Light intensity impacts on growth, molting and oxidative stress of juvenile mud crab *Scylla paramamosain*

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Abstract

An 8 weeks regression study was performed to test the effects of increasing light intensities from darkness to 30 W m^{-2} on growth performance, molting, antioxidant capacity, and gene expression of molting and apoptosis-related genes in *Scylla paramamosain*. No significant differences were found in survival rates between treatments (ranging from 71.7 to 87.3 % at the end of the experiment). However, weight gain and specific growth rate over the study period displayed a curvilinear response to light treatments with peak values in crabs exposed to 10 W m^{-2}. Linear (BLM), quadratic (BQM), and 4-parameter saturation kinetic (4-SKM) models show the optimal light intensities for SGR were 12.98, 18.27, and 11.36 W m^{-2}, respectively. The light intensity did not appear to impact molting. However, crabs reared in darkness showed significantly reduced molt frequency (3.51 ± 0.16) and extended intermolt intervals compared to other treatments. Melatonin levels in the eyestalks were significantly higher in crabs exposed to darkness (502.52 ± 56.24 pg mL^{-1}) than light intensities of 10 to 30 W m^{-2} (413.50 ± 32.38 and 384.99 ± 15.56 pg mL^{-1}). Cortisol levels were significantly lower in the 0 and 5 W m^{-2} groups. Light intensity significantly impacted the activity of antioxidant enzymes, with crabs showing a significant increase in total antioxidant capacity (T-AOC) under 10 W m^{-2}, catalase (CAT), and superoxide dismutase (SOD) under 15 W m^{-2} and lower malondialdehyde (MDA). Gene expression of the molt-inhibiting hormone (MIH) was downregulated in eyestalks from crabs exposed to 10 W m^{-2} compared to darkness and 20-30 W m^{-2}. Expression of apoptosis-related genes did not show clear light intensity trends. Taken together, these results suggest light intensity can impact *S. paramamosain* growth, molting, stress levels, and antioxidant capacity. As such, light regimes used in crab farming should be carefully considered to optimize productivity and welfare.

Keywords: Light intensity, Molting, Cortisol, Apoptosis, Antioxidant capacity, *Scylla paramamosain*

1 Introduction

The mud crab (*Scylla paramamosain*) is the most economically important species in China, with 160,616 tonnes produced in 2019, according to the Chinese Fishery Statistical Yearbook (2020). However, farming still relies mainly on caught juveniles from the wild, which raised concerns over the sector's sustainability (Waiho et al., 2018). As a result, the breeding of mud crab in captivity has been researched in recent years (Ma et al., 2010; Ma et al., 2014). However, the early development and growth performances of mud crab remain problematic and lack optimized and standardized husbandry protocols.

Light is an important environmental cue for terrestrial (Nasr et al., 2019; Kang et al., 2020) and aquatic animals (Gao et al., 2021a, b). In fish, photoperiod is a significant environmental signal for entraining most physiological events, including reproduction and migration (Migaud et al., 2010). Light intensity and spectrum also appear to impact fish physiology as shown in European sea bass (*Dicentrarchus labrax*), Senegal sole (*Solea senegalensis*), and haddock (*Melanogrammus aeglefinus*) larvae which performed better when exposed to blue wavelengths (Downing and Litvak, 2001; Villamizar et al., 2011). So far, most research on the physiological effects of light and the light transduction pathways has been focusing on fish species, while mollusks and crustaceans remain little studied. The primary commercially important traits in crustaceans include growth and molting, which can be impacted by light intensity (Wang et al., 2004; Li et al., 2011). In our previous study, low light intensity (1.43 μmol m^{-2} s^{-1} (*ca.* 0.27 W m^{-2})) led to enhanced growth and increased accumulation of unsaturated fatty acids in adult mud crab (Li et al., 2020). However, contrasting results were reported for larvae of *Scylla paramamosain* in which survival post-metamorphosis was increased in crabs exposed to a high light intensity of 5000 lx (Zhang et al., 2011a). Differences in optimal light conditions are most likely species, stage of development, water characteristics, and light technology specific as suggested in other aquatic species (Migaud et al., 2007; Migaud et al., 2010; Villamizar et al., 2011). To date, the effects of light on mud crab physiology remains poorly understood.

An increasing number of studies have shown a relationship between crab molting status and growth increment (Kobayashi, 2012; Yang et al., 2018). Molting and subsequent growth in crab species are regulated by several neurohormones synthesized and secreted from the eyestalks, including the molt-inhibiting hormone (MIH), the crustacean hyperglycemic hormone (CHH), and the mandibular organ-inhibiting hormone (MOIH) (Li et al., 2019; Sook Chung et al., 2020). MIH is synthesized and released by the X-organ sinus gland (XO-SG) complex located within the eyestalks, and it inhibits the synthesis of ecdysteroid (Pamuru et al., 2012). The primary function of MOIH is to suppress the synthesis of methyl farnesoate that stimulates vitellogenesis while CHH regulates carbohydrate metabolism, lipid mobilization, and molting (Santos et al., 1997; Chung and Webster, 2005). Melatonin (N-acetyl-5-methoxy-tryptamine), in addition, the light perception hormone, is remarkably conserved across vertebrates and plays a vital role in the entrainment of circadian and seasonal physiology, albeit not fully elucidated in non-mammalian species (Falcón et al., 2010). However, the role of melatonin in invertebrates and especially crustaceans remains to be characterized. Previous studies suggested that melatonin secreted by the eyestalks in crustaceans and also found in the hemolymph and nervous systems, would interact with retinoic acid receptors, which are involved in glucose homeostasis independently from CHH-induced hyperglycemia, and

is also involved in ovarian maturation and limb regeneration closely associated to molting (Sainath and Reddy, 2010a, b; Sainath et al., 2013; Girish et al., 2015). Administration of melatonin to the edible crab, *Oziotelphusa senex senex*, was shown to stimulate molting suggesting a potential inhibition of eyestalk neuropeptides MIH and MOIH (Sainath and Reddy, 2010b). While the pleiotropic actions of melatonin remain to be elucidated in crabs, melatonin could mediate the effects of light intensity on crab physiology while also acting as a potent antioxidant as already extensively reported in the literature (Maciel et al., 2010).

Suboptimal light conditions can lead to stress and imbalance in the oxidative status of tissues in animals, as demonstrated in previous studies (Lushchak, 2011; Wei et al., 2019). Metabolism and immune defense response generate various reactive oxygen species (ROS) and reactive nitrogen species (RNS), which, when accumulated, could induce oxidative stress and damage proteins, lipids, and DNA, resulting in cell and tissues damage (Yu, 1994; Bogdan et al., 2000; Kohen and Nyska, 2002; Wu et al., 2016; Jin et al., 2017). ROS plays an essential role in cell proliferation, differentiation, signal transduction, and immune defense function (Bogdan et al., 2000; Ermak and Davies, 2002). However, excessive accumulation of ROS may lead to oxidative stress, damage to critical cellular biomolecules, and ultimately can compromise cell functions, as shown in crab species (Guo et al., 2013b, c; Cheng et al., 2020). Animals have evolved to counteract oxidation through various antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxiredoxins (Prx) (Chen et al., 2021). As an end-product of lipid peroxidation, malondialdehyde (MDA) is used as an indicator to reflect the status of oxidative damage in organisms (Liu et al., 2011). In addition, apoptosis is used to remove 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 *caspases 3* can be used as indicators to assess the state of tissue apoptosis (Cheng et al., 2020; Cheng et al., 2021). A recent study showed that exposure to dark and red light suppressed growth, increased oxidative stress response, and apoptosis-related gene expression levels of Pacific white shrimp (*Litopenaeus vannamei*, Boone, 1931) (Fei et al., 2020a, b).

This study aimed to test the effects of increasing light intensities (from darkness to 30 W m^{-2} using LED lighting systems) on growth, molting, antioxidant activity, and apoptosis-related gene expression in *S. paramamosain* and define optimal light intensity range for on growing of juvenile mud crab in aquaculture.

2 Material and methods

2.1 Experimental animal and rearing conditions

Juvenile mud crabs (Initial weight: 20.07 ± 0.37 mg) were obtained from Choupijiang farm (Ningbo

City, Zhejiang province, China). Crabs were transferred to the experimental unit on the Meishan campus of Ningbo University. Prior to the experiment, crabs were acclimatized in polypropylene boxes individually for one week and fed with a commercial diet made of 40 % protein and 6 % lipid (Ningbo Tech-Bank Feed Co. Ltd., Ningbo, China, Table 1). A total of 273 juvenile mud crabs were weighed and randomly distributed to 273 transparent polypropylene boxes and reared individually (14.1 cm × 8.4 cm × 5.0 cm). During the experiment, crabs were fed with a commercial diet daily at 17:00, and the water was replaced every day (8:00 am). Temperature (25 - 27 °C), salinity (23 - 25 ppt), ammonia nitrogen (HACH, 2604545) and nitrite (HACH, 2608345) (< 0.5 mg L^{-1}) and dissolved oxygen was monitored daily by YSI (Proplus, YSI, Yellow Springs, Ohio, USA) (> 6.0 mg L^{-1}).

2.2 Experimental design

Seven light intensities, *i.e.* 0, 5, 10, 15, 20, 25 and 30 W m^{-2} were tested in triplicate (n=3, 39 individuals/replicate) with a photoperiod of 12L : 12D (6:00 - 18:00 light). Light intensities and light spectral composition were set up using a spectroradiometer (EVERFINE Spectroradiometer, Model: PLA-30, Hangzhou, China). The water surface (in the air) light intensity was set to be 0 (0 W m^{-2}), 5 (5.02 ± 0.18 W m^{-2}), 10 (10.36 ± 0.47 W m^{-2}), 15 (14.91 ± 0.50 W m^{-2}), 20 (20.08 ± 0.19 W m^{-2}), 25 (24.87 ± 0.28 W m^{-2}) and 30 (29.89 ± 0.25 W m^{-2}) by adjusting the dimmer and the distance between the LEDs and the water surface and the experimental light spectral composition is shown in Fig. 1. Full-spectrum LEDs (Shenzhen Yamingjie intelligent technology Co. Ltd., Shenzhen, China) were suspended above the rearing tank. To avoid light pollution, tanks were light proofed using black-out cloth.

2.3 Sampling and calculations

At the end of the experiment (8 weeks), the body weight of all the survival crabs (ranging from 28 to 34/replicate depending on treatments) was measured after 24 h starvation. In addition, the hepatopancreas and eyestalk were collected and snap-frozen in liquid nitrogen. Finally, samples were transferred to -80 °C for later analysis. The growth-related parameters were calculated as follows:

Weight gain (WG) = $(W_f - W_i) / W_i$

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

Survival (%) = 100 × (final number of crabs) / (initial number of crabs)

CV_{wg} (Coefficient of Variation of WG) = 100% × (SD / mean)

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

Intermolt interval (IMI) = Date (C_n) – Date (C_{n-1})

Where W_f stands for final body weight (g); W_i stands for initial body weight (g); T for the experimental duration (d); N_n, the number of molting stages; N_t, the total number of survival crabs; C_n, the developmental stage of crab. The full-spectrum light measurement unit conversion was calculated as 1 W m^{-2} = 5.33 μmol m^{-2} s^{-1} (Villamizar et al., 2011).

2.4 Analysis of antioxidant capacity

Three hepatopancreas samples in each replicate from each treatment were randomly selected for antioxidant capacity analysis (total of 9/treatment). Before analysis, samples were homogenized in ice-cold normal saline and centrifuged at 825 g min^{-1} at 4 °C for 15 min. The total antioxidant capacity (T-AOC, A015-2-1) was assessed via the ABTS method, malondialdehyde (MDA, A003-1-2) was measured by thiobarbituric acid (TBA) reaction (Ohkawa et al., 1979), and enzyme activities of superoxide dismutase (SOD, A001-3-2) was measured by WST-1 method (Peskin and Winterbourn, 2000), catalase (CAT, A007-1-1) was tested using the hydrogen peroxide decomposition method (Góth, 1991). The operation steps were according to corresponding commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.5 Measurement of melatonin and cortisol

For mud crab, eyestalks synthesize and secrete most of the hormones. Thus, in the current study, three eyestalks in each replicate from each treatment (total of 9 / treatment) were randomly selected, grounded in glass mortar with liquid nitrogen to test melatonin and cortisol. According to the weight of eyestalk, four parts PBS was added to the centrifuge tube for tissue homogenization. The samples were centrifuged at 825 g min^{-1} at 4 °C for 15 min, and the supernatant was collected. The assays were performed with crab's specific melatonin and cortisol ELISA kit (Enzyme-linked Biotechnology, Shanghai, China). A standard curve was prepared using six standard dilutions of melatonin and cortisol: 0, 5, 10, 20, 40, and 80 pg mL^{-1} and 0, 12.5, 25, 50, 100, and 200 ng mL^{-1}. The assay was conducted in microplates based on the principle of competitive binding: melatonin or cortisol in standards and samples competed with melatonin or cortisol conjugated to horseradish peroxidase for the antibody binding sites in the microtiter wells. Microplates were incubated for 60 min at 37 °C, and unbound components were washed away with buffer. Bound melatonin or cortisol enzyme conjugate was measured by the reaction of the horseradish peroxidase enzyme with the substrate tetramethylbenzidine (TMB). The reaction was carried out at 37 °C for 15 min (stopped by adding 100 μ L of 0.5 M H_2SO_4), and the absorbance at 450 nm was read with an Absorbance Microplate Reader (SpectraMax 190, Molecular Devices, USA) within 15 min.

2.6 Total RNA extraction, cDNA synthesis, and qPCR analysis

Eyestalks secrete MIH, and the hepatopancreas is a vital metabolic and immune organ in crustaceans. Thus, eyestalks and hepatopancreas were selected to test the relative expression of MIH gene and apoptosis-related genes, respectively. In this study, the Trizol Reagent (Invitrogen, USA) was used to extract total RNA from eyestalks and hepatopancreas. The total RNA was treated with RNase-Free DNase (Takara, China) to remove genomic DNA contaminant, then quantified and electrophoresed to test for RNA integrity. The purity and concentration were assessed by Nanodrop-2000. The cDNA was synthesized using HiFiScript cDNA Synthesis Kit (CW Biotech. Co. Lid., Shanghai, China) with 2 μg RNA. Real-time PCR assays were carried out in a quantitative thermal cycler (Bio-Rad CFX96, USA) using SYBR green as a fluorescent dye. The primers used for qPCR are listed in Table 2. The molting inhibiting hormone (*MIH*), tumor suppressor protein p53 (*p53*), *Bcl-2*, *caspase-3*, and cytochrome *c* oxidase IV (*COX IV*) were analyzed with *β-actin* used as the housekeeping gene. All detection for each sample was performed in two replicates. To confirm primer pairs only produced a single product, dissociation curve analysis was performed by heating from 55 °C to 95 °C at the end of the reaction. Expression levels of target genes were normalized to housekeeping gene *β-actin* using the optimized comparative $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

2.7 Analytical models and statistical analysis

All results are presented as the mean ± standard deviation and analyzed by SPSS 25.0 statistical software. Before analysis, raw data were tested for normality of distribution and homogeneity of variance with Kolmogorov - Smirnov test and Levene's test. One-way analysis of variance (One-way ANOVA) was used to test for significant treatment effects. Differences were then analyzed using Tukey multiple comparison post-hoc test. No parametric analyses were performed for SR (after subjected to arcsine square-root transformation), molting frequency, and intermolt interval using Kruskal-Wallis and Mann-Whitney tests. To determine the relationship between MF and SGR, the Pearson correlation analysis was performed. All treatment effects were considered significant at a significance level of $P < 0.05$. In order to estimate the optimal light intensity for growth, SGR was fitted against light intensity using three models (Salze et al., 2018; Guo et al., 2020), *i.e.*, broken-line models with line (BLM), quadratic (BQM), and 4-parameter saturation kinetic models (4-SKM). The analytical models were performed using Excel 2016.

3 Results

238 3. Growth performance, survival, and molting

239 Final weight (W_f), weight gain (WG), and SGR were significantly lower in crabs exposed to
240 complete darkness compared with the other treatments ($P < 0.05$) (Table 3). Figure 2 shows the
241 fitting results of SGR to the light intensity by BLM, BQM, and 4-SKM. BLM, BQM and 4-SKM
242 showed that the optimal light intensity for maximum SGR in mud crab was 12.98, 18.27 and 11.36
243 W m^{-2}, respectively (Fig. 2). In addition, coefficients of variation in weight gain (CV_{WG}) of *S.*
244 *paramamosain* were significantly influenced by light intensity with crabs in 10 and 30 W m^{-2} group
245 showing a lower CV_{WG} than other groups ($P < 0.05$) (Table 3).

246 No significant differences were detected in survival between treatments ranging between 71
247 and 87 %. However, significant differences in molting frequency (MF) and intermolt interval (IMI)
248 were detected between treatments ($P < 0.05$) (Fig. 3A-3E). MF in crabs from 0 W m^{-2} group (3.51
249 ± 0.16) was significantly lower than 5 (4.41 ± 0.19), 10 (4.68 ± 0.16), 15 (4.59 ± 0.04) and 20 W m^{-2} (4.77 ± 0.21) groups ($P < 0.05$), but no differences were detected with 25 and 30 W m^{-2} groups
251 (Fig. 3A). Significant differences in IMI were found with crabs from 0 W m^{-2} group showing the
252 largest IMI in all consecutive molts except for C4-C5 (Fig. 3B-3E). Molt frequency and SGR
253 appeared to be positively correlated (R^2=0.58, Pearson correlation indices of 0.767, $P < 0.05$) (Fig.
254 3F).

255

256 3.2 Eyestalk melatonin and cortisol

257 Melatonin levels in the eyestalks of crabs from the 0 W m^{-2} group were significantly higher than for
258 all treatments except the 5 W m^{-2} group ($P < 0.05$) (Fig. 4A). In addition, melatonin levels in the 5
259 W m^{-2} group were higher than for 25 and 30 W m^{-2} groups. Cortisol levels significantly increased
260 with light intensity ($P < 0.05$) with a sharp increase between 0 and 5 W m^{-2} groups (0.23 ± 0.03 and
261 0.28 ± 0.09 mg mL^{-1}, respectively) compared to all other treatments (>0.94 ng mL^{-1}) (Fig. 4B).

262

263 3.3 Antioxidant capacity

264 Antioxidant capacity was significantly impacted by light intensity (Fig. 5). T-AOC levels in the
265 hepatopancreas of crabs reared under 10 W m^{-2} were significantly higher than those reared under all
266 other treatments except 15 W m^{-2} ($P < 0.05$), but no significant difference was detected between the
267 10 and 15 W m^{-2} ($P > 0.05$) (Fig. 5A). SOD activities in crabs reared under 15 W m^{-2} were
268 significantly higher than all other groups ($P < 0.05$), while crabs reared under 0 W m^{-2} had the lowest
269 SOD levels, although only significantly different from 15 and 20 W m^{-2} groups (Fig. 5B). CAT
270 levels in crabs from 10 and 15 W m^{-2} groups were significantly higher than 0, 5 and 20 W m^{-2} groups
271 ($P < 0.05$), but no significant difference was observed between 0, 5, 20 and 25 W m^{-2} groups ($P >$

0.05) (Fig. 5C). MDA levels were lower in crabs from the 15 W m^{-2} group than 25 and 30 W m^{-2} (Fig. 5D).

3.4 Gene expression

3.4.1 Molt-inhibiting hormone (*MIH*) in eyestalks

The relative expression of the *MIH* gene in eyestalks of crabs reared under 10 W m^{-2} was significantly lower than all other treatments except 5 and 15 W m^{-2} (Fig. 6). *MIH* expression appeared to increase with increasing light intensity from 10 to 30 W m^{-2}.

3.4.2 Apoptosis related genes in hepatopancreas

No significant differences were found in the relative expression levels of *Bcl-2* ($P > 0.05$) between treatments (Fig. 7A). However, the relative expression levels of *p53* were significantly influenced by the light intensity, with the lowest levels found in crabs exposed to 15 and 25 W m^{-2} ($P < 0.05$) (Fig. 7B). The relative expression of *p53* was gradually down-regulated and then up-regulated with the increasing light intensities. Relative expression levels of *COX IV* were also significantly influenced by the light intensity with the highest expression levels detected in the 5 W m^{-2} group, significantly higher than those reared under 0, 15, 20, and 30 W m^{-2} ($P < 0.05$) (Fig. 7C). Expression levels of *Caspase 3* were significantly higher in crabs reared under 10 W m^{-2} than those reared under 5, 15, and 30 W m^{-2} ($P < 0.05$) (Fig. 7D).

4 Discussion

In the present study, light intensities ranging from 0 to 30 W m^{-2} did not affect survival ranging from 71 to 87 %. Similar results were observed in overwintering *S. paramamosain,* in which light intensity did not affect survival (Li et al., 2020). However, a significant impact on the growth performance of juvenile *S. paramamosain* was observed. The optimal light intensity for growth was extrapolated to be 11.36-18.27 W m^{-2} based on the regression analysis between SGR and light intensity. However, the best growth performance was previously found in crabs exposed to a much lower light intensity of 1.43 μmol m^{-2} s^{-1} (*ca.* 0.53 W m^{-2}) (Li et al., 2020). The likely explanation for the contrasting results is the difference in stage of development between the two studies (juveniles in the present study vs. adult *ca.* 290 g in Li et al., 2020) concerning the ecological shift between pelagic to benthic habitats in adults mud crab (Wang et al., 2019). Similar results were obtained in swimming crab, *Portunus trituberculatus*, larvae with light sensitivity changing between the zoea and megalopa larval phases compared to juvenile crabs (Dou et al., 2021).

Although no significant difference in MF was detected among light treatment groups in this

study, a positive relationship was detected between MF and SGR (except for the darkness treatment). In addition, light intensity significantly affected *MIH* gene expression in the eyestalks of *S. paramamosain*. Molting in crustaceans, which consists in the shedding of the rigid exoskeleton, is primarily controlled by ecdysteroids and MIH secreted by Y-organs, X-organs, and Sinus gland (XO-SG) (Imayavaramban et al., 2007). Besides, molting in crustaceans is mainly controlled by ecdysteroids (Mykles, 2011). MIH is known to inhibit ecdysteroid synthesis (Watson and Spaziani, 1985; Huang et al., 2015) and regulate the duration of the molting cycle (Takuji et al., 2005).

The relationship between molting and light intensity has not been fully explored yet. Previous studies showed that constant light intensity did not affect MF of *Litopenaeus vannamei* (Guo et al., 2012) and Chinese mitten crab (Li et al., 2011) while fluctuating and periodic light intensity changes promoted the growth of *Litopenaeus vannamei* by increasing MF (Guo et al., 2012, 2013a). However, light intensity was shown to impact the WG of *Penaeus merguiensis* without apparent effects on MF or IMI (Hoang et al., 2003). *MIH* RNAi in *Macrobrachium nipponense* led to a significant reduction in IMI and increased body weight increment after molting (Qiao et al., 2018). Besides, a study in the red swamp crayfish (*Procambarus clarkia*) found that SNPs mapped on the 5' -flanking region of the *MIH* gene correlated with growth with GG genotype exhibiting superior growth than CG genotype (Xu et al., 2019). In addition, a negative correlation between carapace length and width increases induced by molting and *MIH* gene expression has been reported in the Chinese mitten crab (Liu et al., 2021), which confirms the relationships between the expression of *MIH* levels, molting, and growth. The present study indicates that light intensity may play an essential role in growth by regulating molting.

In the present study, melatonin levels in the eyestalks of *S. paramamosain* were significantly elevated in crabs exposed to darkness compared to light intensities of 10 W m^{-2} and above, and a light sensitivity threshold was detected between 5 and 10 W m^{-2} treatment groups. As a well-studied light perception hormone, melatonin synchronizes and entrains circadian rhythmicity with a wide range of biological functions in animals (Falcon et al., 2010; Saha et al., 2019; Song et al., 2020). In crustaceans, melatonin suppresses nitric oxide synthase activity leading to a reduction in nitric oxide as shown in *Gecarcinus lateralis* crabs (Kim et al., 2004; Lee et al., 2007), which is also thought to be the mode of action of the MIH - mediated inhibition of ecdysteroidogenesis (Nakatsuji et al., 2009). Furthermore, melatonin injection promoted the molting activity of freshwater crab, *Oziotelphusa senex senex,* leading to precocious molting in crabs (Sainath and Reddy, 2010b). Melatonin has also been reported to be a potent antioxidant and to promote limb regeneration by up-regulating the expression of growth-related genes (Zhang et al., 2018). Melatonin synthesis and secretion are regulated by the day-night cycle in light intensity (Falcón et al., 2010; Mcintyre et al.,

2010). Interestingly, in this study, while melatonin levels and relative expression of *MIH* remained the same in crabs exposed to darkness or 5 W m^{-2}, SGR and MF were significantly higher in the 5 W m^{-2} group. These results could suggest that the inferior growth performance in crabs under darkness may be due to the absence of circadian rhythmicity.

Most animals are sensitive to photoperiodic changes from early developmental stages, and their development and physiology are entrained by daily changes in illumination, resulting in circadian rhythms at molecular, biochemical, and cellular levels (Zhao et al., 2019). While the circadian system of crustaceans has not been characterized yet (Chabot and Watson, 2014), light / dark cycles were found to entrain important life cycle events, including molting in crabs (Li et al., 2019). For example, in American lobster (*Homarus americanus*), molting became arrhythmic under continuous light (Waddy and Aiken, 1999). In addition, growth was reduced under constant darkness as shown in blue swimmer crab (*Portunus pelagicus*) larvae (Andrés et al., 2010), Pacific white shrimp (*Litopenaeus vannamei*) (Fleckenstein et al., 2019), and spiny lobster (*Sagmariasus verreauxi*) (Fitzgibbon and Battaglene, 2012). Therefore, this hypothesis is further supported by the fact that crabs under darkness had the highest weight gain coefficient of variation among all groups, indicating that molting weight gain was reduced in the absence of light cues.

Cortisol is an important and conserved stress hormone used as an indicator of stress in animals, including crustaceans (Yong et al., 2020). As a critical catabolic hormone, cortisol increases the availability of blood glucose, free fatty acids, and amino acids (Christiansen et al., 2007) and is usually associated with depressed growth performance (Tataranni et al., 1996). In the present study, crabs in higher light intensities (10 – 20 W m^{-2}) also had higher SGR and cortisol simultaneously than darkness or low intensity (5 W m^{-2}). One explanation for these results is that cortisol could have anabolic effects by mobilizing energy to meet the increased metabolic demand to maintain homeostasis (Mommsen et al., 1999; Elverson and Wilson, 2005). Consequently, increased cortisol could stimulate food intake through interactions with feeding regulators and eventually promote growth (Bernier et al., 2004; Kang and Kim, 2013). Meanwhile, data on cortisol responses to chronic stress are scarce in crabs as for most aquatic animals (Aerts et al., 2015). Thus, while cortisol is a good indicator of acute stress, it may not reflect a state of chronic stress due to allostatic overload and desensitization.

Excessive accumulation of ROS may cause oxidative damage, induce disease, and lead to death in animals. Aerobic animals have evolved various antioxidant enzymes such as SOD, CAT, and Prx to protect cells from ROS damage (Wu et al., 2020), and antioxidant capacity is one of the most important factors affecting growth performance (Ding et al., 2020). Previous studies have shown that antioxidant enzymes can be activated rapidly following mild acute stress or challenge (Wang et

al., 2009; Duan et al., 2015). However, chronic stress or over-production and residuals of ROS could cause oxidative damage, suppressing antioxidant enzyme activity (Sun et al., 2012; Lin et al., 2018). In the present study, crabs reared under 15 W m^{-2} displayed significantly higher SOD, CAT, T-AOC, and lower MDA levels. As the end product of lipid peroxidation caused by free radicals, MDA directly reflects the degree of oxidative damage (Gao et al., 2016). These results suggest crabs reared under light intensities of 10 to 15 W m^{-2} had a higher antioxidant ability, and suboptimal light intensity may induce hepatopancreas oxidative stress.

Apoptosis is an essential physiological process to remove excess, damaged, or potentially dangerous cells such as virus-infected cells (Sahtout et al., 2001; Xian et al., 2013). Previous studies have shown that apoptosis can be induced by a variety of factors in crustaceans, including nitrite exposure (Cheng et al., 2020), lipopolysaccharide challenge (Xian et al., 2013), temperature reduction (Li et al., 2014), and ultraviolet light (Fei et al., 2020b). The Bcl-2 family proteins are essential regulators of intrinsic apoptosis, which protect cells from apoptosis. However, in this study, the relative expression of *Bcl-2* did not change under different light intensity treatments. The *p53* is a crucial transcription factor for cell cycle arrest, cellular senescence, and apoptosis. It can be activated by various stressors such as DNA damage, UV radiation, hypoxia, and nucleotide deprivation (Vogelstein et al., 2000; Cheng et al., 2020). In the present study, the lowest gene expression levels of *p53* were detected in crabs from the 15, 20, and 25 W m^{-2} groups, suggesting that *p53* expression is transcriptionally regulated by light intensity. In the mitochondrial-mediated apoptosis pathway, cytochrome *c* is released from mitochondria into the cytoplasm, triggers caspase activation, and eventually leads to apoptosis (Yang et al., 1997). Cytochrome c works together with dATP, apoptosis activating factor-1 and procaspase-9, to form the apoptosome. In this study, the relative expression of *COX IV* was significantly up-regulated in the 5 W m^{-2} group, indicating that the light intensity in this group appeared to induce apoptosis. Mitochondria produce ROS and release different proteins into the cytosol to scavenge the extra ROS (Giannattasio et al., 2008). Thus, high expression of *COX IV* could be triggered by the accumulation of ROS induced by light intensity. In the pathway initiated by mitochondria, caspase activation is triggered by the increase of mitochondrial membrane permeability and the release of cytochrome *c* (Liu et al., 1996). Caspase-9 and caspase-3 could be activated by apoptosome formed by cytochrome *c*. Caspase-3 and other effector caspases cleave death substrates, leading to apoptosis (Guo et al., 2020). The current study showed a consistent pattern of *caspase 3* expression with *COX IV*, further suggesting that light intensity induced apoptosis. Similarly, ultraviolet light exposure activated chitobiase (a chitinolytic enzyme) and caspase-3, leading to apoptosis, impaired molting, and reduced growth performance of zooplankton (Wolinski et al., 2020).

5 Conclusions

This study investigated the effect of light intensity on the growth performance, molting, antioxidant capacity, and apoptosis-related gene expression of *S. paramamosain*. Light did not significantly impact survival but significantly affected growth performance and molting of *S. paramamosain.* The observed effects could be mediated through hormonal, antioxidant, and apoptosis pathways (Fig. 8), although many more studies are required to describe and understand these pathways concerning environmental conditions. Importantly, results suggest the optimal light intensity for growth of juvenile *S. paramamosain* is between 11.36 and 18.27 W m^{-2} at the water surface. Thus, the supplementary full-spectrum artificial light source could improve the production parameters of juvenile *S. paramamosain*. These new results contribute to understand better optimal light conditions for the farming of mud crab and provide scientific hypotheses for further studies to characterize light regulation in crustaceans.

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Zhao, J., Warman, G.R., Stanewsky, R., Cheeseman, J.F., 2019. Development of the molecular circadian clock and its light sensitivity in *Drosophila Melanogaster*. J. Biol. Rhythm. 34, 272-282.

706 Table 1. Nutrient contents of basal diet (air-dry basis).

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708 Table 2. Primers used for qPCR in this study.

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710 Table 3. Effects of light intensity on the growth performance, survival rate, and molting performance
711 of juvenile *Scylla paramamosain* (mean initial weight 20.07 mg).

712

713 Table 1.

Items	composition (%)
Crude protein	≥40.0
Crude lipid	≥6.0
Crude fiber	≤5.0
Ash	≤18.0
Moisture	≤12.0
Total phosphorus	≥1.2
Lysine	≥2.0

714

715 Table 2.

Gene	Sequence (5'-3')	Reference
β-actin	F: GAGCGAGAAATCGTTCGTGAC R: GGAAGGAAGGCTGGAAGAGAG	(Xu et al., 2019)
MIH	F: CCGCGCTAACTCCAGATTTT R: TTGCCAGTATCGGTGTGAGA	JQ855710.2
p53	F: AAGCAAGTCAATGAACGCTATGTG R: AATGGGCTGCGAAGGACG	(Cheng et al., 2020)
caspase-3	F: ACGAAGTGAGGGGATTATGCC R: CAGCCCATCCAGCGAGC	
Bcl-2	F: GAAGTGGACCTGGAAAGTAA R: GCTCACAGGGAGAAGCATAG	MK426684.1
cytochrome *c* oxidase IV	F: GGCGAGGAAGGGATAC R: GGAAGTCAACACGGTCATA	FJ774694.1

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722 Table 3.

Treatment	0	5	10	15	20	25	30
Light intensity ($W\ m^2$)	0	5.02 ± 0.18	10.36 ± 0.47	14.91 ± 0.50	20.08 ± 0.19	24.87 ± 0.28	29.89 ± 0.25
Initial Weight (mg)	20.07 ± 0.37						
W_f (g)	0.85 ± 0.02^a	1.21 ± 0.01^b	1.33 ± 0.09^b	1.31 ± 0.17^b	1.32 ± 0.08^b	1.26 ± 0.03^b	1.18 ± 0.03^b
WG	41.32 ± 0.89^a	58.98 ± 0.65^b	64.83 ± 4.71^b	63.76 ± 8.32^b	64.65 ± 4.19^b	61.56 ± 1.60^b	57.51 ± 1.72^b
SGR (%/day)	6.55 ± 0.01^a	7.21 ± 0.07^b	7.43 ± 0.13^b	7.31 ± 0.26^b	7.42 ± 0.13^b	7.33 ± 0.03^b	7.21 ± 0.03^b
CV_{WG} %	40.83 ± 5.11^b	27.58 ± 7.15^{ab}	22.13 ± 7.14^a	30.26 ± 8.64^{ab}	25.89 ± 5.31^{ab}	25.10 ± 6.15^{ab}	23.29 ± 4.09^a
Survival (%)	87.33 ± 4.04	87.33 ± 4.04	82.33 ± 4.62	82.33 ± 4.62	79.67 ± 4.62	87.00 ± 8.66	71.67 ± 4.62

723 Different letters denote significant differences between treatments ($P < 0.05$).

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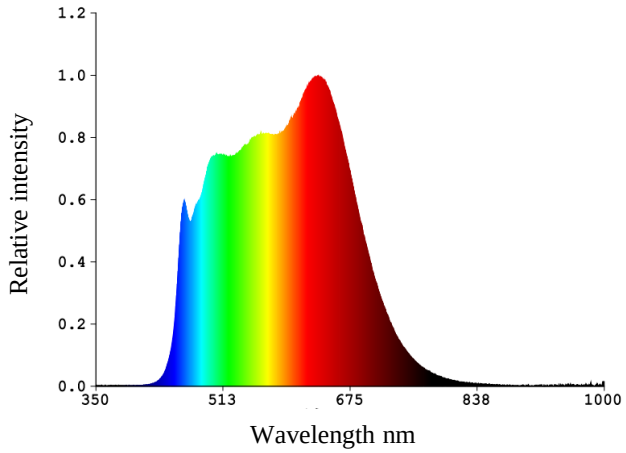


Fig. 1 The spectrum of the experimental LED light source.

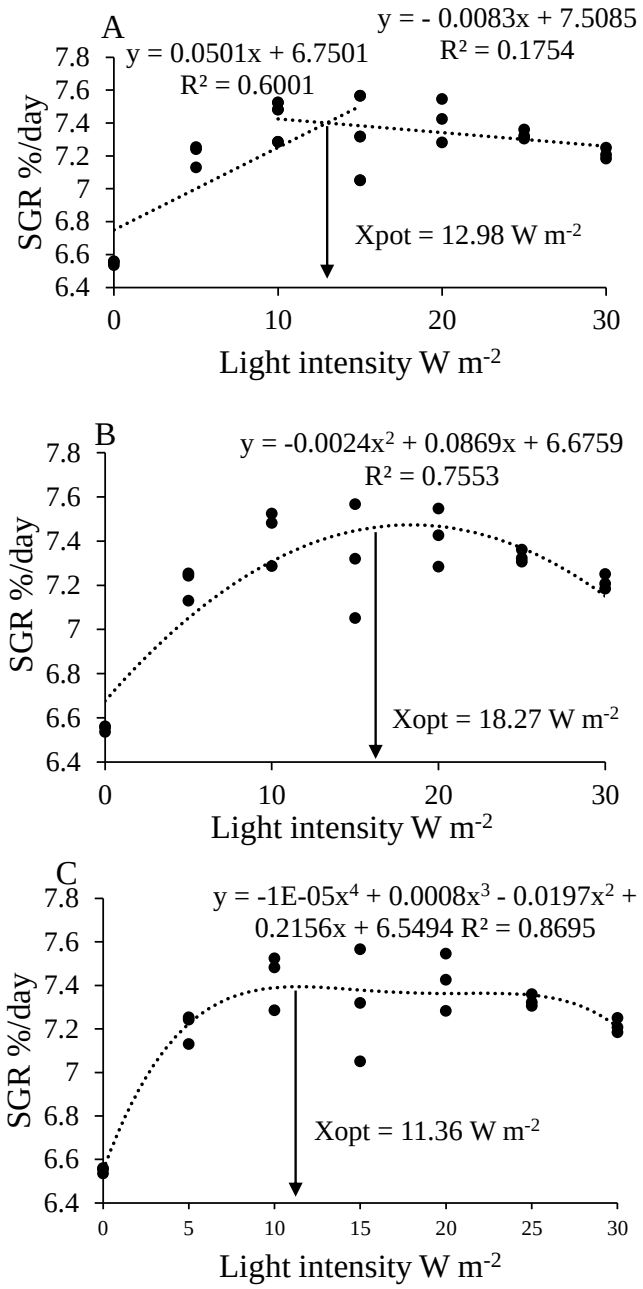
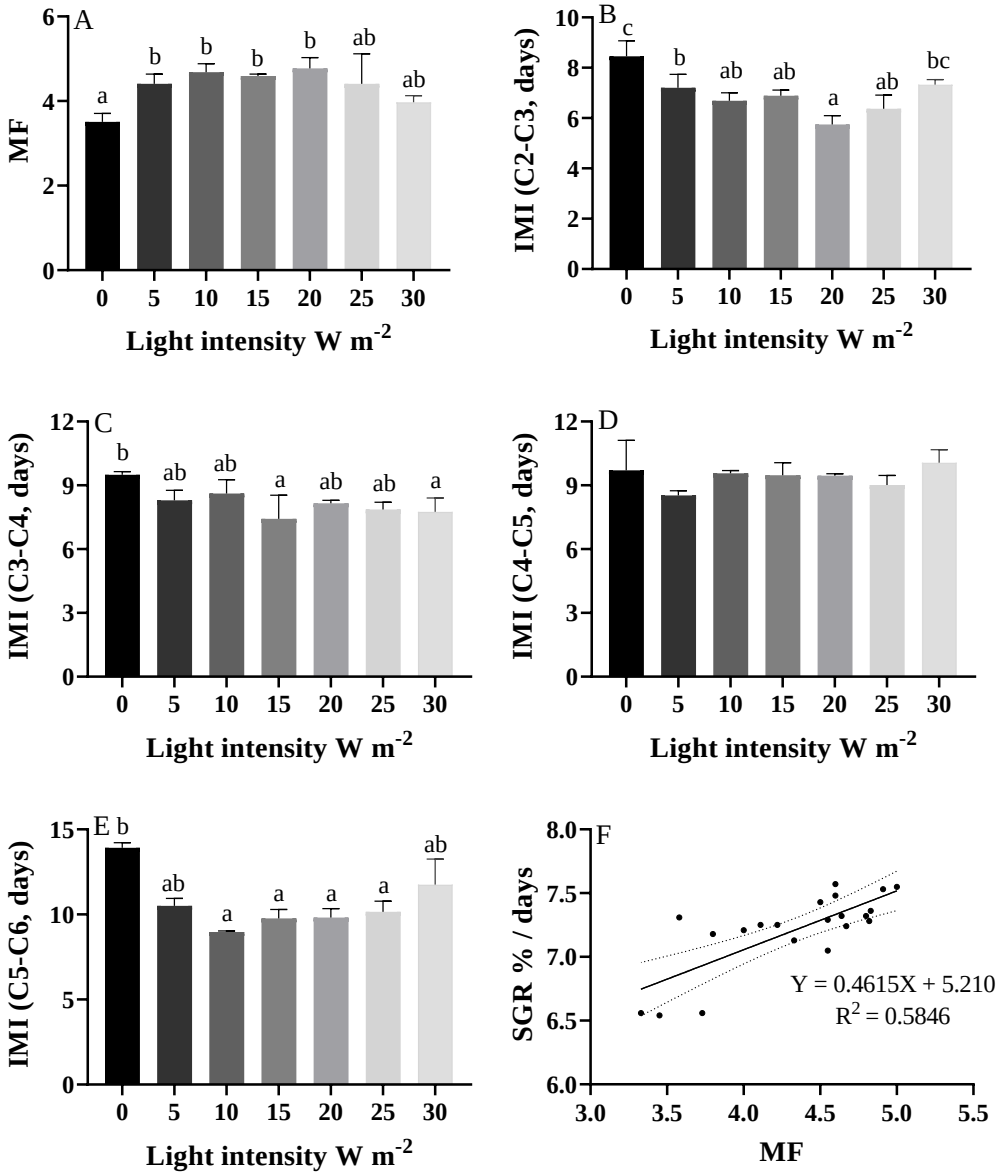


Fig. 2 Relationship between light intensity and specific growth rate (SGR) in mud crab based on linear (BLM) (A), quadratic (BQM) (B), and 4-parameter saturation kinetic (4-SKM) (C) models, where Xopt represents the optimal light intensity for the maximum SGR.



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768 Fig. 3 Molting frequency (MF) (A) and intermolt interval (IMI) (B-E) in *S. paramamosain* reared
769 under increasing light intensities (0 to 30 W m^{-2}) and the relationship of MF and SGR (F). Molt
770 frequency and SGR appeared to be positively correlated (R^2=0.58, Pearson correlation indices of
771 0.767, $P < 0.05$). Values are expressed as means ± SD (n = 3). Different superscripts denote
772 significant differences between treatments ($P < 0.05$). Dashed lines represent the 95% estimate of
773 the confidence interval.

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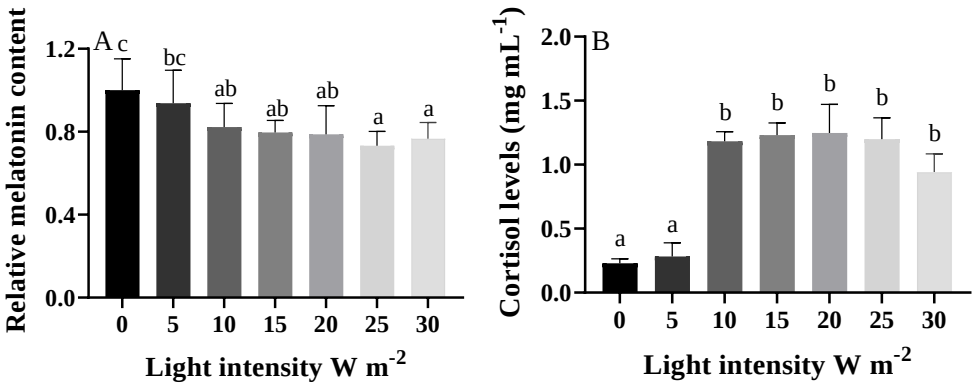


Fig. 4 Relative melatonin content (A) and cortisol levels (B) in eyestalks of *S. paramamosain* reared under increasing light intensities (0 to 30 W m^{-2}). Values are expressed as means ± SD (n = 3). Different superscripts denote significant differences between treatments ($P < 0.05$).

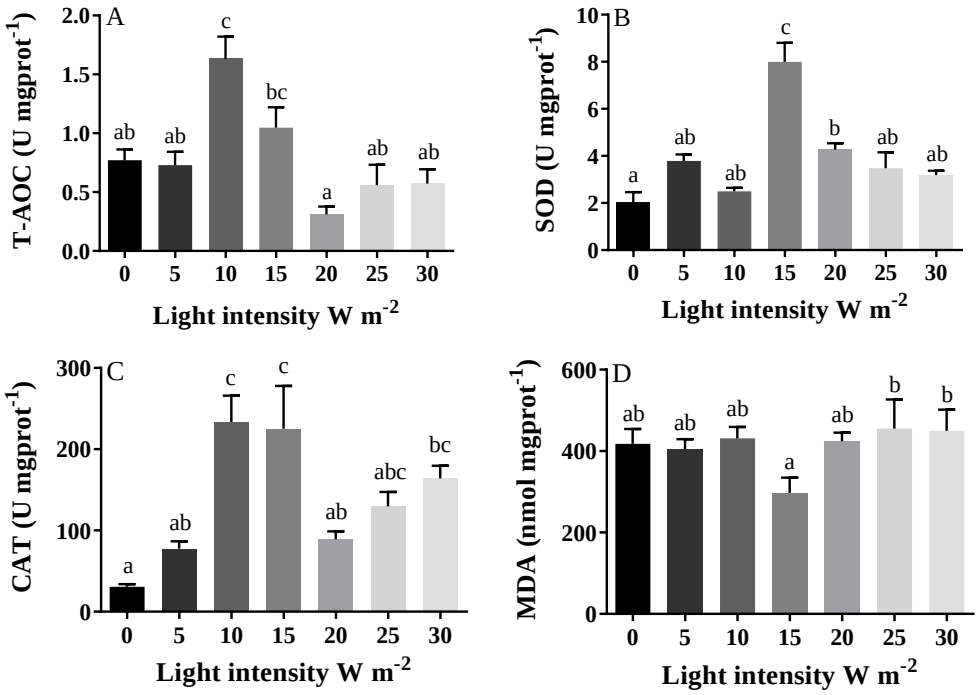


Fig. 5 Total antioxidant capacity (T-AOC) (A), superoxide dismutase (SOD) (B), catalase (CAT) (C), andand malondialdehyde (MDA) (D) contents in the hepatopancreas of *S. paramamosain* reared under increasing light intensities (0 to 30 W m^{-2}). Values are expressed as means ± SD (n = 3). Different superscripts denote significant differences between treatments ($P < 0.05$).

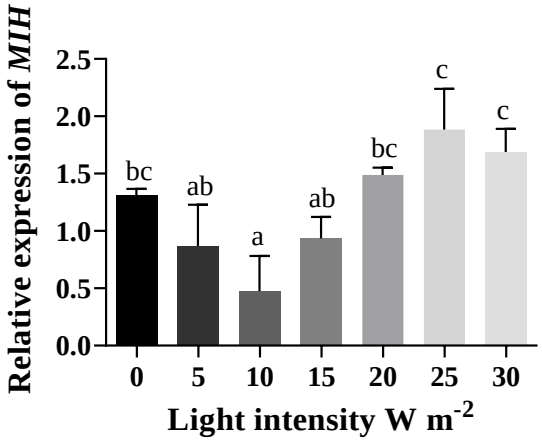


Fig. 6 Gene expression of molt-inhibiting hormone (*MIH*) in the eyestalk of *S. paramamosain* reared under increasing light intensities (0 to 30 W m^{-2}). Values are expressed as means ± SD (n = 3). Different superscripts denote significant differences between treatments ($P < 0.05$).

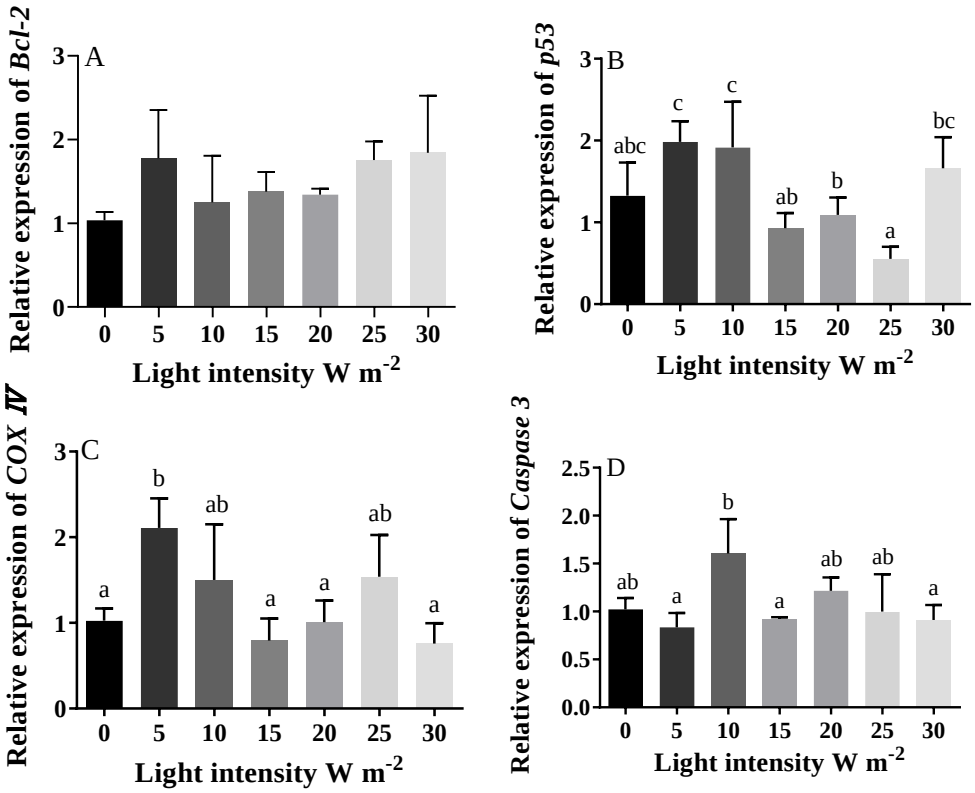
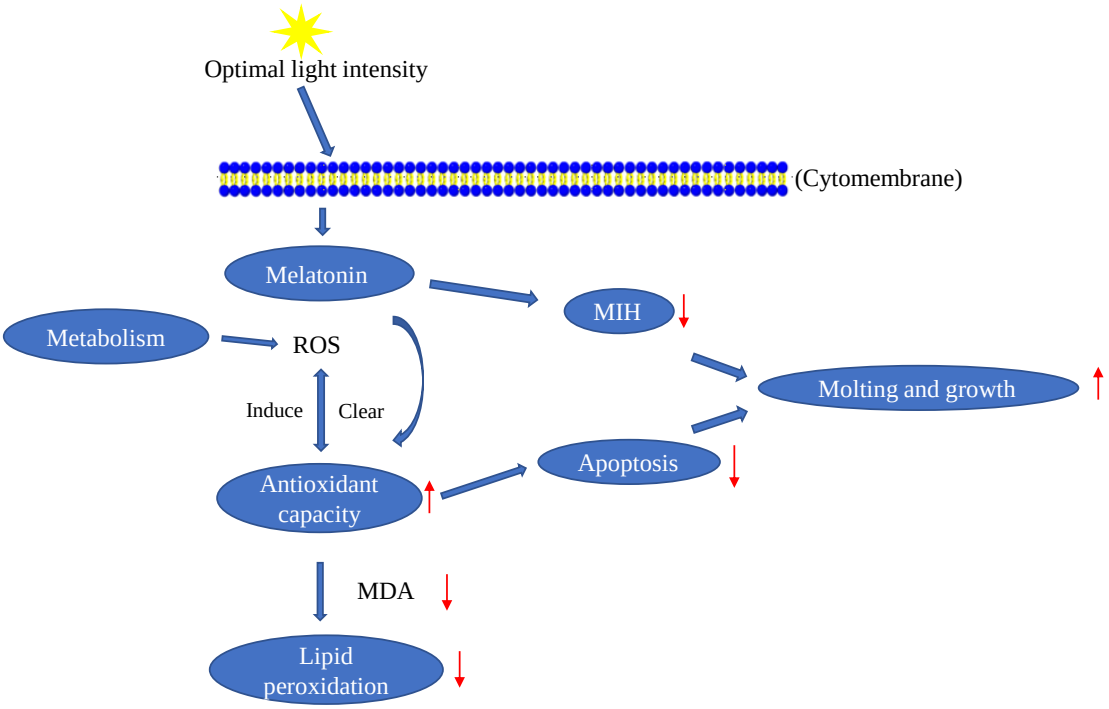


Fig. 7 Gene expression of apoptosis-related genes *Bcl-2* (A), *p53* (B), *COX Ⅳ* (C) and *Caspase 3* (D) in hepatopancreases of *S. paramamosain* reared under increasing light intensities (0 to 30 W m^{-2}). Values are expressed as means ± SD (n = 3). Different superscripts denote significant differences between treatments ($P < 0.05$).



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889 Fig. 8 A possible mechanism for how light intensities affect the growth and molting of *S.*
890 *paramamosain* through hormonal, antioxidant and apoptosis pathways.

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