



Investigation of the Light Intensity Effect on Growth, Molting, Hemolymph Lipid, and Antioxidant Capacity of Juvenile Swimming Crab *Portunus trituberculatus*

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An eight-week experiment was conducted to evaluate the effects of eight light intensities (0, 0.83, 1.61, 8.17, 15.89, 31.38, 63.69, and 124.31 $\mu\text{mol m}^{-2} \text{s}^{-1}$) on the survival, growth, antioxidant, molting-related and apoptosis-related gene expression of juvenile swimming crab *Portunus trituberculatus*. The results showed that the survival rate of crabs was the highest under the light intensities of 8.17 and 15.89 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Under this light intensity, the crabs had the lowest hemolymph glucose (GLU) but the highest triglyceride (TG) content. The crabs in these treatments also had the highest total antioxidant capacity (T-AOC), superoxide dismutase (SOD), catalase (CAT) activities, and the lowest malondialdehyde (MDA) content. The growth performance (including weight gain and specific growth rate) and molting frequency of crabs increased with increasing light intensity. The improved growth performance and molting of 8.17 and 15.89 $\mu\text{mol m}^{-2} \text{s}^{-1}$ groups were also accompanied by up-regulated of the retinoid-X receptor (*rxr*), ecdysone receptor (*ecr*), nuclear receptor E75 (*e75*) gene expression, and down-regulated molt-inhibiting hormone (*mih*) gene expression. In addition, suboptimal light intensity (0, 0.83, 63.69, and 124.31 $\mu\text{mol m}^{-2} \text{s}^{-1}$) significantly up-regulated the expression of apoptosis-related genes including c-Jun N-terminal kinase (*jnk*), tumor suppressor p53 (*p53*), and B-cell lymphoma-2 (*bcl-2*). Taken together, the suitable light intensity for the juvenile *P. trituberculatus* was estimated to be 9.96 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Keywords: LED, molting, antioxidant, apoptosis, swimming crab

INTRODUCTION

The swimming crab *Portunus trituberculatus* is an essential economic species naturally distributed from Southeast Asia to the Indian Ocean (Dai et al., 1986). In 2019, the global captured *P. trituberculatus* reached 460,580 tons, making it the highest fishing yield of crustaceans in the world (FAO, 2021). China contributes more than 95% of the capture yield of *P. trituberculatus* (Liu et al.,

2013), and this proportion is increasing year by year. However, with the decline of fishery resources and strict supervision of the government departments, the captured output of *P. trituberculatus* has dropped from 542,337 tons in 2015 to 424,630 tons in 2020 (Fisheries Bureau of Agriculture Ministry of China, 2016–2021). However, the decline in capture did not promote the aquaculture production of *P. trituberculatus* for the seed of swimming crab is mainly produced in earth-pond (Wang et al., 2019), of which the yield is low because of the uncontrollable environment, creating a gap between seed supplies and farmer's demand. As a result, indoor seedling production has been tried in recent years.

Researchers have shown that indoor nurseries could improve the survival, growth, and reproduction of the crabs by manipulating various environmental factors, including light (Li et al., 2020a; Li et al., 2020b; Chen et al., 2021; Dou et al., 2021), tank color (Shi et al., 2019; Wang et al., 2019), tank bottom area (Yu et al., 2022), water temperature (Yuan et al., 2017; Syafaat et al., 2020), and salinity (Nurdiani and Zeng, 2007; Shentu et al., 2015). Light is a compound environmental factor including three characteristics: light spectrum, light intensity, and photoperiod (Boeuf and Le Bail, 1999). As an important abiotic factor, the light intensity has been reported to affect crustacean survival (Coyle et al., 2011; Dou et al., 2021), molting (Chen et al., 2021), growth (Moss et al., 1999; Hoang et al., 2003; Li et al., 2011; Li et al., 2020a), behavior (Gardner and Maguire, 1998; Haché et al., 2015) and reproduction (Hoang et al., 2002a; Hoang et al., 2002b). Aquatic animals are sensitive to changes in light intensity, and the optimal light intensity varies significantly among species and developmental stages (Villamizar et al., 2011; Ruchin, 2021). In fish, a recent review by Ruchin (2021) summarized the effect of light intensities from 0–27300 lx on fish development, growth, physiological and biochemical processes. There are only a few studies regarding the impact of light intensities on crabs (Li et al., 2011; Li et al., 2020a; Li et al., 2020b; Li et al., 2020c; Chen et al., 2021; Dou et al., 2021; Li et al., 2021). For the mud crab *Scylla paramamosain*, the suitable light intensity for juvenile crabs ranged from 11.36–18.27 W m⁻² (ca. 60.55–97.38 μmol m⁻² s⁻¹) (The conversion method is based on Villamizar et al., 2011) (Chen et al., 2021). For adults, it was only 1.43 μmol m⁻² s⁻¹ (Li et al., 2020a). As to larval *P. trituberculatus*, the suitable light intensity was 124.31–195.31 μmol m⁻² s⁻¹ (Dou et al., 2021), but the light requirement for juvenile swimming crabs is still unknown.

Many studies have found that suboptimal light intensity adversely affects crustaceans, from larval development to broodstock reproduction. For the larvae and juveniles, suboptimal light intensity resulted in high mortality, poor growth, and delayed development (Gardner and Maguire, 1998; Li et al., 2011; Dou et al., 2021; Chen et al., 2021). In adults, the suboptimal light intensity could lead to premature maturation, reducing spawning and fecundity (Hoang et al., 2002a; Li et al., 2011; Li et al., 2020b). Some scholars attribute the adverse effects partly to the stress caused by suboptimal light intensity (Chen et al., 2021; Dou et al., 2021; Ruchin, 2021). Under stressful conditions, organisms will produce large

amounts of reactive oxygen species (ROS), such as superoxide radical (O₂•⁻), hydroxyl radical (•OH), and hydrogen (H₂O₂) (Suzuki and Mittler, 2006; Paital and Chainy, 2012). Excessive accumulation of ROS could cause oxidative damage to cells and tissues of crabs (Lin et al., 2017; Zhang et al., 2019). In response to ROS, crabs developed an efficient antioxidant system, including antioxidant enzymes represented by superoxide dismutase (SOD) and catalase (CAT) to remove excess ROS (Dong et al., 2009; Chen et al., 2021). In addition, when cells are damaged by oxidative stress, the organism will clear the damaged cells by apoptosis to maintain homeostasis (Xian et al., 2013; Chen et al., 2021). Apoptosis is a complex process regulated by multiple signals, and the process remains obscure in arthropods (including crustaceans) (Menze et al., 2010). However, some apoptosis-related proteins such as c-Jun N-terminal kinase (JNK), B-cell lymphoma-2 (Bcl-2), and tumor suppressor p53 (p53) were found to regulate apoptosis in crustaceans (Menze et al., 2010; Chen et al., 2021).

In the present study, the Light Emitting Diodes (LED) were used to create eight different light intensities to investigate the effects of light intensities on growth, molting, antioxidant capacity, and apoptosis-related gene expression in the swimming crab *P. trituberculatus*. This work aims to identify optimal light conditions for *P. trituberculatus* produced in land-based hatcheries and nurseries to boost juvenile outputs.

MATERIAL AND METHOD

Experimental Crabs

The *P. trituberculatus* were purchased from commercial nurseries (Ningbo, China). First, the megalopa was accommodated in several tanks lined with woolen mesh (as a shelter to reduce cannibalism). The tanks were continuously oxygenated, and the water was changed by 50% daily. Megalopa was fed with *Artemia tibetiana* until they metamorphosed to 1st crablet instar (Crablet 1, C1). The Crablets were immediately taken out and kept in a transparent square tank (17 cm × 11.5 cm × 6.8 cm) until they developed into C2. The healthy and lively juvenile crabs (initial body weight of 11.20 mg) were randomly selected for the experiment.

Experimental Design and Management

This experiment was conducted in June and July 2020 at the Intelligent Aquaculture Laboratory, School of Marine Sciences, Ningbo University (Ningbo, China). Full-spectrum LED lights (Shenzhen Yamingjie intelligent technology Co. Ltd., Shenzhen, China) were used as the light source. According to Dou et al. (2021) and Chen et al. (2021), eight light intensities, *i.e.*, 0, 0.83, 1.61, 8.17, 15.89, 31.38, 63.69, and 124.31 μmol m⁻² s⁻¹ were used for the experiment. Light intensities were achieved by adjusting the dimmer and the distance between the LEDs and the water surface. Each light intensity treatment had four replicates, with ten crablets cultured for each replicate. Every crablet was kept individually in a transparent square tank as described in 2.1. The photoperiod during the experiment was 12L:12D (light on at 6:00

am). All the tanks were placed on the shelf with shading cloth to avoid external light pollution.

Crablets were fed with *Artemia tibetiana* at 8:00 am and 5:00 pm daily, with an extra meal [fed a commercial diet (Ningbo Tech-Bank Feed Co. Ltd., Ningbo, China, Crude protein \geq 40.0, Crude lipid \geq 6.0)] at 12:00 pm. During the experiment, the molting and dead crabs were counted every day, and the water quality parameters were: the temperature at $26 \pm 1^\circ\text{C}$, salinity at 24 ± 1 psu, pH at 8.5, dissolved oxygen $> 6.0 \text{ mg L}^{-1}$, $\text{NH}_3\text{-N} < 0.06 \text{ mg L}^{-1}$, $\text{NO}_2\text{-N} < 0.1 \text{ mg L}^{-1}$. The experimental protocol of the present study was reviewed and approved by the Animal Care and Use Committee of Ningbo University.

Sample Collection

After eight weeks, all surviving crabs were starved for 24 hours, then anesthetized with ice and weighed. The hemolymph was collected from the heart with a disposable needle, and the eyestalk and hepatopancreas were separated. All samples were frozen in liquid nitrogen and stored at -80°C until analysis.

Hemolymph Index Analysis

The hemolymph glucose (Glu, A154-2-1) was determined by the hexokinase method (Deeg et al., 1980), triglyceride (TG, A110-1-1), and total cholesterol (TCHO, A111-1-1) were determined by the GPO-PAP method (Henkel and Stoltz, 1982). All the hemolymph indexes were determined by commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Antioxidant Index Analysis

Antioxidative capacity in the hepatopancreas was also measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Briefly, the hepatopancreas was homogenized in pre-cooled normal saline (0.9% NaCl, 4°C) at a ratio of 1:9 (W: V), then centrifuged at 2500 rpm for 10 minutes at 4°C , and the supernatant was taken for antioxidant capacity determination. Total protein concentration (TP, A045-2) of the supernatant was determined by the Coomassie Brilliant Blue

method (Bradford, 1976), the total antioxidant capacity (T-AOC, A015-3-1) was determined by the FRAP method (Benzie and Strain, 1996), the concentration of malondialdehyde (MDA, A003-2-2) was measured by thiobarbituric acid reaction (Ohkawa et al., 1979), the activity of superoxide dismutase (SOD, A001-1-2) was measured by the xanthine oxidase method (Peskin and Winterbourn, 2000), catalase (CAT, A007-1-1) was tested using the hydrogen peroxide decomposition method (Góth, 1991).

Gene Expression Analysis

Total RNA in hepatopancreas and eyestalk was extracted using a commercial kit (R401-01, Vazyme, Nanjing, China) by the TRIzol method. RNA quality was checked by ultramicrospectrophotometer (A260:A280 nm) and 1% gel electrophoresis. Then, RNA was reverse transcribed to cDNA using a commercial kit (CW2569M, CWBIO, Beijing, China).

The qPCR system (LightCycler480, Roche, USA) was used to analyze the relative expression level of target genes. A total 20 μl reaction system (2 \times MagicSYBR Mixture, 10 μl ; cDNA, 1 μl ; each primer (10 μM), 0.4 μl ; ddH₂O, 8.2 μl) was prepared using a commercial kit (CW3008H, CWBIO, Beijing, China). The specific primers used in this study are shown in **Table 1**. The reaction program was set as follows: 95°C for 30 s; 40 cycles at 95°C for 5 s, 60°C for 30 s; and 95°C for 15 s. The relative expression level of the target gene was normalized by $\beta\text{-actin}$ and calculated by the equation of $2^{-\Delta\Delta\text{CT}}$ (Livak and Schmittgen, 2001).

Calculations

The calculation formula used in this study is as follows:

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

Weight gain (WG, %) = $100 \times (\text{final body weight} - \text{initial body weight}) / \text{initial body weight}$,

Special growth rate (SGR, % day^{-1}) = $100 \times (\ln \text{final weight} - \ln \text{initial weight}) / 56 \text{ days}$,

TABLE 1 | The specific primers used for qPCR in this study.

Gene	Sequence (5' to 3')	Size (bp)	Access number
<i>jnk</i>	F: TAGACTTCGGACTTGCTCG R: GGTC AATATGATCTGTGCC	195	MK287992
<i>p53</i>	F: GAAAGTCGCCATCGTGTA R: CCAACAACAGTCCCAGAG	338	MH155953
<i>bcl-2</i>	F: TCCTCCATAGCGTCCCTTACCT R: CCAGCAGGATTCTAAGGAC	251	MH155954
<i>ecr</i>	F: TAAGTGATGACGACTCGGATGC R: ACGAGCAAGCCTTTAGCAGTG	150	KC354381
<i>rxr</i>	F: AGCGTCAGAGGACAAAAGGC R: TGGTCCAGTGGCTGCTCAT	136	KF061043
<i>e75</i>	F: CGAGAGCCTAGTGATGTA R: ATGAGTGATGAGCGAGTA	118	KM016226
<i>mih</i>	F: TATCAAGTGCAGGAACCTCAG R: GGAACATACAAGCCTAAACA	110	EU869539
$\beta\text{-actin}$	F: CGAAACCTTCAACACTCCCG R: GATAGCGTGAGGAAGGGCATA	154	FJ641977

jnk, c-Jun N-terminal kinase; *p53*, tumor suppressor *p53*; *bcl-2*, B-cell lymphoma-2; *ecr*, ecdysone receptor; *rxr*, retinoid X receptor; *e75*, nuclear receptor *E75*; *mih*, molt-inhibiting hormone.

Molting frequency (MF) = Σ ((the stage of crab - 1) \times the number of molting stages)/the total number of survival crabs.

Statistical Analysis

First, the survival rate data was converted into a continuous probability distribution (arcsine square-root transformation). Then, all data were checked for homogeneity (Levene's test) and normality (Kolmogorov-Smirnov test). If the data was homogeneous and normally distributed, One-way ANOVA was used to test for significant treatment effects followed by Duncan's multiple comparison *post hoc* test; otherwise, a nonparametric test (Kruskal-Wallis tests) was applied. $P < 0.05$ was considered statistically different between treatments. All statistical processing was performed in SPSS 26.0.

RESULT

Survival and Growth

The survival of juvenile *P. trituberculatus* was significantly affected by light intensity (Figure 1). As the light intensity increased from 0 to 15.89 $\mu\text{mol m}^{-2} \text{s}^{-1}$, the survival rate of crabs increased from 52.50% to 75.00%; then as the light intensity further increased to 124.31 $\mu\text{mol m}^{-2} \text{s}^{-1}$, the survival rate decreased to 57.50%.

The growth performance of juvenile *P. trituberculatus* was significantly improved by light intensity (Figures 1, 2). With the increase in light intensity, the WG, SGR, and MF of crabs increased significantly. Based on the linear fit of the survival, the suitable light intensity for the juvenile *P. trituberculatus* was 9.96 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 3).

Hemolymph Indicators

The GLU levels in the hemolymph of the juvenile *P. trituberculatus* showed a U shape trend with increasing light intensity. Significantly low levels of GLU were observed at 1.61 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 8.17 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and 15.89 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity groups (Figure 4A).

The TG levels in the hemolymph of the juvenile *P. trituberculatus* was parabolic. Significantly high levels of TG were also observed at 1.61 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 8.17 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and 15.89 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity groups (Figure 4B).

High light intensity significantly increased TCHO levels in the hemolymph of juvenile *P. trituberculatus*, and significantly high levels of TCHO were observed at 63.69 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 124.31 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity groups (Figure 4C).

Antioxidant Capacity

In this study, the antioxidant capacity of juvenile *P. trituberculatus* was significantly affected by light intensity (Figure 5). The content of MDA in the hepatopancreas decreased with the increasing light intensity. The lowest value was observed in the 15.89 $\mu\text{mol m}^{-2} \text{s}^{-1}$ group (Figure 5A). The T-AOC, SOD, and CAT activity in hepatopancreas showed opposite trends compared to MDA content, and high levels of T-AOC and SOD, CAT activities were observed in 8.17 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 15.89 $\mu\text{mol m}^{-2} \text{s}^{-1}$ groups (Figures 5B–D).

Gene Expression

Light intensity significantly affected apoptosis-related genes expression (Figure 6). High light intensity (63.69 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 124.31 $\mu\text{mol m}^{-2} \text{s}^{-1}$) significantly up-regulated the expression of the *jnk* gene (Figure 6A). The expression levels of *p53* and *bcl-2* genes showed a U shape trend (63.69 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 124.31 $\mu\text{mol m}^{-2} \text{s}^{-1}$) (Figures 6B, C).

Molting-related genes were also significantly affected by light intensity (Figure 7). With the increasing light intensity, the gene expression of ecdysone receptor (*ecr*), retinoid-X receptor (*rxr*), and nuclear receptor E75 (*e75*) was significantly up-regulated (Figures 7A–C). On the contrary, the molt-inhibiting hormone (*mih*) gene decreased with increasing light intensity (except for the 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$ group) (Figure 7D).

DISCUSSION

Light intensity manipulation is widely used in aquaculture to obtain greater productivity (Villamizar et al., 2011; Ruchin, 2021). In this study, the survival rate of juvenile *P. trituberculatus* was parabolic with the increasing light intensity. A linear fit based on survival showed that the suitable light intensity for the survival of the juvenile *P. trituberculatus* was 9.96 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The value is considerably lower compared with the optimal light intensity for *P. trituberculatus* larvae, for a previous study suggested that the highest survival rates were detected for Zoea IV larvae and megalopae at 195.31 and 124.31 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Dou et al., 2021). The substantial decline in the light requirement of the juvenile *P. trituberculatus* could be attributed to their adaption to the ecological shift from pelagic to benthic habitats. However, the light intensity did not affect the survival of the juvenile *S. paramamosain* (Li et al., 2020a; Li et al., 2020b; Chen et al., 2021), probably because *S. paramamosain* are intertidal benthos (Walton et al., 2006; Yu et al., 2022) and may have broader adaptations to light intensity.

Darkness or low light intensities have been identified as a stressor (Wang et al., 2013). Low light intensities (including darkness) were found to cause oxidative damage in juvenile *S. paramamosain* (Chen et al., 2021) and gibel carp *Carassius auratus gibelio* (Wei et al., 2019). Wagner and Congleton (2004) divided blood indicators into four categories: (i) total protein, cholesterol, calcium, and alkaline phosphatase were considered as nutritional indicators; (ii) alanine aminotransferase, aspartate aminotransferase, and creatine kinase were considered as tissue damage indicators; (iii) triacylglycerol lipase and triglycerides were considered as lipid metabolism indicators; (iv) cortisol, glucose, Na^+ , and Cl^- were considered as stress indicators. Blood GLU is widely used as a stress indicator to describe the stress state in aquatic animals (Leclercq et al., 2014; Qi et al., 2016). In this study, significantly higher hemolymph GLU levels were found in the 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 0.83 $\mu\text{mol m}^{-2} \text{s}^{-1}$ groups. In addition to GLU, previous studies have found that the blood TG and TCHO levels are also raised in response to increased energy demand due to stressful situations (Montero et al., 1999; Qi et al., 2016). However, high hemolymph TG and TCHO levels were not observed in the

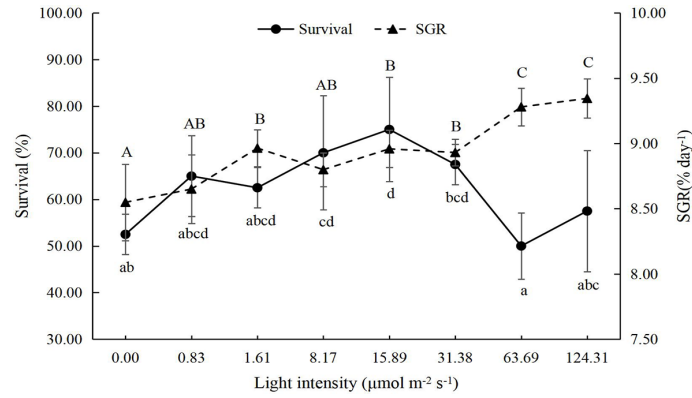


FIGURE 1 | Effect of different light intensities on the survival and special growth rate (SGR) of juvenile *Portunus trituberculatus* (mean \pm SD, n = 4). Different lowercase and capital letters indicate significant differences ($P < 0.05$) in survival and SGR, respectively.

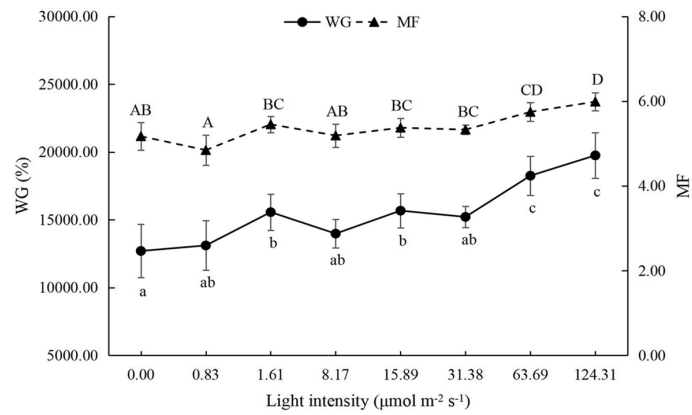


FIGURE 2 | Effect of different light intensities on the weight gain (WG) and molting frequency (MF) of juvenile *Portunus trituberculatus* (mean \pm SD, n = 4). Different lowercase and capital letters indicate significant differences ($P < 0.05$) in WG and MF, respectively.

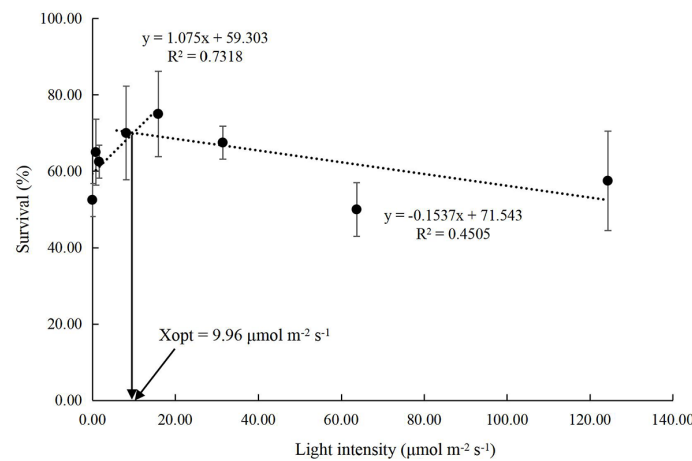


FIGURE 3 | Relationship between light intensity and survival in juvenile *Portunus trituberculatus* (mean \pm SD, n = 4) based on linear models, where X_{opt} represents the optimal light intensity for the maximum survival.

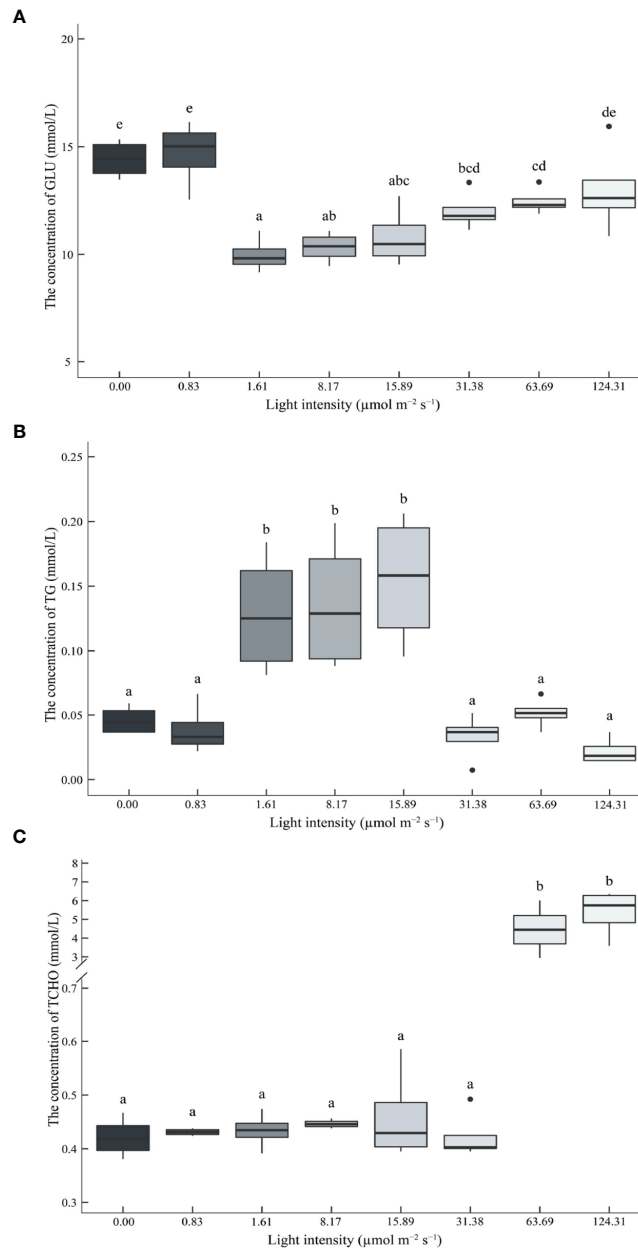


FIGURE 4 | Effect of different light intensities on the glucose (GLU) (A), triglyceride (TG) (B), and total cholesterol (TCHO) (C) contents in the hemolymph of juvenile *Portunus trituberculatus*. Data (n = 4) with different letters significantly differ ($P < 0.05$) among treatments.

dark and low light intensity groups, suggesting lower levels of lipid metabolism in these treatments. Further, the photoperiod (light-dark cycle) acts as a primary environmental cue (zeitgeber) to guide the oscillation of the endogenous biological clock to maintain the normal metabolism and growth of the organism (Ashton et al., 2022). In this study, higher mortality in the dark group may also be attributed to circadian rhythm disruption (Ishibashi et al., 2013; Castejon et al., 2018; Pereira-Davison and Callan, 2018).

On the other hand, as the light intensity increased to $31.38 \mu\text{mol m}^{-2} \text{s}^{-1}$, the survival of crabs started to decrease, and significantly lower survival rates were observed in the $63.69 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $124.31 \mu\text{mol m}^{-2} \text{s}^{-1}$ groups. High light intensity has been reported as a stressor in crabs (Coyle et al., 2011; Chen et al., 2021). Also, in this study, although there was no statistical difference, high hemolymph GLU levels were observed in the high light intensity groups. Moreover, significantly lower TG levels were also observed in the high

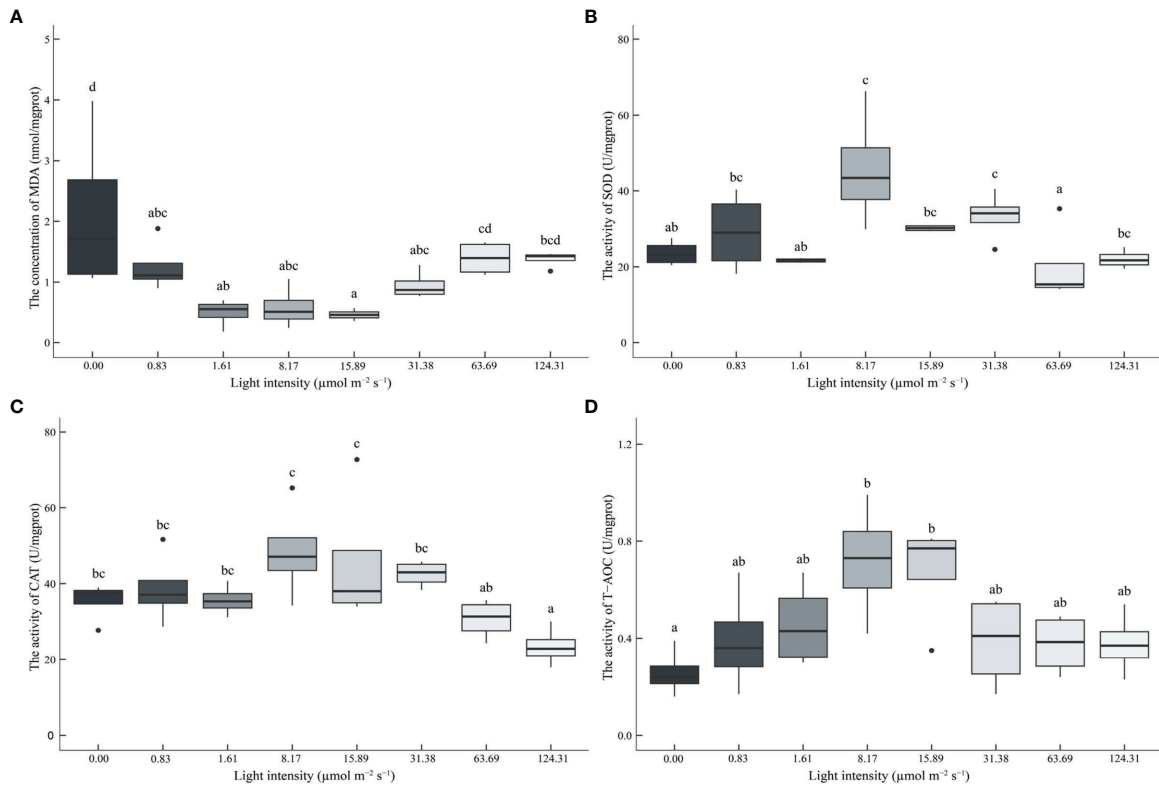


FIGURE 5 | Effect of different light intensities on the malondialdehyde (MDA) (A) contents, superoxide dismutase (SOD) (B), catalase (CAT) (C), and total antioxidant capacity (T-AOC) (D) in the hepatopancreas of juvenile *Portunus trituberculatus*. Data (n=4) with different letters significantly differ ($P < 0.05$) among treatments.

light intensity group, which may be attributed to the impaired lipid metabolism caused by stress (Roychowdhury et al., 2020). In contrast to the low light intensity group, high light intensity significantly increased hemolymph TCHO levels. Although there is no standard for a normal hemolymph TCHO range of *P. trituberculatus*, hemolymph TCHO levels < 1 mmol/L were commonly observed in most studies (including different experimental treatments and different growth stages of the crabs) (Han et al., 2015; Wang et al., 2016; Ding et al., 2017; Sun et al., 2017; Han et al., 2018). Thus, the significantly high TCHO levels (> 4 mmol/L) in the $63.69 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $124.31 \mu\text{mol m}^{-2} \text{s}^{-1}$ groups may suggest a disorder in cholesterol metabolism.

In this study, the growth performance of *P. trituberculatus* was also significantly affected by light intensity. In general, the growth of aquatic animals will decrease under stress (Wei et al., 2019; Yu et al., 2022). Unexpectedly, in this study, the growth of crabs significantly increased with the increase of light intensity. A possible reason is that stress elevates hemolymph cortisol levels, increases food intake and energy metabolism, and produces anabolic effects that ultimately promote growth (Mommsen et al., 1999; Elverson and Wilson, 2005; Kang and Kim, 2013; Chen et al., 2021). Similarly, Gardner and Maguire (1998) attributed the shortened intermolt duration of Australian giant crab *Pseudocarcinus gigas* at brighter light (500 lx vs. 2 lx) to

increased feeding. However, in Chinese mitten crabs *Eriocheir sinensis*, different light intensities did not affect digestive enzyme activity under overfeeding conditions (Li et al., 2011). Therefore, future study is needed to determine whether high light intensity promotes growth performance by increasing feeding. In addition, our previous study found that suitable light intensity significantly increased the survival of the larval *P. trituberculatus* but did not affect development (Dou et al., 2021), implying that the effect of light intensity on crabs' survival and growth varies according to the growth stage.

Crustacean weight gain is phased and closely related to molting (Devaraj and Natarajan, 2006; Chang et al., 2012). Previous studies observed a positive correlation between crab body weight and MF (Chen et al., 2021; Liu et al., 2021), and the same phenomenon was also observed in this study. The molting behavior of crabs is regulated by a pair of antagonistic hormones, ecdysteroids (Mykles, 2011) and molt-inhibiting hormone (MIH) (Huang et al., 2015). The ecdysteroid binds to EcR and RXR to form a heterodimer, activates the expression of the downstream gene *e75*, and finally regulates the molting process (Mykles, 2011; Song et al., 2017). On the other hand, MIH inhibits crustaceans' molting by inhibiting the synthesis of ecdysteroids (Chang and Mykles, 2011). In the present study, the expression of *ecr*, *rxr*, and *e75* genes was up-regulated with increasing light intensity. In contrast, *mih* gene expression levels

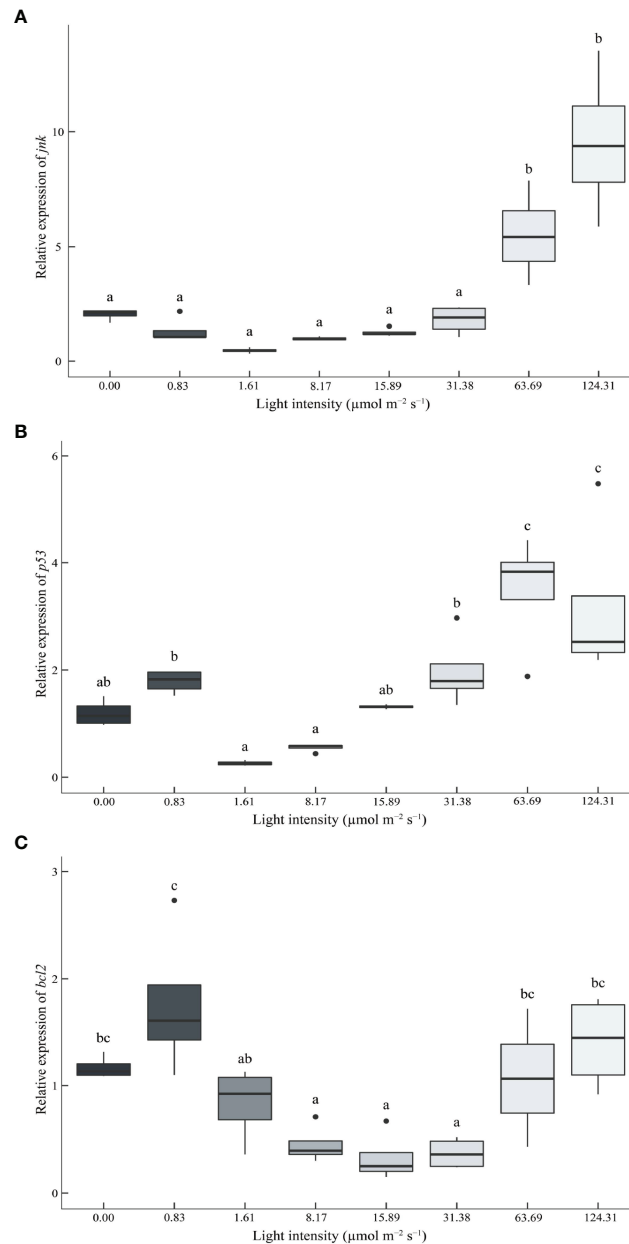


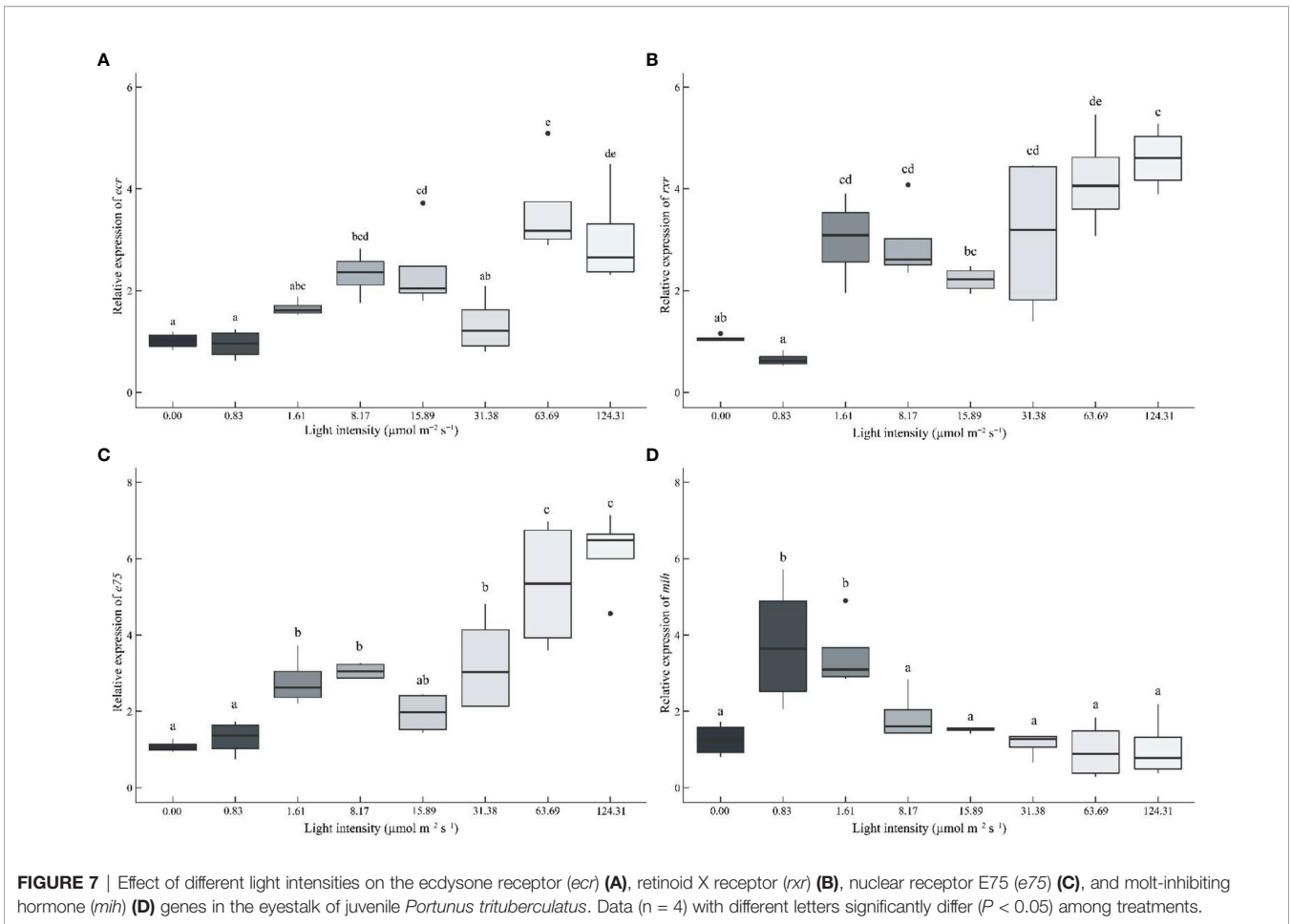
FIGURE 6 | Effect of different light intensities on the c-Jun N-terminal kinase (*jnk*) (A), tumor suppressor p53 (*p53*) (B), and B-cell lymphoma-2 (*bcl-2*) (C) genes in the hepatopancreas of juvenile *Portunus trituberculatus*. Data (n = 4) with different letters significantly differ ($P < 0.05$) among treatments.

were significantly down-regulated (except for the dark group), matching the MF. In addition, some researchers attributed the high MF under high light intensity to the activation of molt-stimulating hormone (MSH) and crustacean hyperglycemic hormones (CHH) synthesis (Hoang et al., 2003; Li et al., 2011).

The adverse stimulation would induce the excessive production and accumulation of ROS in crustaceans (Paital and Chainy, 2012; Duan et al., 2016). In the present study, SOD and CAT activities and T-AOC in the hepatopancreas of *P. trituberculatus* were parabolic with increasing light intensity. However, the level of MDA, an indicator of oxidative damage (Draper and Hadley,

1990; Rio et al., 2005), showed an opposite trend, indicating *P. trituberculatus* has a robust antioxidant capacity under the light intensity range of 8.17-15.89 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

To maintain homeostasis, organisms clear the damaged cells through programmed cell death, a process known as apoptosis (Xian et al., 2013; Chen et al., 2021). This process is regulated by a series of signals and genes in crustaceans (Menze et al., 2010). JNK, Bcl-2, and p53 are essential protein and transcription factors in the apoptosis signaling pathway that regulates the process of apoptosis (Cadet et al., 2000; Fridman and Lowe, 2003; Liu and Lin, 2005; Menze et al., 2010). In this study, suboptimal



light intensity significantly increased the expression levels of these genes, suggesting cellular damage (Cheng et al., 2020; Fei et al., 2020). Similarly, suboptimal light intensity also up-regulated *p53* gene expression in *S. paramamosain* (Chen et al., 2021).

CONCLUSION

The present study explored the effect of light intensity on the survival, growth, antioxidant, molting-related and apoptosis-related gene expression of juvenile swimming crab *P. trituberculatus*. Results revealed that low and high light intensities might act as stressors, reflected by the lower survival rate, higher GLU and MDA levels, and upregulation of apoptotic genes expression (*jnk*, *p53*, and *bcl-2*). On the other hand, high light intensity stimulated the expression of molting-related genes (*ecr*, *rxr*, *e75*) while suppressing *mih* gene expression, consequently boosting molting and growth performance. However, compared with growth, survival could be a more significant economic indicator for the young crabs in aquaculture. Therefore, the optimal light intensity for juvenile *P. trituberculatus* was estimated to be $9.96 \mu\text{mol m}^{-2} \text{s}^{-1}$ in terms of survival rate.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

HX and JD performed the experiment, analyzed the data, and draft the MS. QW and CSh designed the experiment and review and edited the MS. All authors participated in the revision of this MS by providing comments. All authors contributed to the article and approved the submitted version.

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REFERENCES

- Ashton, A., Foster, R. G., and Jagannath, A. (2022). Photic Entrainment of the Circadian System. *Int. J. Mol. Sci.* 23 (2), 729. doi: 10.3390/ijms23020729
- Benzie, I. F. F., and Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (Frap) as a Measure of "Antioxidant Power": The Frap Assay. *Anal. Biochem.* 1996 (1), 70–76. doi: 10.1006/abio.1996.0292
- Boeuf, G., and Le Bail, P. Y. (1999). Does Light Have an Influence on Fish Growth? *Aquaculture* 177 (1-4), 129–152. doi: 10.1016/S0044-8486(99)00074-5
- Bradford, M. M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* 72 (1-2), 248–254. doi: 10.1016/0003-2697(76)90527-3
- Cadet, J. L., Harrington, B., and Ordonez, S. (2000). Bcl-2 Overexpression Attenuates Dopamine-Induced Apoptosis in an Immortalized Neural Cell Line by Suppressing the Production of Reactive Oxygen Species. *Neurosci.* 35, 228–233. doi: 10.1002/(SICI)1098-2396(20000301)35:3<228::AID-YN8>3.0.CO;2-#
- Castejon, D., Rotllant, G., Gimenez, L., Torres, G., and Guerao, G. (2018). Influence of Temperature and Light Regime on the Larval Development of the Common Spider Crab *Maja Brachydactyla* Bals 1922 (Brachyura: Majidae). *Aquacult. Res.* 49 (11), 3548–3558. doi: 10.1111/are.13820
- Chang, E. S., and Mykles, D. L. (2011). Regulation of Crustacean Molting: A Review and Our Perspectives. *Gen. Comp. Endocrinol.* 172, 323–330. doi: 10.1016/j.ygcen.2011.04.003
- Chang, Y. J., Sun, C. L., Chen, Y., and Yeh, S. Z. (2012). Modelling the Growth of Crustacean Species. *Rev. Fish Biol. Fish.* 22 (1), 157–187. doi: 10.1007/s11160-011-9228-4
- Cheng, C. H., Su, Y. L., Ma, H. L., Deng, Y. Q., Feng, J., Chen, X. L., et al. (2020). Effect of Nitrite Exposure on Oxidative Stress, DNA Damage and Apoptosis in Mud Crab (*Scylla Paramamosain*). *Chemosphere* 239, 124668. doi: 10.1016/j.chemosphere.2019.124668
- Chen, S., Migaud, H., Shi, C., Song, C., Wang, C., Ye, Y., et al. (2021). Light Intensity Impacts on Growth, Molting and Oxidative Stress of Juvenile Mud Crab *Scylla Paramamosain*. *Aquaculture* 545, 737159. doi: 10.1016/j.aquaculture.2021.737159
- Coyle, S. D., Bright, L. A., Wood, D. R., Neal, R. S., and Tidwell, J. H. (2011). Performance of Pacific White Shrimp, *Litopenaeus Vannamei*, Reared in Zero-Exchange Tank Systems Exposed to Different Light Sources and Intensities. *J. World Aquacult. Soc.* 42 (5), 687–695. doi: 10.1111/j.1749-7345.2011.00512.x
- Dai, A. Y., Yang, S. L., and Song, Y. Z. (1986). *Marine Crabs in China Sea* (Beijing: Marine Publishing Company), 194–196.
- Deeg, R., Kraemer, W., and Ziegenhorn, J. (1980). Kinetic Determination of Serum Glucose by Use of the Hexokinase/Glucose-6-Phosphate Dehydrogenase Method. *Clin. Chem. Lab. Med.* 18, 49–52. doi: 10.1515/ccm.1980.18.1.49
- Devaraj, H., and Natarajan, A. (2006). Molecular Mechanisms Regulating Molting in a Crustacean. *FEBS J.* 273 (4), 819–846. doi: 10.1111/j.1742-4658.2006.05117.x
- Ding, L. Y., Fu, H. Y., Hou, Y. M., Jin, M., Sun, P., and Zhou, Q. C. (2017). Effects of Starvation and Feeding on Blood Chemistry, Fatty Acid Composition and Expression of Vitellogenin and Fatty Acid-Binding Protein Genes in Female Swimming Crab *Portunus Trituberculatus* Broodstock. *Fish Sci.* 83, 455–464. doi: 10.1007/s12562-017-1075-3
- Dong, C. H., Zhao, J. M., Song, L. S., Wang, L. L., Qiu, L. M., Zheng, P. L., et al. (2009). The Immune Responses in Chinese Mitten Crab *Eriocheir Sinensis* Challenged With Double-Stranded RNA. *Fish Shellfish Immunol.* 26 (3), 438–442. doi: 10.1016/j.fsi.2009.01.005
- Dou, J., Zhang, G. L., Shi, C., Song, C. B., Mu, C. K., Ye, Y. F., et al. (2021). High-Intensity Light of Full-Spectrum LED Promotes Survival Rate But Not Development of the Larval Swimming Crab *Portunus Trituberculatus*. *Aquacult. Eng.* 93, 102158. doi: 10.1016/j.aquaeng.2021.102158
- Draper, H. H., and Hadley, M. (1990). Malondialdehyde Determination as Index of Lipid Peroxidation. *Methods Enzymol.* 186, 421–431. doi: 10.1016/0076-6879(90)86135-I
- Duan, Y. F., Zhang, Y., Dong, H. B., and Zhang, J. S. (2016). Effect of Desiccation on Oxidative Stress and Antioxidant Response of the Black Tiger Shrimp *Penaeus Monodon*. *Fish Shellfish Immunol.* 58, 10–17. doi: 10.1016/j.fsi.2016.09.004
- Elverson, C. A., and Wilson, M. E. (2005). Cortisol: Circadian Rhythm and Response to a Stressor. *N. Newborn Infant Nurs. Rev.* 5 (4), 159–169. doi: 10.1053/j.nainr.2005.09.002
- FAO. (2021). *FAO Yearbook. Fishery and Aquaculture Statistics 2019* (Rome: Food and Agriculture Organization of the United Nations). doi: 10.4060/cb1213t
- Fei, F., Liu, B., Gao, X., Wang, X., Liu, Y., and Bin, H. (2020). Effects of Supplemental Ultraviolet Light on Growth, Oxidative Stress Responses, and Apoptosis-Related Gene Expression of the Shrimp *Litopenaeus Vannamei*. *Aquaculture* 520, 735013. doi: 10.1016/j.aquaculture.2020.735013
- Fisheries Bureau of Agriculture Ministry of China. (2016–2021). *2016–2021 China Fishery Statistical Yearbook* (Beijing, China: China Agriculture Press), 22, 38.
- Fridman, J. S., and Lowe, S. W. (2003). Control of Apoptosis by P53. *Oncogene* 22, 9030–9040. doi: 10.1038/sj.onc.1207116
- Gardner, C., and Maguire, G. B. (1998). Effect of Photoperiod and Light Intensity on Survival, Development and Cannibalism of Larvae of the Australian Giant Crab *Pseudocarcinus Gigas* (Lamarck). *Aquaculture* 165 (1-2), 51–63. doi: 10.1016/S0044-8486(98)00245-2
- Góth, L. (1991). A Simple Method for Determination of Serum Catalase Activity and Revision of Reference Range. *Clin. Chimica Acta* 196, 143–151. doi: 10.1016/0009-8981(91)90067-M
- Haché, R., Mallet, M. D., and Dumas, A. (2015). Effects of Light Regime on Larvae Survival, Growth and Settling Behavior of Hatchery Reared American Lobster (*Homarus Americanus*). *Aquaculture* 443, 31–39. doi: 10.1016/j.aquaculture.2015.02.028
- Han, T., Wang, J. T., Li, X. Y., Yang, Y. X., Wang, J. X., Hu, S. X., et al. (2015). Effects of Dietary Cholesterol Levels on the Growth, Molt Performance, and Immunity of Juvenile Swimming Crab, *Portunus Trituberculatus*. *Israeli J. Aquacult-Bamidgeh* 67, 1191. doi: 10.46989/001c.20696
- Han, T., Wang, J. T., Li, X. Y., Yang, Y. X., Yang, M., Tian, H. L., et al. (2018). Effects of Dietary Phospholipid and Cholesterol Levels on Growth and Fatty Acid Composition of Juvenile Swimming Crab, *Portunus Trituberculatus*. *Aquacult. Nutr.* 24 (1), 164–172. doi: 10.1111/anu.12544
- Henkel, E., and Stoltz, M. (1982). A Newly Drafted Colour Test for the Determination of Triglycerides Convenient for Manual and Mechanized Analysis (Glycerolphosphate-Oxidase-PAP Method). *Fresenius' Z. für Analytische Chemie* 311, 451–452. doi: 10.1007/BF00481817
- Hoang, T., Barchiesi, M., Lee, S. Y., Keenan, C. P., and Marsden, G. E. (2003). Influences of Light Intensity and Photoperiod on Moulting and Growth of *Penaeus Merquiensis* Cultured Under Laboratory Conditions. *Aquaculture* 216, 343–354. doi: 10.1016/S0044-8486(02)00460-X
- Hoang, T., Lee, S. Y., Keenan, C. P., and Marsden, G. E. (2002a). Ovarian Maturation of the Banana Prawn, *Penaeus Merquiensis* De Man Under Different Light Intensities. *Aquaculture* 208 (1-2), 159–168. doi: 10.1016/S0044-8486(01)00713-X
- Hoang, T., Lee, S. Y., Keenan, C. P., and Marsden, G. E. (2002b). Effect of Light Intensity on Maturation and Spawning of Ablated Female *Penaeus Merquiensis*. *Aquaculture* 209 (1–4), 347–358. doi: 10.1016/S0044-8486(01)00814-6
- Huang, H., Fu, C., Chen, X., Gong, J., Huang, X., and Ye, H. (2015). Molt-Inhibiting Hormone (MIH) Gene From the Green Mud Crab *Scylla Paramamosain* and its Expression During the Molting and Ovarian Cycle. *Aquacult. Res.* 46 (11), 2665–2675. doi: 10.1111/are.12421

- Ishibashi, Y., Honryo, T., Miyashita, S., and Oda, S. (2013). Suitable Photoperiod and Light Intensity of Laboratory-Reared Juvenile Pacific Bluefin Tuna *Thunnus Orientalis*. *Aquacult. Sci.* 61 (4), 399–402. doi: 10.11233/aquaculturesci.61.399
- Kang, D. Y., and Kim, H. C. (2013). Influence of Density and Background Color to Stress Response, Appetite, Growth, and Blind-Side Hypermelanosis of Flounder, *Paralichthys Olivaceus*. *Fish Physiol. Biochem.* 39, 221–232. doi: 10.1007/s10695-012-9693-2
- Leclercq, E., Davie, A., and Migaud, H. (2014). The Physiological Response of Farmed Ballan Wrasse (*Labrus Bergylta*) Exposed to an Acute Stressor. *Aquaculture* 434, 1–4. doi: 10.1016/j.aquaculture.2014.07.017
- Li, X. W., Li, Z. J., Liu, J. S., Zhang, T. L., and Zhang, C. W. (2011). Effects of Light Intensity on Molting, Growth, Precocity, Digestive Enzyme Activity, and Chemical Composition of Juvenile Chinese Mitten Crab *Eriocheir Sinensis*. *Aquacult. Int.* 19 (2), 301–311. doi: 10.1007/s10499-010-9414-8
- Lin, Y., Huang, J. J., Dahms, H. U., Zhen, J. J., and Ying, X. P. (2017). Cell Damage and Apoptosis in the Hepatopancreas of *Eriocheir Sinensis* Induced by Cadmium. *Aquat. Toxicol.* 190, 190–198. doi: 10.1016/j.aquatox.2017.07.008
- Liu, J., and Lin, A. N. (2005). Role of JNK Activation in Apoptosis: A Double-Edged Sword. *Cell Res.* 15 (1), 36–42. doi: 10.1038/sj.cr.7290262
- Liu, S., Sun, J. S., and Hurtado, L. A. (2013). Genetic Differentiation of *Portunus Trituberculatus*, the World's Largest Crab Fishery, Among its Three Main Fishing Areas. *Fish Res.* 148, 38–46. doi: 10.1016/j.fishres.2013.08.003
- Liu, S., Wang, X., Bu, X., Zhang, C., Qiao, F., Qin, C., et al. (2021). Influences of Dietary Vitamin D3 on Growth, Antioxidant Capacity, Immunity and Molting of Chinese Mitten Crab (*Eriocheir Sinensis*) Larvae. *J. Steroid Biochem. Mol. Biol.* 210, 105862. doi: 10.1016/j.jsbmb.2021.105862
- Livak, K. J., and Schmittgen, T. D. (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods* 25 (4), 402–408. doi: 10.1006/meth.2001.1262
- Li, N., Zhou, J. M., He, C. Y., Fu, Y. Y., Wang, C. L., Liu, L., et al. (2021). GC-MS-Based Metabolomics Reveal That Light Intensity During Indoor Overwintering Affects the Metabolism of *Scylla Paramamosain*. *Aquacult. Res.* 52, 1013–1025. doi: 10.1111/are.14956
- Li, N., Zhou, J. M., Wang, H., Mu, C. K., Shi, C., Liu, L., et al. (2020c). Transcriptome Analysis of Genes and Pathways Associated With Metabolism in *Scylla Paramamosain* Under Different Light Intensities During Indoor Overwintering. *BMC Genomics* 21, 775. doi: 10.1186/s12864-020-07190-w
- Li, N., Zhou, J., Wang, H., Wang, C., Mu, C., Shi, C., et al. (2020a). Effects of Light Intensity on Growth Performance, Biochemical Composition, Fatty Acid Composition and Energy Metabolism of *Scylla Paramamosain* During Indoor Overwintering. *Aquacult. Rep.* 18, 100443. doi: 10.1016/j.aqrep.2020.100443
- Li, N., Zhou, J., Wang, H., Wang, C., Mu, C., Shi, C., et al. (2020b). Effect of Light Intensity on Digestion and Immune Responses, Plasma Cortisol and Amino Acid Composition of *Scylla Paramamosain* During Indoor Overwintering. *Aquacult. Res.* 51, 5005–5014. doi: 10.1111/are.14836
- Menze, M. A., Fortner, G., Nag, S., and Hand, S. C. (2010). Mechanisms of Apoptosis in Crustacea: What Conditions Induce Versus Suppress Cell Death? *Apoptosis* 15 (3), 293–312. doi: 10.1007/s10495-009-0443-6
- Mommsen, T. P., Vijayan, M. M., and Moon, T. W. (1999). Cortisol in Teleosts: Dynamics, Mechanisms of Action, and Metabolic Regulation. *Rev. Fish Biol. Fish* 9, 211–268. doi: 10.1023/A:1008924418720
- Montero, D., Izquierdo, M., Tort, L., Robaina, L. E., and Vergara, J. M. (1999). High Stocking Density Produces Crowding Stress Altering Some Physiological and Biochemical Parameters in Gilthead Seabream, *Sparus Aurata*, Juveniles. *Fish Physiol. Biochem.* 20, 53–60. doi: 10.1023/A:1007719928905
- Moss, G. A., Tong, L. J., and Illingworth, J. (1999). Effects of Light Intensity and Food Density on the Growth and Survival of Early-Stage Phyllosoma Larvae of the Rock Lobster *Jasus Edwardsii*. *Mar. Freshwater Res.* 50, 129–134. doi: 10.1071/MF98112
- Mykles, D. L. (2011). Ecdysteroid Metabolism in Crustaceans. *J. Steroid Biochem. Mol. Biol.* 127, 196–203. doi: 10.1016/j.jsbmb.2010.09.001
- Nurdiani, R., and Zeng, C. S. (2007). Effects of Temperature and Salinity on the Survival and Development of Mud Crab, *Scylla Serrata* (Forsskal), Larvae. *Aquacult. Res.* 38 (14), 1529–1538. doi: 10.1111/j.1365-2109.2007.01810.x
- Ohkawa, H., Ohishi, N., and Yagi, K. (1979). Assay for Lipid Peroxides in Animal Tissues by Thiobarbituric Acid Reaction. *Anal. Biochem.* 95 (2), 351–358. doi: 10.1016/0003-2697(79)90738-3
- Paital, B., and Chainy, G. B. N. (2012). Effects of Salinity on O-2 Consumption, ROS Generation and Oxidative Stress Status of Gill Mitochondria of the Mud Crab *Scylla Serrata*. *Comp. Biochem. Physiol. C-Toxicol. Pharmacol.* 155 (2), 228–237. doi: 10.1016/j.cbpc.2011.08.009
- Pereira-Davison, E., and Callan, C. K. (2018). Effects of Photoperiod, Light Intensity, Turbidity and Prey Density on Feed Incidence and Survival in First Feeding Yellow Tang (*Zebrasoma Flavescens*) (Bennett). *Aquacult. Res.* 49 (2), 890–899. doi: 10.1111/are.13535
- Peskin, A. V., and Winterbourn, C. C. (2000). A Microtiter Plate Assay for Superoxide Dismutase Using a Water-Soluble Tetrazolium Salt (WST-1). *Clin. Chim. Acta* 293 (1-2), 157–166. doi: 10.1016/S0009-8981(99)00246-6
- Qi, C. L., Xie, C. X., Tang, R., Qin, X., Wang, D. Y., and Li, D. P. (2016). Effect of Stocking Density on Growth, Physiological Responses, and Body Composition of Juvenile Blunt Snout Bream, *Megalobrama Amblycephala*. *J. World Aquacult. Soc.* 47 (3), 358–368. doi: 10.1111/jwas.12278
- Rio, D. D., Stewart, A. J., and Pellegrini, N. (2005). A Review of Recent Studies on Malondialdehyde as Toxic Molecule and Biological Marker of Oxidative Stress. *Nutr. Metab. Cardiovasc. Dis.* 15, 316–328. doi: 10.1016/j.numecd.2005.05.003
- Roychowdhury, P., Aftabuddin, M., and Pati, M. K. (2020). Thermal Stress Altered Growth Performance and Metabolism and Induced Anaemia and Liver Disorder in Labeo Rohita. *Aquacult. Res.* 51 (4), 1406–1414. doi: 10.1111/are.14486
- Ruchin, A. B. (2021). Effect of Illumination on Fish and Amphibian: Development, Growth, Physiological and Biochemical Processes. *Rev. Aquacult.* 13 (1), 567–600. doi: 10.1111/raq.12487
- Shentu, J. K., Xu, Y. J., and Ding, Z. N. (2015). Effects of Salinity on Survival, Feeding Behavior and Growth of the Juvenile Swimming Crab, *Portunus Trituberculatus* (Miers 1876). *Chin. J. Oceanol. Limnol.* 33 (3), 679–684. doi: 10.1007/s00343-015-4218-3
- Shi, C., Wang, J., Peng, K., Mu, C., Ye, Y., and Wang, C. (2019). The Effect of Tank Colour on Background Preference, Survival and Development of Larval Swimming Crab *Portunus Trituberculatus*. *Aquaculture* 504, 454–461. doi: 10.1016/j.aquaculture.2019.01.032
- Song, Y., Villeneuve, D. L., Toyota, K., Lguchi, T., and Tollefsen, K. E. (2017). Ecdysone Receptor Agonism Leading to Lethal Molting Disruption in Arthropods: Review and Adverse Outcome Pathway Development. *Environ. Sci. Technol.* 51 (8), 4142–4157. doi: 10.1021/acs.est.7b00480
- Sun, P., Jin, M., Ding, L. Y., Lu, Y., Yuan, Y., Ma, H. N., et al. (2017). Effect of Dietary Soybean Lecithin and Cholesterol on Growth, Antioxidant Status and Fatty Acid Composition of Juvenile Swimming Crab, *Portunus Trituberculatus*. *Israeli J. Aquacult-Bamidgheh* 69, 1421. doi: 10.46989/001c.20856
- Suzuki, N., and Mittler, R. (2006). Reactive Oxygen Species and Temperature Stresses: A Delicate Balance Between Signaling and Destruction. *Physiol. Plantarum* 126 (1), 45–51. doi: 10.1111/j.0031-9317.2005.00582.x
- Syafaat, M. N., Mohammad, S., Azra, M. N., Ma, H. Y., Abol-Munafi, A. B., and Ikhwanuddin, M. (2020). Effect of Water Temperature on Survival, Growth and Molting Cycle During Early Crablet Instar of Mud Crab, *Scylla Paramamosain* (Estampado). *Thalassas* 36 (2), 543–551. doi: 10.1007/s41208-020-00233-9
- Villamizar, N., Blanco-Vives, B., Migaud, H., Davie, A., Carboni, S., and Sanchez-Vazquez, F. J. (2011). Effects of Light During Early Larval Development of Some Aquacultured Teleosts: A Review. *Aquaculture* 315 (1-2), 86–94. doi: 10.1016/j.aquaculture.2010.10.036
- Wagner, T., and Congleton, J. L. (2004). Blood Chemistry Correlates of Nutritional Condition, Tissue Damage, and Stress in Migrating Juvenile Chinook Salmon (*Oncorhynchus Tshawytscha*). *Can. J. Fish Aquat. Sci.* 61 (7), 1066–1074. doi: 10.1139/F04-050
- Walton, M. E., Le Vay, L., Truong, L. M., and Ut, V. N. (2006). Significance of Mangrove-Mudflat Boundaries as Nursery Grounds for the Mud Crab, *Scylla Paramamosain*. *Mar. Biol.* 149, 1199–1207. doi: 10.1007/s00227-006-0267-7
- Wang, T., Cheng, Y. Z., Liu, Z. P., Yan, S. H., and Long, X. H. (2013). Effects of Light Intensity on Growth, Immune Response, Plasma Cortisol and Fatty Acid Composition of Juvenile *Epinephelus Coioides* Reared in Artificial Seawater. *Aquaculture* 414, 135–139. doi: 10.1016/j.aquaculture.2013.08.004

- Wang, J. T., Han, T., Li, X. Y., Hu, S. X., Jiang, Y. D., and Wang, C. L. (2016). Effects of Dietary Phosphatidylcholine (PC) Levels on the Growth, Molt Performance and Fatty Acid Composition of Juvenile Swimming Crab, *Portunus Trituberculatus*. *Anim. Feed Sci. Technol.* 216, 225–233. doi: 10.1016/j.anifeedsci.2016.03.023
- Wang, J., Peng, K., Lu, H., Li, R., Song, W., Liu, L., et al. (2019). The Effect of Tank Colour on Growth Performance, Stress Response and Carapace Colour of Juvenile Swimming Crab *Portunus Trituberculatus*. *Aquacult. Res.* 50, 2735–2742. doi: 10.1111/are.14224
- Wei, H., Li, H. D., Xia, Y., Liu, H. K., Han, D., Zhu, X. M., et al. (2019). Effects of Light Intensity on Phototaxis, Growth, Antioxidant and Stress of Juvenile Gibel Carp (*Carassius Auratus Gibelio*). *Aquaculture* 501, 39–47. doi: 10.1016/j.aquaculture.2021.737159
- Xian, J. A., Miao, Y. T., Li, B., Guo, H., and Wang, A. L. (2013). Apoptosis of Tiger Shrimp (*Penaeus Monodon*) Haemocytes Induced by *Escherichia Coli* Lipopolysaccharide. *Comp. Biochem. Phys. A* 164, 301–306. doi: 10.1016/j.cbpa.2012.10.008
- Yuan, Q., Wang, Q. D., Zhang, T. L., Li, Z. J., and Liu, J. S. (2017). Effects of Water Temperature on Growth, Feeding and Molting of Juvenile Chinese Mitten Crab *Eriocheir Sinensis*. *Aquaculture* 468, 169–174. doi: 10.1016/j.aquaculture.2016.10.007
- Yu, K. J., Shi, C., Liu, X. Z., Ye, Y. F., Wang, C. L., Mu, C. K., et al. (2022). Tank Bottom Area Influences the Growth, Molting, Stress Response, and Antioxidant Capacity of Juvenile Mud Crab *Scylla Paramamosain*. *Aquaculture* 548, 737705. doi: 10.1016/j.aquaculture.2021.737705
- Zhang, C., Zhang, Q., Pang, Y. Y., Song, X. Z., Zhou, N., Wang, J., et al. (2019). The Protective Effects of Melatonin on Oxidative Damage and the Immune System of the Chinese Mitten Crab (*Eriocheir Sinensis*) Exposed to Deltamethrin. *Sci. Total Environ.* 653, 1426–1434. doi: 10.1016/j.scitotenv.2018.11.063

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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