C2) **(B)**, Molting interval (C2-C3) **(C)**, Molting interval (C3-C4) **(D)**, Molting interval (C4-C5) **(E)** and Molting interval (C5-C6) **(F)** of *S. paramamosain* reared under the different spectra. Values are expressed as means ± SD (n = 3, all survival crab per replicate). F, Full spectrum; V, Violet; B, Blue; C, Cyan; G, Green; Y, Yellow; R, Red. Different superscripts denote significant differences between treatments ($P < 0.05$).

and the relationship between the animal spectral sensitivity and its behavior, depending on its stage of development, is not well understood. Spectral sensitivity of mud crab *Scylla serrata* was shown to peak at around 500 nm (Yang et al., 1985). A study in the swimming crab (*Portunus trituberculatus*) suggested that crabs are more sensitive to yellow and red light by looking at the microvillus of photoreceptors and number of organelles (Luo et al., 2006). However, a growth experiment showed feeding rate was suppressed in *P. trituberculatus* exposed to yellow light in contrast to blue which appeared to promote feeding but surprisingly growth did not correlate with feeding response (Wang et al., 2014). The visual sensitivity, background color preference, and growth and development are clearly not consistent, as observed in *P. trituberculatus* larvae (Shi et al., 2019) but also in fish species like barramundi (*Lates calcarifer*) (Ullmann et al., 2011). Light perception, sensitivity and biological efficiency change during the ontogenic development of any species and in crabs, it was suggested that juveniles may rely more on chemoreception to locate and identify their preys rather than light at the larval stage (Zimmer-Faust, 1989). This would explain mismatches reported between light sensitivity and physiological effects such as feeding and growth response. Growth effects identified under specific light conditions may be mediated through changes in feed conversion ratio as reported in fish species rather than feeding rate (Taylor et al., 2006; Karakatsouli et al., 2010). Characterizing the effect of light spectrum on crustacean physiology is therefore complex and will depend on species, stage of development but also light irradiance and photoperiod.

In the present study, the highest molting frequency was found in full-spectrum and cyan groups. The relative expression of *mih* gene was significantly upregulated in violet and yellow groups

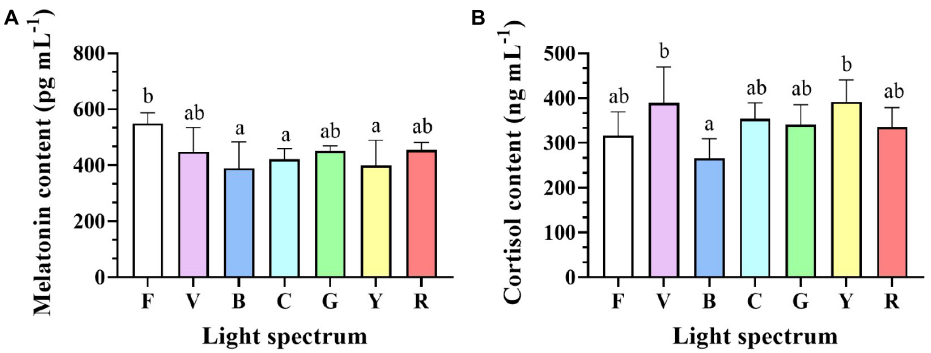

FIGURE 5 | Melatonin **(A)** and cortisol **(B)** levels in eyestalks of *S. paramamosain* reared under various light spectrum. Values are expressed as means ± SD (n = 3, 2 individuals per replicate). F, Full spectrum; V, Violet; B, Blue; C, Cyan; G, Green; Y, Yellow; R, Red. Different superscripts denote significant differences between treatments ($P < 0.05$).

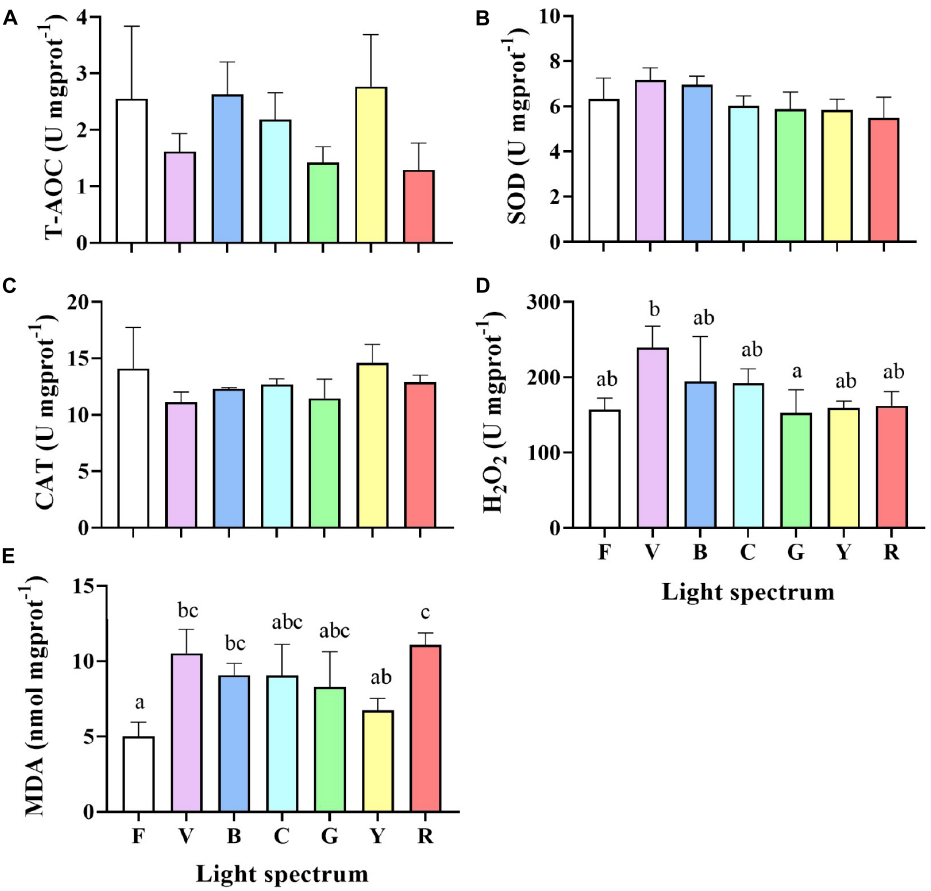

FIGURE 6 | The total antioxidant capacity (T-AOC) **(A)**, superoxide dismutase (SOD) **(B)**, catalase (CAT) **(C)**, hydrogen peroxide (H_2O_2) **(D)** and malondialdehyde (MDA) **(E)** contents in the hepatopancreas of *S. paramamosain* reared under the various light spectrum. Values are expressed as means ± SD (n = 3, 2 individuals per replicate). F, Full spectrum); V, Violet; B, Blue; C, Cyan, G, Green; Y, Yellow; R, Red. Different superscripts denote significant differences between treatments ($P < 0.05$).

(3.1- and 2.4-fold, respectively, compared to full-spectrum group), and these two groups had the lowest molting frequency, suggesting spectrum may play an important role in growth by regulating molting in crabs. Similar results were also observed in *Litopenaeus vannamei*, with higher molting frequency in shrimp exposed to green rather than blue light (Guo et al., 2011). Molting in crustaceans can also be suppressed by stress factors (Mykles et al., 2010). The highest cortisol levels found in

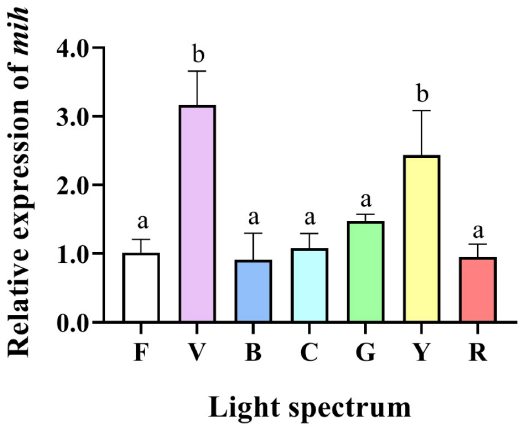

FIGURE 7 | Gene expression of molt-inhibiting hormone (*mih*) gene in the eyestalk of *S. paramamosain* reared under the various light spectrum. Values are expressed as means ± SD (n = 3, 2 individuals per replicate). F, Full spectrum; V, Violet; B, Blue; C, Cyan; G, Green; Y, Yellow; R, Red. Different superscripts denote significant differences between treatments ($P < 0.05$).

the present study were in crabs reared under violet and yellow light. As a stress indicator, cortisol is correlated to mortality and growth suppression (Tataranni et al., 1996). In crustaceans, the crustaceans hyperglycemic hormone (CHH) secreted by X-organ and sinus gland complex (XO-SG) has similar effects than cortisol and corticosterone, by regulating the conversion of glycogen into glucose (Elwood et al., 2009; Liu et al., 2019). Although CHH and MIH are different in structure, yet they appear to have similar functions in inhibiting ecdysteroid synthesis (Chung and Webster, 2003). Thus, higher cortisol levels could lead to delayed molting and subsequently reduced growth in crabs exposed to violet and yellow lights.

Heat shock proteins (HSPs) belong to a conserved class of molecular chaperones that play essential roles in growth, development, and stress response in all living organisms (Nie et al., 2017). HSPs are used as biomarkers of stress response, especially HSP70 and HSP90 which are involved in stress and immune responses (Fu et al., 2011). As a cochaperone for HSP70, HSP40 is in the regulation of ATP hydrolysis to maintain normal physiological functions and alleviate stress-related responses (Fan et al., 2003). In the present study, the relative expression of *hsp40* in the violet group was significantly higher. Moreover, higher expression of *hsp70* was detected in blue and yellow groups, and the relative expression of *hsp90* was upregulated in all narrow bandwidth light treatments. These results suggest that monochromatic light may lead to stress in crabs.

Melatonin, the light perception hormone, is very conserved across animals and plays an important role in the entrainment of circadian rhythmicity although the underlying pathways remain unclear (Migaud et al., 2007b; McStay et al., 2014; Saha et al., 2019; Song Y. et al., 2020). In crustaceans, melatonin injection was shown to induce ecdysteroidogenesis and stimulate

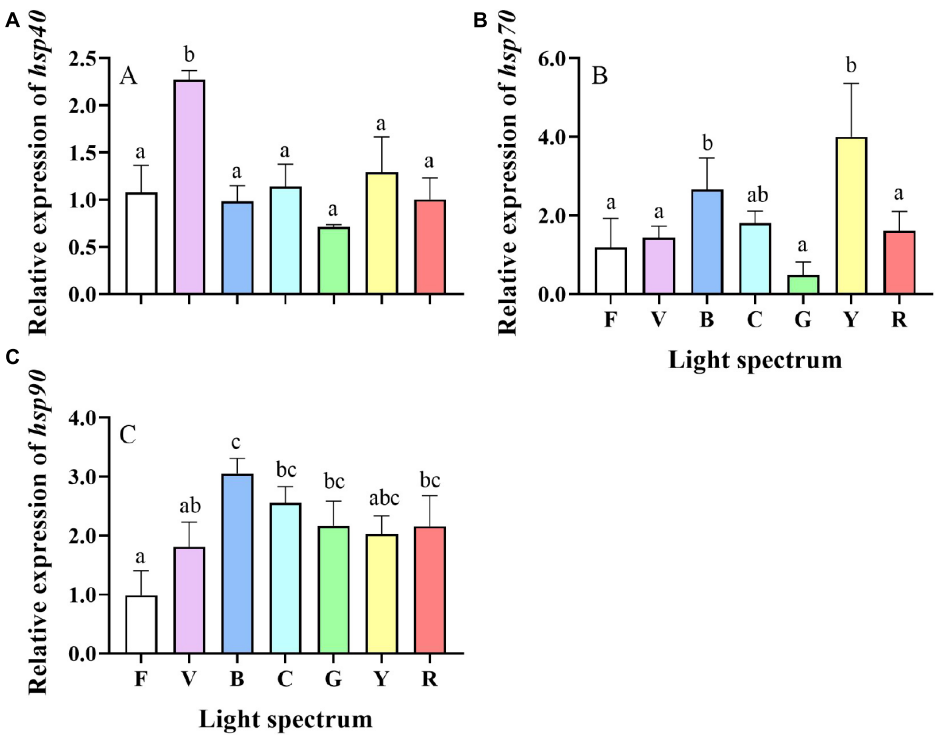

FIGURE 8 | Gene expression of heat shock protein 40, 70, and 90 [*hsp40* **(A)**, *hsp70* **(B)**, and *hsp90* **(C)**] in the hepatopancreas of *S. paramamosain* reared under the various light spectrum. Values are expressed as means ± SD (n = 3, 2 individuals per replicate). F, Full spectrum; V, Violet; B, Blue; C, Cyan; G, Green; Y, Yellow; R, Red. Different superscripts denote significant differences between treatments ($P < 0.05$).

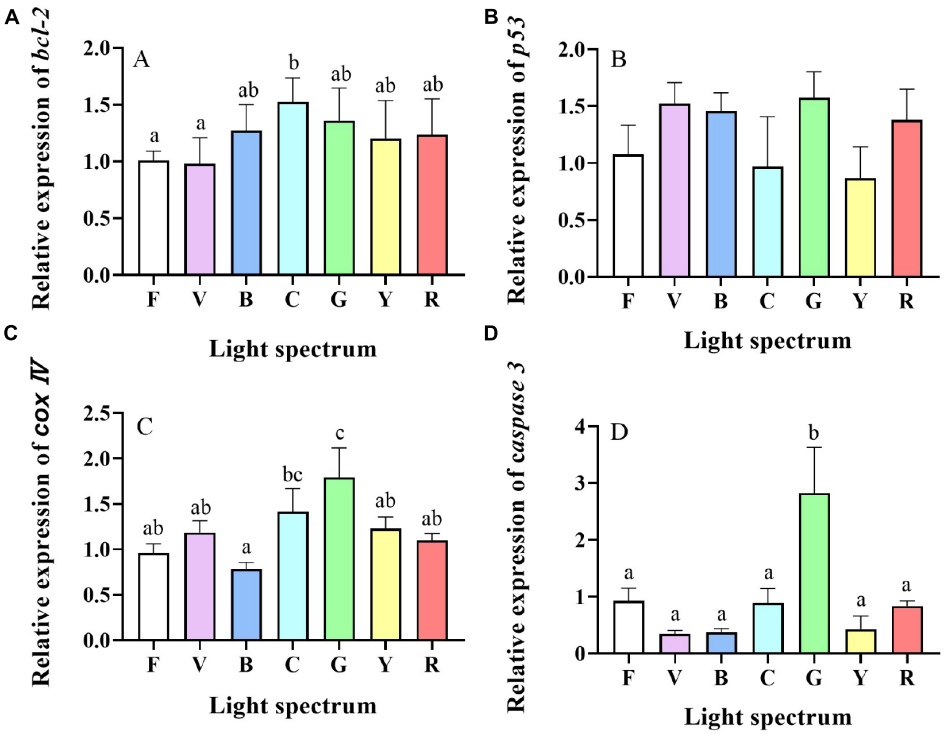

FIGURE 9 | Gene expression of apoptosis-related genes [*bcl-2* **(A)**, *p53* **(B)**, *cox* IV **(C)** and *caspase 3* **(D)**] in hepatopancreases of S. paramamosain reared under the various light spectrum. Values are expressed as means ± SD (n = 3, 2 individuals per replicate). F, Full spectrum; V, Violet; B, Blue; C, Cyan; G, Green; Y, Yellow; R, Red. Different superscripts denote significant differences between treatments ($P < 0.05$).

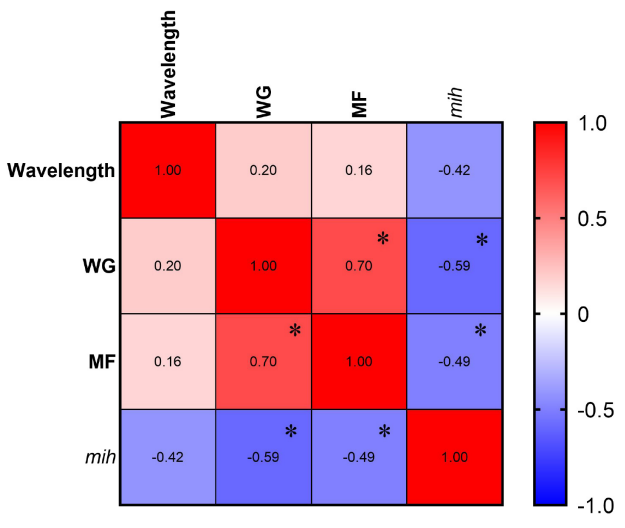

FIGURE 10 | Correlation analyses among wavelength and WG, MF, and relative expression of *mih* (n = 3, 2 individuals per replicate of *mih*, and n = 3 replicate; and 34, 31, 37, 37, 35, 32, and 34 individuals per treatment of WG and MF. The "*" indicates $P < 0.05$.

the synthesis of methyl farnesoate which is involved in the regulation of molting, leading to increased molting frequency in *Scylla serrata* (Sainath and Reddy, 2010b; Girish et al., 2015). Furthermore, melatonin was shown to suppress nitric oxide synthase activity and reduce nitric oxide (Kim et al., 2004; Lee et al., 2007), which are thought to inhibit ecdysteroidogenesis mediated by MIH (Nakatsuji et al., 2009). The present study showed that short-wavelength light (blue and cyan) suppressed melatonin contents of *S. paramamosain* eyestalks. Similar results were also observed in ornamental cleaner shrimp (*Lysmata amboinensis*) in which melatonin content in eyestalks of shrimp reared under blue light (455 nm) was significantly reduced compared to red (630 nm) and white (control) spectral treatments (Choi et al., 2019). However, a lower melatonin level was not shown in this study to impact on molting frequency.

Antioxidant capacity refers to compounds capable of protecting cells against oxidative stress involving ROS and RNS (reactive nitrogen species) that are by-products of the metabolism and immune system. Antioxidant capacity plays an essential role in maintaining homeostasis of oxidation/reduction by antioxidant enzymes such as SOD, CAT, and glutathione peroxidase (GPX) (Karadag et al., 2009; Wu et al., 2020). Excessive accumulation of ROS may cause oxidative damage, induce disease, and lead to death in animals. MDA, the end product of lipid peroxidation by ROS, is an essential indicator that reflects oxidative damage status (Chen et al., 2021b). While no significant differences in T-AOC, SOD, and CAT were detected between light treatments, H_2O_2 and MDA were significantly higher in hepatopancreas of crabs exposed to violet light, suggesting a higher oxidative stress under this spectrum.

Correlation analysis showed that the content of H_2O_2 was negatively correlated with wavelength. These results contrast with previously published data obtained in goldfish (*Carassius auratus*) and bay scallop (*Argopecten irradians*) that showed a reduction in H_2O_2 content under short wavelength lights (Kim et al., 2014; Song J. A. et al., 2020). Contrasting results between studies may be due to differences between species and stage of development, light characteristics of the respective habitats and tissues analyzed. Thus, further studies are required to understand the mechanisms behind such distinctions.

In the present study, *bcl-2* gene expression in cyan, and the *cox IV* and *caspase 3* in green were upregulated. Bcl-2 is the founding member of the Bcl-2 family that can block apoptosis by preventing the release of cytochrome *c* into the cytoplasm to suppress the activation of the caspase cascade (Chen et al., 2019). The *p53* is a crucial transcription factor for cell cycle arrest, cellular senescence, and apoptosis (Nuñez-Hernandez et al., 2018). Cytochrome *c* and cytochrome c oxidase (COX) could catalyze the terminal reaction of the mitochondrial electron transport chain, reducing oxygen to water by transfers electrons to molecular oxygen (Kadenbach et al., 2004; Hüttemann et al., 2012). The results from the present study suggest that cyan light could inhibit, while green light could promote apoptosis. On the contrary, previous studies in white leg shrimp and olive flounder (*Paralichthys olivaceus*) showed that green light could reduce oxidative stress and apoptosis via the mitochondria-mediated caspase-dependent pathway (Kim et al., 2016; Fei et al., 2020a). Unlike *P. vannamei* and *P. olivaceus*, *S. paramamosain* is an intertidal organism that migrates between that migrates between different tidal zones. Light requirements of mud crab may therefore be more complex. Thus, the results may suggest that cyan light (a mixture of blue and green light) and full-spectrum (a mixture of all spectrum) may have some advantage over other treatments in terms of stress and apoptosis for mud crab.

CONCLUSION

In summary, this study evaluated the effects of light spectrum on the growth performance, molting, antioxidant capacity, stress response, and apoptosis-related gene expression of *S. paramamosain*. The results suggested that crabs under full-spectrum, cyan and blue light performed better, while violet light appeared to reduce molting and growth of *S. paramamosain* via hormonal, oxidative stress, and apoptosis pathways. Notably, the current results suggested that the optimal light wavelength for SGR is 449.97 nm. Although more studies are required to describe and understand the underlying pathways behind light induced effects, these new results contribute to the optimization of rearing environment for mud crab farming while providing scientific hypotheses for further studies to characterize light perception and biological efficiency in crustaceans. Violet light should be avoided in the hatchery and nursery of *S. paramamosain* while blue, cyan, and full-spectrum light are recommended for juvenile on growing.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

SC: methodology, data curation, visualization, and writing—original draft. CS: conceptualization and review and editing. HM: writing—review and editing. CbS: resources and review and editing. CM: review and editing and supervision. YY: review and editing. CW: funding acquisition. ZR: resources. All authors contributed to the article and approved the submitted version.

FUNDING

The work was supported by the National Natural Science Foundation of China (Grant Nos. 32172994 and 31972783), 2025 Technological Innovation for Ningbo (No. 2019B10010), Collaborative Promotion Program of Zhejiang Provincial Agricultural Technology of China (No. 2020XTTGSC03), Zhejiang Thousand Talents Plan awarded to HM, Ministry of Agriculture of China and China Agriculture Research System (No. CARS48), the Special research funding from the Marine Biotechnology and Marine Engineering Discipline Group in Ningbo University (No. 422004582), and K. C. Wong Magna Fund in Ningbo University.

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