

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

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33 **Abstract**

34 An 8 weeks regression study was performed to test the effects of increasing light intensities
35 from darkness to 30 W m⁻² on growth performance, molting, antioxidant capacity, and gene
36 expression of molting and apoptosis-related genes in *Scylla paramamosain*. No significant
37 differences were found in survival rates between treatments (ranging from 71.7 to 87.3 % at
38 the end of the experiment). However, weight gain and specific growth rate over the study
39 period displayed a curvilinear response to light treatments with peak values in crabs
40 exposed to 10 W m⁻². Linear (BLM), quadratic (BQM), and 4-parameter saturation kinetic (4-SKM)
41 models show the optimal light intensities for SGR were 12.98, 18.27, and 11.36 W m⁻², respectively.
42 The light intensity did not appear to impact molting. However, crabs reared in darkness showed
43 significantly reduced molt frequency (3.51 ± 0.16) and extended intermolt intervals compared to
44 other treatments. Melatonin levels in the eyestalks were significantly higher in crabs exposed to
45 darkness (502.52 ± 56.24 pg mL⁻¹) than light intensities of 10 to 30 W m⁻² (413.50 ± 32.38 and
46 384.99 ± 15.56 pg mL⁻¹). Cortisol levels were significantly lower in the 0 and 5 W m⁻² groups. Light
47 intensity significantly impacted the activity of antioxidant enzymes, with crabs showing a
48 significant increase in total antioxidant capacity (T-AOC) under 10 W m⁻², catalase (CAT),
49 and superoxide dismutase (SOD) under 15 W m⁻² and lower malondialdehyde (MDA). Gene
50 expression of the molt-inhibiting hormone (MIH) was downregulated in eyestalks from crabs
51 exposed to 10 W m⁻² compared to darkness and 20-30 W m⁻². Expression of apoptosis-
52 related genes did not show clear light intensity trends. Taken together, these results suggest
53 light intensity can impact *S. paramamosain* growth, molting, stress levels, and antioxidant
54 capacity. As such, light regimes used in crab farming should be carefully considered to
55 optimize productivity and welfare.

56

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

59

60 **1 Introduction**

61 The mud crab (*Scylla paramamosain*) is the most economically important species in China, with
62 160,616 tonnes produced in 2019, according to the Chinese Fishery Statistical Yearbook (2020).
63 However, farming still relies mainly on caught juveniles from the wild, which raised concerns over
64 the sector's sustainability (Waiho et al., 2018). As a result, the breeding of mud crab in captivity has
65 been researched in recent years (Ma et al., 2010; Ma et al., 2014). However, the early development
66 and growth performances of mud crab remain problematic and lack optimized and standardized
67 husbandry protocols.

68 Light is an important environmental cue for terrestrial (Nasr et al., 2019; Kang et al., 2020)
69 and aquatic animals (Gao et al., 2021a, b). In fish, photoperiod is a significant environmental signal
70 for entraining most physiological events, including reproduction and migration (Migaud et al., 2010).
71 Light intensity and spectrum also appear to impact fish physiology as shown in European sea bass
72 (*Dicentrarchus labrax*), Senegal sole (*Solea senegalensis*), and haddock (*Melanogrammus*
73 *aeglefinus*) larvae which performed better when exposed to blue wavelengths (Downing and Litvak,
74 2001; Villamizar et al., 2011). So far, most research on the physiological effects of light and the
75 light transduction pathways has been focusing on fish species, while mollusks and crustaceans
76 remain little studied. The primary commercially important traits in crustaceans include growth and
77 molting, which can be impacted by light intensity (Wang et al., 2004; Li et al., 2011). In our previous
78 study, low light intensity ($1.43 \mu\text{mol m}^{-2} \text{s}^{-1}$ (ca. 0.27 W m^{-2})) led to enhanced growth and increased
79 accumulation of unsaturated fatty acids in adult mud crab (Li et al., 2020). However, contrasting
80 results were reported for larvae of *Scylla paramamosain* in which survival post-metamorphosis was
81 increased in crabs exposed to a high light intensity of 5000 lx (Zhang et al., 2011a). Differences in
82 optimal light conditions are most likely species, stage of development, water characteristics, and
83 light technology specific as suggested in other aquatic species (Migaud et al., 2007; Migaud et al.,
84 2010; Villamizar et al., 2011). To date, the effects of light on mud crab physiology remains poorly
85 understood.

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

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

109 Suboptimal light conditions can lead to stress and imbalance in the oxidative status of tissues
110 in animals, as demonstrated in previous studies (Lushchak, 2011; Wei et al., 2019). Metabolism and
111 immune defense response generate various reactive oxygen species (ROS) and reactive nitrogen
112 species (RNS), which, when accumulated, could induce oxidative stress and damage proteins, lipids,
113 and DNA, resulting in cell and tissues damage (Yu, 1994; Bogdan et al., 2000; Kohen and Nyska,
114 2002; Wu et al., 2016; Jin et al., 2017). ROS plays an essential role in cell proliferation,
115 differentiation, signal transduction, and immune defense function (Bogdan et al., 2000; Ermak and
116 Davies, 2002). However, excessive accumulation of ROS may lead to oxidative stress, damage to
117 critical cellular biomolecules, and ultimately can compromise cell functions, as shown in crab
118 species (Guo et al., 2013b, c; Cheng et al., 2020). Animals have evolved to counteract oxidation
119 through various antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and
120 peroxiredoxins (Prx) (Chen et al., 2021). As an end-product of lipid peroxidation, malondialdehyde
121 (MDA) is used as an indicator to reflect the status of oxidative damage in organisms (Liu et al.,
122 2011). In addition, apoptosis is used to remove the excess, damaged, necrotic, and potentially
123 dangerous cells (Wyllie et al., 1980). The expression levels of apoptosis-related genes such as *Bcl-*
124 *2*, *p53*, and *caspases 3* can be used as indicators to assess the state of tissue apoptosis (Cheng et al.,
125 2020; Cheng et al., 2021). A recent study showed that exposure to dark and red light suppressed
126 growth, increased oxidative stress response, and apoptosis-related gene expression levels of Pacific
127 white shrimp (*Litopenaeus vannamei*, Boone, 1931) (Fei et al., 2020a, b).

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

132

133 **2 Material and methods**

134 2.1 Experimental animal and rearing conditions

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

136 City, Zhejiang province, China). Crabs were transferred to the experimental unit on the Meishan
137 campus of Ningbo University. Prior to the experiment, crabs were acclimatized in polypropylene
138 boxes individually for one week and fed with a commercial diet made of 40 % protein and 6 % lipid
139 (Ningbo Tech-Bank Feed Co. Ltd., Ningbo, China, Table 1). A total of 273 juvenile mud crabs were
140 weighed and randomly distributed to 273 transparent polypropylene boxes and reared individually
141 (14.1 cm × 8.4 cm × 5.0 cm). During the experiment, crabs were fed with a commercial diet daily
142 at 17:00, and the water was replaced every day (8:00 am). Temperature (25 - 27 °C), salinity (23 -
143 25 ppt), ammonia nitrogen (HACH, 2604545) and nitrite (HACH, 2608345) (< 0.5 mg L⁻¹) and
144 dissolved oxygen was monitored daily by YSI (Proplus, YSI, Yellow Springs, Ohio, USA) (> 6.0
145 mg L⁻¹).

146

147 2.2 Experimental design

148 Seven light intensities, *i.e.* 0, 5, 10, 15, 20, 25 and 30 W m⁻² were tested in triplicate (n=3, 39
149 individuals/replicate) with a photoperiod of 12L : 12D (6:00 - 18:00 light). Light intensities and light
150 spectral composition were set up using a spectroradiometer (EVERFINE Spectroradiometer, Model:
151 PLA-30, Hangzhou, China). The water surface (in the air) light intensity was set to be 0 (0 W m⁻²),
152 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),
153 25 (24.87 ± 0.28 W m⁻²) and 30 (29.89 ± 0.25 W m⁻²) by adjusting the dimmer and the distance
154 between the LEDs and the water surface and the experimental light spectral composition is shown
155 in Fig. 1. Full-spectrum LEDs (Shenzhen Yamingjie intelligent technology Co. Ltd., Shenzhen,
156 China) were suspended above the rearing tank. To avoid light pollution, tanks were light proofed
157 using black-out cloth.

158

159 2.3 Sampling and calculations

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

165
$$\text{Weight gain (WG)} = (W_f - W_i) / W_i$$

166
$$\text{Specific growth rate (SGR, \% day}^{-1}\text{)} = 100 \times (\text{Ln } W_f - \text{Ln } W_i) / t$$

167
$$\text{Survival (\%)} = 100 \times (\text{final number of crabs}) / (\text{initial number of crabs})$$

168
$$\text{CV}_{\text{wg}} \text{ (Coefficient of Variation of WG)} = 100\% \times (\text{SD} / \text{mean})$$

169
$$\text{Molting frequency (MF)} = \Sigma((C_n - 1) \times N_n) / N_t$$

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

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

175

176 2.4 Analysis of antioxidant capacity

177 Three hepatopancreas samples in each replicate from each treatment were randomly selected for
178 antioxidant capacity analysis (total of 9/treatment). Before analysis, samples were homogenized in
179 ice-cold normal saline and centrifuged at 825 g min^{-1} at $4 \text{ }^\circ\text{C}$ for 15 min. The total antioxidant
180 capacity (T-AOC, A015-2-1) was assessed via the ABTS method, malondialdehyde (MDA, A003-
181 1-2) was measured by thiobarbituric acid (TBA) reaction (Ohkawa et al., 1979), and enzyme
182 activities of superoxide dismutase (SOD, A001-3-2) was measured by WST-1 method (Peskin and
183 Winterbourn, 2000), catalase (CAT, A007-1-1) was tested using the hydrogen peroxide
184 decomposition method (Góth, 1991). The operation steps were according to corresponding
185 commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

186

187 2.5 Measurement of melatonin and cortisol

188 For mud crab, eyestalks synthesize and secrete most of the hormones. Thus, in the current study,
189 three eyestalks in each replicate from each treatment (total of 9 / treatment) were randomly selected,
190 grounded in glass mortar with liquid nitrogen to test melatonin and cortisol. According to the weight
191 of eyestalk, four parts PBS was added to the centrifuge tube for tissue homogenization. The samples
192 were centrifuged at 825 g min^{-1} at $4 \text{ }^\circ\text{C}$ for 15 min, and the supernatant was collected. The assays
193 were performed with crab's specific melatonin and cortisol ELISA kit (Enzyme-linked
194 Biotechnology, Shanghai, China). A standard curve was prepared using six standard dilutions of
195 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} .
196 The assay was conducted in microplates based on the principle of competitive binding: melatonin
197 or cortisol in standards and samples competed with melatonin or cortisol conjugated to horseradish
198 peroxidase for the antibody binding sites in the microtiter wells. Microplates were incubated for 60
199 min at $37 \text{ }^\circ\text{C}$, and unbound components were washed away with buffer. Bound melatonin or cortisol
200 enzyme conjugate was measured by the reaction of the horseradish peroxidase enzyme with the
201 substrate tetramethylbenzidine (TMB). The reaction was carried out at $37 \text{ }^\circ\text{C}$ for 15 min (stopped
202 by adding $100 \text{ } \mu\text{L}$ of $0.5 \text{ M H}_2\text{SO}_4$), and the absorbance at 450 nm was read with an Absorbance
203 Microplate Reader (SpectraMax 190, Molecular Devices, USA) within 15 min.

204

205 2.6 Total RNA extraction, cDNA synthesis, and qPCR analysis

206 Eyestalks secrete MIH, and the hepatopancreas is a vital metabolic and immune organ in crustaceans.
207 Thus, eyestalks and hepatopancreas were selected to test the relative expression of MIH gene and
208 apoptosis-related genes, respectively. In this study, the Trizol Reagent (Invitrogen, USA) was used
209 to extract total RNA from eyestalks and hepatopancreas. The total RNA was treated with RNase-
210 Free DNase (Takara, China) to remove genomic DNA contaminant, then quantified and
211 electrophoresed to test for RNA integrity. The purity and concentration were assessed by Nanodrop-
212 2000. The cDNA was synthesized using HiFiScript cDNA Synthesis Kit (CW Biotech. Co. Lid.,
213 Shanghai, China) with 2 μ g RNA. Real-time PCR assays were carried out in a quantitative thermal
214 cycler (Bio-Rad CFX96, USA) using SYBR green as a fluorescent dye. The primers used for qPCR
215 are listed in Table 2. The molting inhibiting hormone (*MIH*), tumor suppressor protein p53 (*p53*),
216 *Bcl-2*, *caspase-3*, and cytochrome *c* oxidase IV (*COX IV*) were analyzed with β -*actin* used as the
217 housekeeping gene. All detection for each sample was performed in two replicates. To confirm
218 primer pairs only produced a single product, dissociation curve analysis was performed by heating
219 from 55 °C to 95 °C at the end of the reaction. Expression levels of target genes were normalized to
220 housekeeping gene β -*actin* using the optimized comparative $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen,
221 2001).

222

223 2.7 Analytical models and statistical analysis

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

236

237 **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 $W\ m^{-2}$
250 (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 $0.28 \pm 0.09\ mg\ mL^{-1}$, 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 ($P >$

272 0.05) (Fig. 5C). MDA levels were lower in crabs from the 15 W m⁻² group than 25 and 30 W m⁻²
273 (Fig. 5D).

274

275 3.4 Gene expression

276 3.4.1 Molt-inhibiting hormone (*MIH*) in eyestalks

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

280

281 3.4.2 Apoptosis related genes in hepatopancreas

282 No significant differences were found in the relative expression levels of *Bcl-2* ($P > 0.05$) between
283 treatments (Fig. 7A). However, the relative expression levels of *p53* were significantly influenced
284 by the light intensity, with the lowest levels found in crabs exposed to 15 and 25 W m⁻² ($P < 0.05$)
285 (Fig. 7B). The relative expression of *p53* was gradually down-regulated and then up-regulated with
286 the increasing light intensities. Relative expression levels of *COX IV* were also significantly
287 influenced by the light intensity with the highest expression levels detected in the 5 W m⁻² group,
288 significantly higher than those reared under 0, 15, 20, and 30 W m⁻² ($P < 0.05$) (Fig. 7C). Expression
289 levels of *Caspase 3* were significantly higher in crabs reared under 10 W m⁻² than those reared under
290 5, 15, and 30 W m⁻² ($P < 0.05$) (Fig. 7D).

291

292 4 Discussion

293 In the present study, light intensities ranging from 0 to 30 W m⁻² did not affect survival ranging from
294 71 to 87 %. Similar results were observed in overwintering *S. paramamosain*, in which light
295 intensity did not affect survival (Li et al., 2020). However, a significant impact on the growth
296 performance of juvenile *S. paramamosain* was observed. The optimal light intensity for growth was
297 extrapolated to be 11.36-18.27 W m⁻² based on the regression analysis between SGR and light
298 intensity. However, the best growth performance was previously found in crabs exposed to a much
299 lower light intensity of 1.43 μmol m⁻² s⁻¹ (ca. 0.53 W m⁻²) (Li et al., 2020). The likely explanation
300 for the contrasting results is the difference in stage of development between the two studies
301 (juveniles in the present study vs. adult ca. 290 g in Li et al., 2020) concerning the ecological shift
302 between pelagic to benthic habitats in adults mud crab (Wang et al., 2019). Similar results were
303 obtained in swimming crab, *Portunus trituberculatus*, larvae with light sensitivity changing between
304 the zoea and megalopa larval phases compared to juvenile crabs (Dou et al., 2021).

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

306 study, a positive relationship was detected between MF and SGR (except for the darkness treatment).
307 In addition, light intensity significantly affected *MIH* gene expression in the eyestalks of *S.*
308 *paramamosain*. Molting in crustaceans, which consists in the shedding of the rigid exoskeleton, is
309 primarily controlled by ecdysteroids and MIH secreted by Y-organs, X-organs, and Sinus gland
310 (XO-SG) (Imayavaramban et al., 2007). Besides, molting in crustaceans is mainly controlled by
311 ecdysteroids (Mykles, 2011). MIH is known to inhibit ecdysteroid synthesis (Watson and Spaziani,
312 1985; Huang et al., 2015) and regulate the duration of the molting cycle (Takuji et al., 2005).

313 The relationship between molting and light intensity has not been fully explored yet. Previous
314 studies showed that constant light intensity did not affect MF of *Litopenaeus vannamei* (Guo et al.,
315 2012) and Chinese mitten crab (Li et al., 2011) while fluctuating and periodic light intensity changes
316 promoted the growth of *Litopenaeus vannamei* by increasing MF (Guo et al., 2012, 2013a).
317 However, light intensity was shown to impact the WG of *Penaeus merguensis* without apparent
318 effects on MF or IMI (Hoang et al., 2003). *MIH* RNAi in *Macrobrachium nipponense* led to a
319 significant reduction in IMI and increased body weight increment after molting (Qiao et al., 2018).
320 Besides, a study in the red swamp crayfish (*Procambarus clarkia*) found that SNPs mapped on the
321 5' -flanking region of the *MIH* gene correlated with growth with GG genotype exhibiting superior
322 growth than CG genotype (Xu et al., 2019). In addition, a negative correlation between carapace
323 length and width increases induced by molting and *MIH* gene expression has been reported in the
324 Chinese mitten crab (Liu et al., 2021), which confirms the relationships between the expression of
325 *MIH* levels, molting, and growth. The present study indicates that light intensity may play an
326 essential role in growth by regulating molting.

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

340 2010). Interestingly, in this study, while melatonin levels and relative expression of *MIH* remained
341 the same in crabs exposed to darkness or 5 W m⁻², SGR and MF were significantly higher in the 5
342 W m⁻² group. These results could suggest that the inferior growth performance in crabs under
343 darkness may be due to the absence of circadian rhythmicity.

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

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

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

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

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

408

409 **5 Conclusions**

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

421

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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).

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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

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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: GAAGTGGACCTGGAAAAGTAA R: GCTCACAGGGAGAAGCATAG	MK426684.1
cytochrome <i>c</i> oxidase IV	F: GGCGAGGAAGGGATAC R: GGAAGTCAACACGGTCATA	FJ774694.1

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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 ($P < 0.05$).

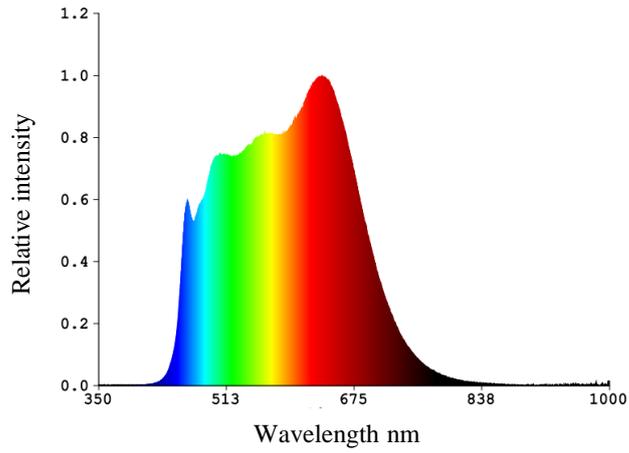
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730 Fig. 1 The spectrum of the experimental LED light source.

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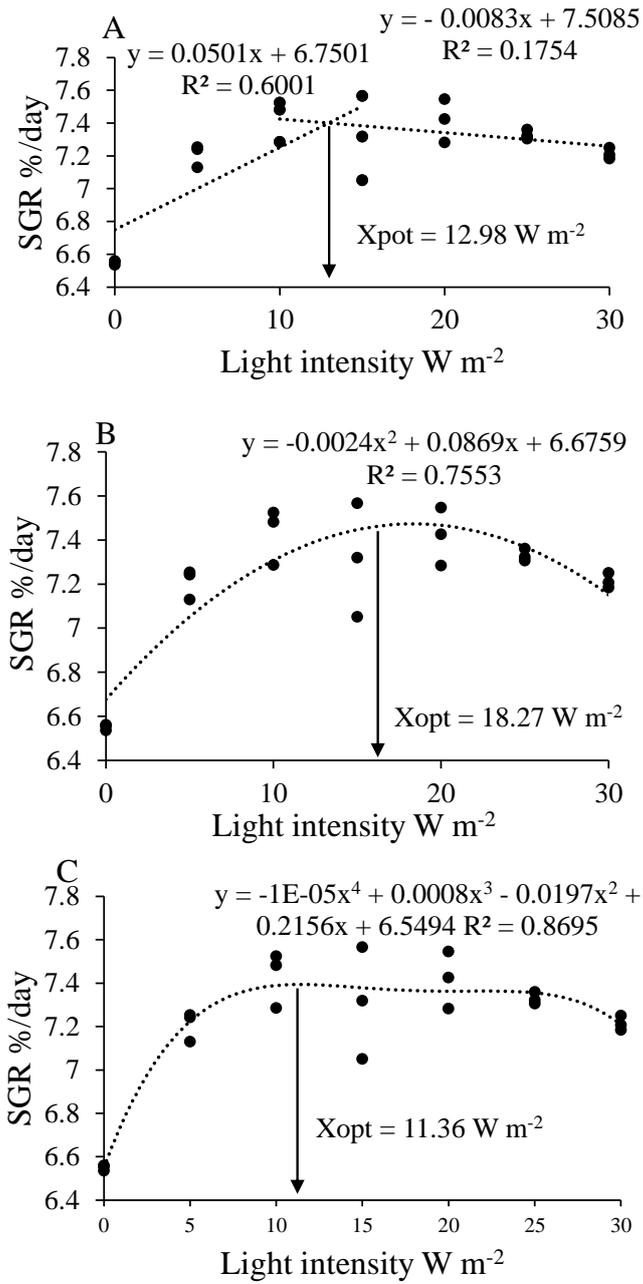
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Fig. 2 Relationship between light intensity and specific growth rate (SGR) in mud crab based on
 757 linear (BLM) (A), quadratic (BQM) (B), and 4-parameter saturation kinetic (4-SKM) (C) models,
 758 where X_{opt} represents the optimal light intensity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

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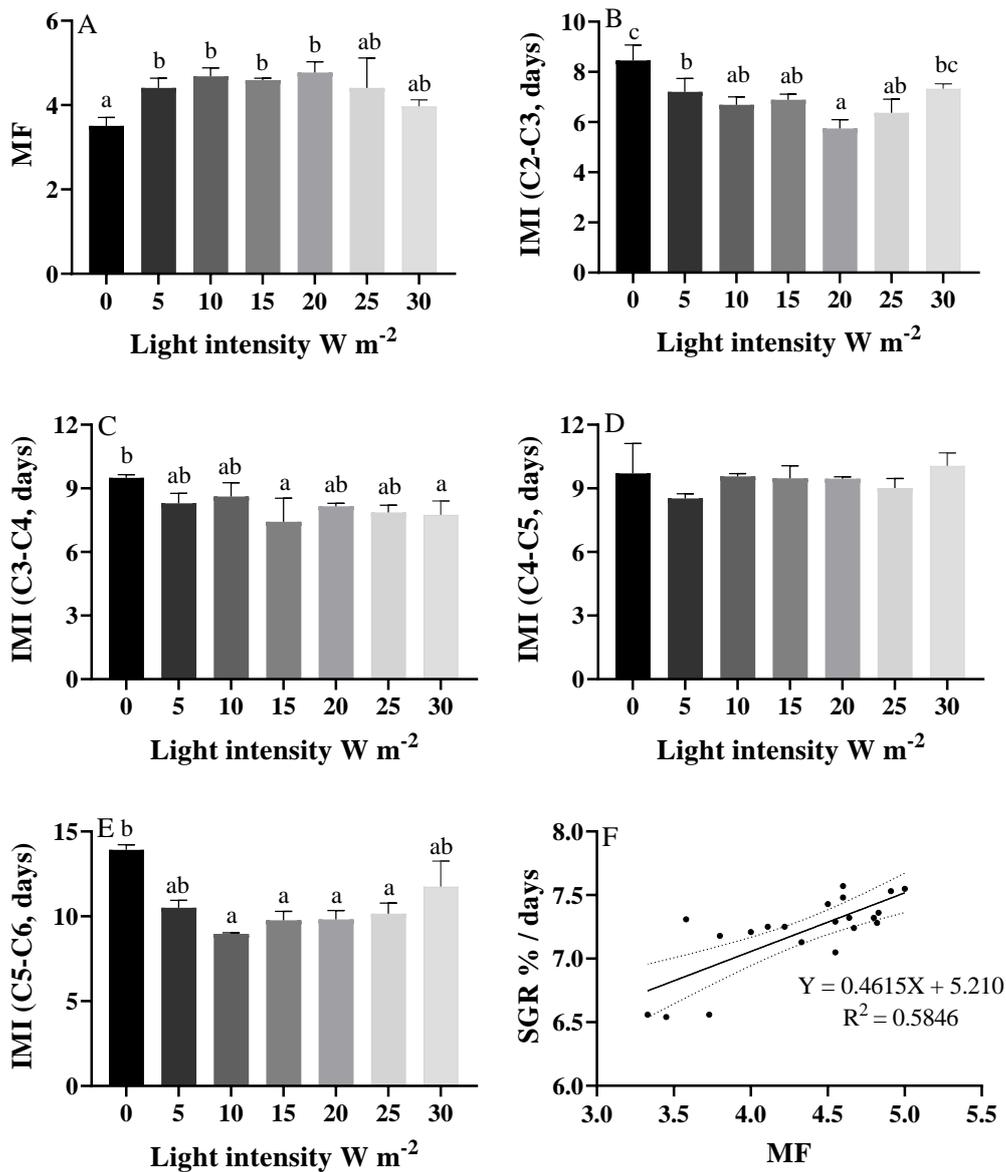
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768 Fig. 3 Molting frequency (MF) (A) and intermolt interval (IMI) (B-E) in *S. paramamosain* reared
 769 under increasing light intensities (0 to 30 W m⁻²) and the relationship of MF and SGR (F). Molt
 770 frequency and SGR appeared to be positively correlated ($R^2=0.58$, Pearson correlation indices of
 771 0.767, $P < 0.05$). Values are expressed as means \pm 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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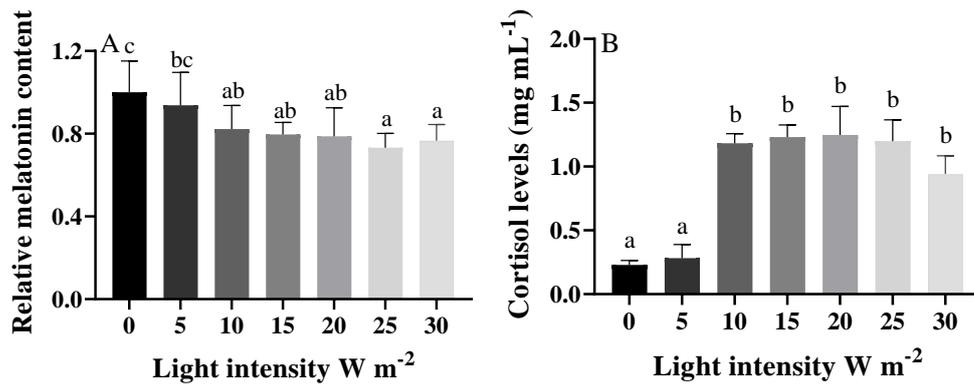
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781 Fig. 4 Relative melatonin content (A) and cortisol levels (B) in eyestalks of *S. paramamosain* reared
 782 under increasing light intensities (0 to 30 W m⁻²). Values are expressed as means ± SD (n = 3).
 783 Different superscripts denote significant differences between treatments ($P < 0.05$).

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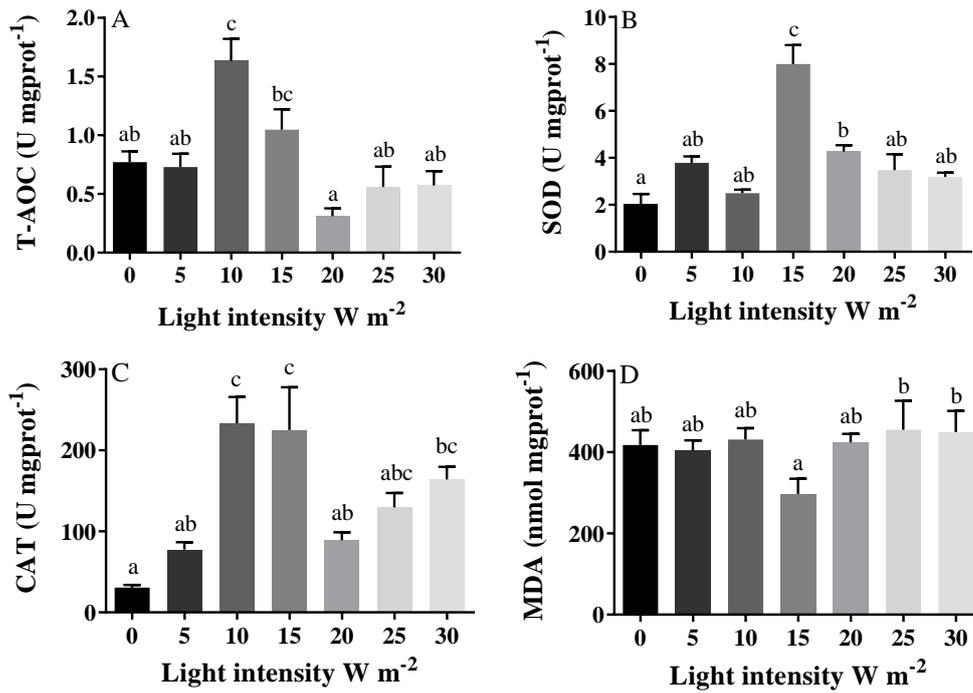
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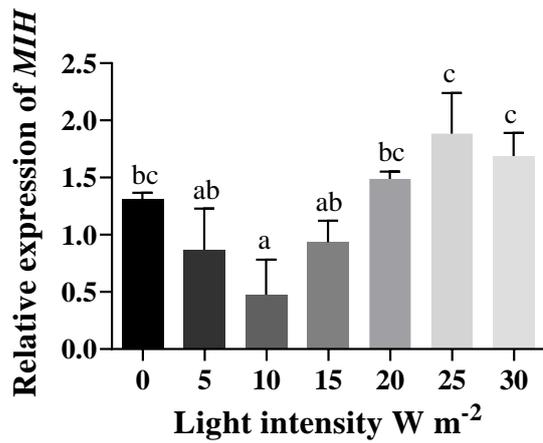
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 813 Fig. 5 Total antioxidant capacity (T-AOC) (A), superoxide dismutase (SOD) (B), catalase (CAT)
 814 (C), and malondialdehyde (MDA) (D) contents in the hepatopancreas of *S. paramamosain*
 815 reared under increasing light intensities (0 to 30 W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

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838 Fig. 6 Gene expression of molt-inhibiting hormone (*MIH*) in the eyestalk of *S. paramamosain* reared
 839 under increasing light intensities (0 to 30 W m⁻²). Values are expressed as means ± SD (n = 3).

840 Different superscripts denote significant differences between treatments ($P < 0.05$).

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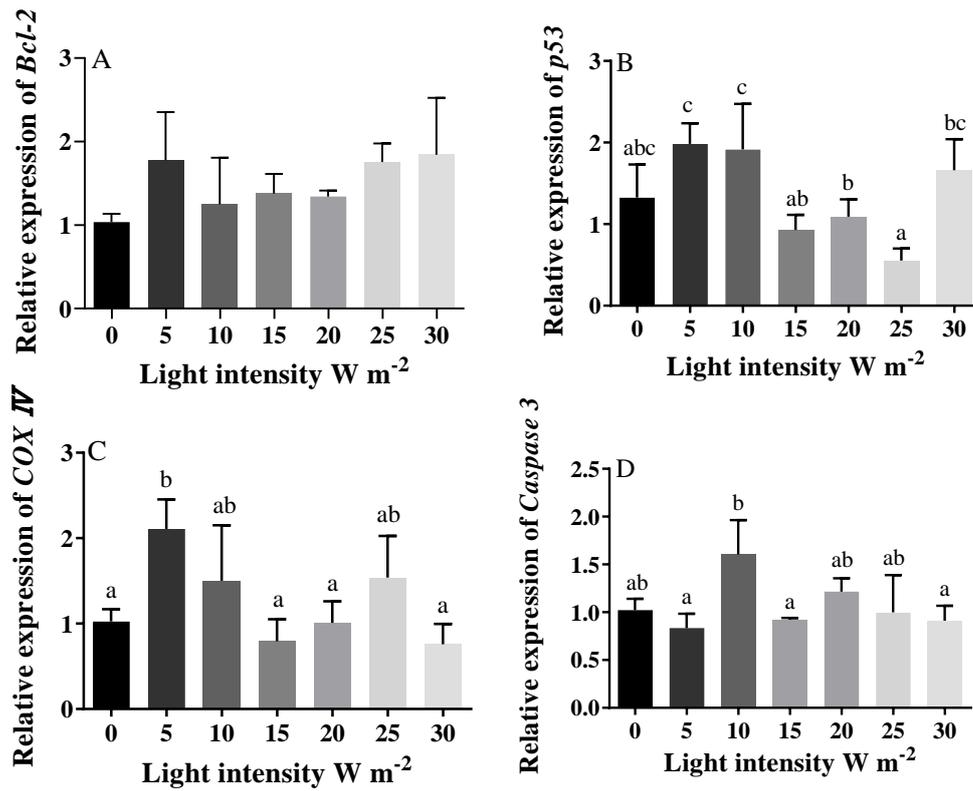
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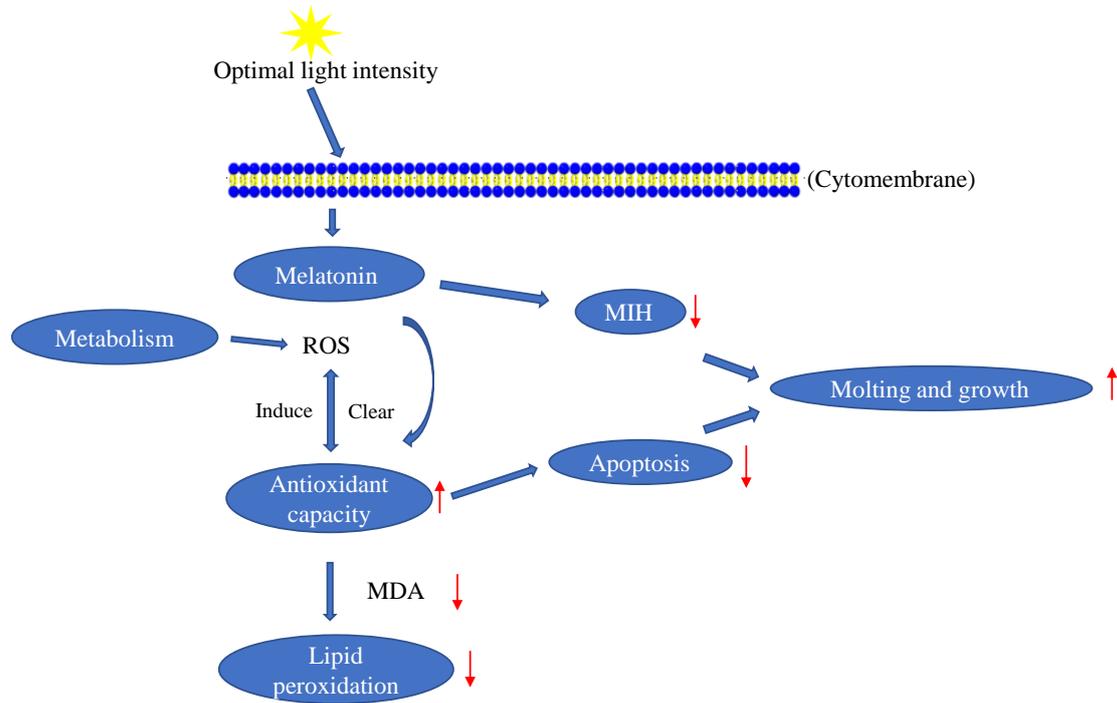
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 866 Fig. 7 Gene expression of apoptosis-related genes *Bcl-2* (A), *p53* (B), *COX IV* (C) and *Caspase 3*
 867 (D) in hepatopancreases of *S. paramamosain* reared under increasing light intensities (0 to 30 W m⁻²). Values are expressed as means ± SD (n = 3). Different superscripts denote significant differences
 868 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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