



Effects of light intensities on the growth and physiology of *Megalobrama pellegrini* larvae and juveniles

Feifan Ma^{a,b,1}, Qin Li^{a,b,1}, Xiaogang Lin^{a,b}, Wenyu Li^{a,b}, Yonghua Zheng^{a,b}, Hongyu Tang^{a,b,*}

^a College of Fisheries, Southwest University, Chongqing 400715, China

^b Key Laboratory of Freshwater Fish Reproduction and Development (Ministry of Education), Key Laboratory of Aquatic Science of Chongqing, 400715, China

ARTICLE INFO

Keywords:

Megalobrama pellegrini
Light intensity
Growth
Enzyme activity
Growth-related gene

ABSTRACT

Light intensity significantly influences multiple aspects of fish growth and physiology. To investigate the effects of light intensity on early-stage growth and physiology of *Megalobrama pellegrini* and determine the optimal light intensity for their early development in a recirculating aquaculture system (RAS), we conducted a rearing experiment exposing *M. pellegrini* larvae to four light intensities: 0 Lx, 100 Lx, 500 Lx, and 2500 Lx. The results demonstrated that the growth performance was maximized at 2500 Lx and was significantly higher than other groups ($P < 0.05$). Quadratic regression analysis indicated that the optimal light intensity range for growth performance was between 1911.9 and 2139.6 Lx. The expression of growth-related genes under different light intensities mirrored changes in body weight and total length. Specifically, *GH*, *GHRa*, and *IGF-2* mRNA expression levels increased at 2500 Lx. High light intensity (2500 Lx) promoted the digestion of starch and protein by increasing the activities of α -amylase (AMS) and trypsin (TPS) while enhancing phosphatase metabolism, which was conducive to growth. Conversely, low light intensity impaired fat digestion, reduced phosphatase metabolism, and concurrently elevated antioxidant enzyme activity, leading to increased oxidative stress and adversely affecting growth. In conclusion, a light intensity range of 1911.9–2139.6 Lx is recommended as the optimal light environment for rearing *M. pellegrini* larvae and juveniles in RAS. The findings contribute to optimizing light conditions for the breeding of black bream seedlings and provide a theoretical foundation for enhancing early-stage rearing practices of *M. pellegrini* in RAS.

1. Introduction

Light is a critical environmental factor in nature, comprising three key components: light spectrum, light intensity, and photoperiod. Light intensity, an important and complex ecological environment factor, significantly influences fish growth, feeding behavior, and development (Hu et al., 2023; Stuart and Drawbridge, 2011). Fish that rely on vision for feeding exhibit species-specific and stage-specific light thresholds and optimal ranges (Lee et al., 2017). Appropriate light intensity levels can markedly enhance fish appetite and growth rate (Wang et al., 2023). For instance, *Oreochromis niloticus* exposed to a light intensity of 2000 Lx exhibited significantly better growth performance than those exposed to 1000 Lx and 3000 Lx (Wang et al., 2023); *Anoplopoma fimbria* larvae in large tanks demonstrated improved growth under lower light intensities (Lee et al., 2017). Conversely, light intensities outside the optimal range

may impair fish growth performance, survival rates, and feeding situation, cause retinal damage, and induce oxidative stress responses (Vera and Migaud, 2009; Wei et al., 2019). Photoreceptors in the retina and skin first detect light signals, and then these signals are processed through pathways in the hypothalamus to the pituitary gland. This process ultimately influences critical physiological functions in fish, such as growth, antioxidant responses, and stress adaptation (Falcón et al., 2010). In fish, as in other vertebrates, growth is regulated by the growth hormone/insulin-like growth factor-I (GH/IGF) axis (Zheng et al., 2024). While current research has mainly focused on how spectral composition and photoperiod affect the transcriptional regulation of GH/IGF-axis genes (Fei et al., 2024; Fu et al., 2022a,b), the impact of light intensity on the transcriptional dynamics within this axis has been less thoroughly investigated. Therefore, it is essential to study how light intensity affects the expression of GH/IGF-axis genes. Light intensity

* Corresponding author at: College of Fisheries, Southwest University, Chongqing 400715, China.

E-mail address: thy1970@163.com (H. Tang).

¹ These authors contributed equally to this work and should be considered co-first authors.

also exerts a significant regulatory influence on multiple physiological functions in fish (Villamizar et al., 2011), modulating the activities of various enzymes, including digestive enzymes, metabolic enzymes, and antioxidant enzymes (Li et al., 2022). Therefore, it is essential to apply appropriate light intensities during different developmental stages of fish. Identifying the optimal light intensity for fish can promote their growth and survival, reduce deformity rates, improve stress responses, and regulate their physiological states. However, current research on light intensity's effects on fish mostly centers on adult stages, whereas investigations into the larvae and juvenile stages remain relatively limited.

Megalobrama pellegrini (Tchang, 1930), commonly known as the black bream, belongs to Cypriniformes, Cyprinidae, Cultrinae, and *Megalobrama*. It is a fish species of significant economic importance in the upper reaches of the Yangtze River. Black bream exhibits substantial aquaculture potential as a variety with multiple desirable breeding traits. Long-term limitations in outdoor breeding environmental conditions and other factors have hindered black bream mass production. So, it is imperative to achieve controllable, scientific, and stable intensive breeding within a specifically designed indoor recirculating water system (RAS). Information regarding the optimal light intensity for its early developmental stages remains limited. Therefore, determining the appropriate light intensity during these critical early stages is essential for accelerating the industrial development of *M. pellegrini* aquaculture.

In this experiment, the larvae and juveniles of black bream were exposed to different light intensities in RAS for rearing. The growth, expression levels of growth-related genes, and activities of multiple enzymes were measured. We aimed to determine the optimal light intensity level for the early developmental stages of *M. pellegrini* by investigating the effects of different light intensity levels on growth and physiology. The outcomes of this research will offer crucial insights for optimizing the lighting regime during the artificial seedling cultivation process of black bream, thereby providing a theoretical foundation for industrialized breeding and enhancement of breeding welfare.

2. Materials and methods

2.1. Experimental fish and feeding trial

This experiment adhered to the Guide for the Care and Use of Laboratory Animals: Eighth Edition (2011). *M. pellegrini* larvae were obtained from Dongping Aquatic Products Co., LTD, Chongqing, China. The larvae at 5 days after hatching (DAH), with an average size of 6.70 ± 0.19 mm, were saltwater-treated (5000 ppm) and then reared in RAS (Fig. 1) at about 1300 fish per tank (about 40 fish/L). The RAS consisted of glass tanks (40 cm × 32 cm × 25 cm; 32 L volume) with controlled water velocities: < 1 cm/s at 5–15 DAH, 1–2 cm/s at 16–30 DAH, and 2–4 cm/s at 31–60 DAH.

The four experimental groups in this experiment were set up with different light intensities (0 Lx, 100 ± 5 Lx, 500 ± 25 Lx, and 2500 ± 50 Lx), and the photoperiod was set up as 12D: 12 L, with three replicates set up for each group. The illumination system employed 6000–6500 K cool-white LED strips (Yaluodi, 10 W/m) mounted 35 cm above the water surface. These emitted dual light peaks at 450 nm (blue) and 555 nm (green). Light intensity was modulated using Yaluodi dimmers. Matte black KT boards were positioned between experimental groups to prevent light crosstalk. The intensity of light in each experimental group was checked periodically during the experiment with an illuminometer.

The fish were reared to 60 DAH under constant water conditions, with the following parameters maintained: water temperature = 27.7 ± 1.9 °C, dissolved oxygen ≥ 5 mg/L, $\text{NH}_4^+ \leq 0.1$ mg/L, and $\text{NO}_2\text{-N} \leq 0.02$ mg/L. The larvae were fed to apparent satiation three times a day (9:00, 13:00, and 17:00). At 6–9 DAH, *M. pellegrini* was fed with fresh *Artemia salina* larvae and the mash feed (crude protein ≥ 40.0 , crude fat ≥ 3.5). At 10–34 DAH, only mash feed was provided for nourishment. At 35–60 DAH, the mash feed was replaced with pelleted feed (crude protein ≥ 36.0 , crude fat ≥ 3.0). Following each feeding period, the remaining bait was removed. The fish were examined daily to ascertain any mortalities.

2.2. Sampling and analysis

2.2.1. Sampling

The larvae were sampled from the tank at 6, 7, 9, 12, 15, 18, 21, 25,

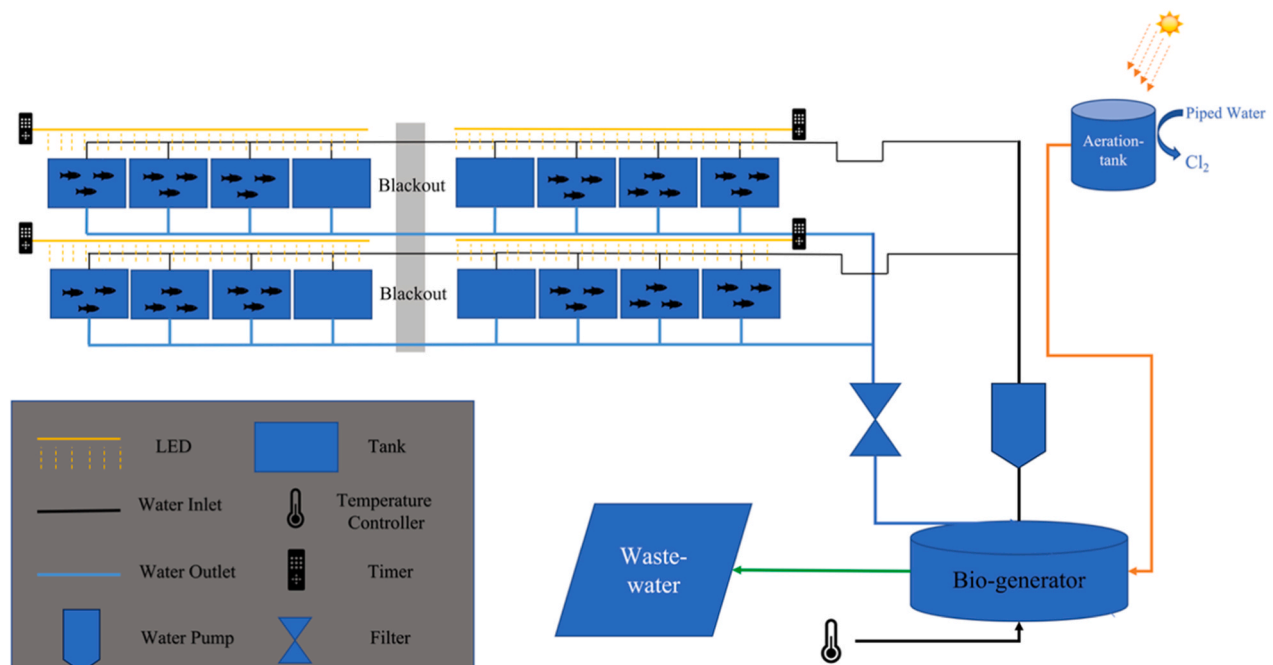


Fig. 1. The diagram of the recirculating water aquaculture system.

30, 35, 40, 45, 50, 55, and 60 DAH before feeding in the morning. Individual samples were anesthetized with tricaine methanesulfonate (MS-222, 120 mg/L). Five larvae were taken from each tank at each sampling point to measure growth data.

Meanwhile, samples of enzyme activity were collected at each sampling time point (the entire fish was collected before 40 DAH, and only the abdominal samples were collected afterward). The samples were placed in enzyme-free centrifuge tubes, ensuring each contained a sample weight greater than 0.2 g. They were then stored temporarily in liquid nitrogen and transferred to a -80°C freezer for preservation.

At 60 DAH, a random selection of juveniles was removed from each tank before the morning feeding and anesthetized with 200 mg/L MS222 to quantify growth-related gene expression. Then, the samples were placed into enzyme-free centrifuge tubes, with each tube containing a sample weight greater than 0.2 g. After adding RNA sample preservation solution (Shanghai Sangong), the samples were temporarily stored in liquid nitrogen and transferred to an ultra-low temperature refrigerator at -80°C for further experiments.

2.2.2. Growth

We used a stereomicroscope to measure the total length of the larvae before 12 DAH and used vernier calipers afterward. The body weight was measured by a microbalance (0.0001 g).

The growth parameters were calculated as follows:
Weight Growth Rate (WGR, %) = $(W_2 - W_1) / W_1$
Length Growth Rate (LGR, %) = $(L_2 - L_1) / L_1$
Daily Weight Gain (DWG, mg/d) = $(W_2 - W_1) / (T_2 - T_1)$
Daily Length Gain (DLG, mm/d) = $(L_2 - L_1) / (T_2 - T_1)$
Specific Growth Rate for Weight (SGRW, %/d) = $100 \times (\ln W_2 - \ln W_1) / (T_2 - T_1)$
Specific Growth Rate for Length (SGRL, %/d) = $100 \times (\ln L_2 - \ln L_1) / (T_2 - T_1)$

In these calculations, W_1 represents the weight at 6 DAH and W_2 at 60 DAH. L_1 represents the total length at 6 DAH and L_2 at 60 DAH. T_1 and T_2 represent 6 DAH and 60 DAH, respectively.

2.2.3. RT-qPCR analysis

We used the Eastep® Super Total RNA Extraction Kit (Promega, USA) to extract total RNA from *M. Pellegrini*. The concentration of RNA was determined using a NanoPhotometer N50 ultra-micro spectrophotometer. To analyze gene expression levels, the GoScript Reverse Transcription Kit (Promega, USA) was used for cDNA synthesis. Following the manufacturer's protocol, fluorescence quantitative reactions were performed with the CFX connect instrument (BIO-RAD, USA) and GoTaq® qPCR Master Mix (Promega, USA). Specific primers were designed using Primer-BLAST (Table 1) and produced by Sangon Biotech (Shanghai) Co., Ltd. Relative expression levels were calculated using the $2^{-\Delta\Delta\text{CT}}$ method.

2.2.4. Analysis of enzyme activity

Before the experiment, samples were homogenized by adding a 1:9 ratio of pre-cooled physiological saline at 4°C . Then, to obtain the supernatant, they were centrifuged at 4°C for 10 min at 2500 rpm. Subsequently, dilutions were carried out using physiological saline in proportion to the activity of different enzymes within the tissue. The enzymatic activity was assessed using test kits obtained from Nanjing

Jiancheng Bioengineering Institute. Enzyme activity was calculated from the change in absorbance.

2.3. Statistical analysis

All data from the experiment were recorded and processed using Microsoft Excel 2016. Results were expressed as mean $\pm$ standard error. A one-way ANOVA was used to determine whether there was a significant difference ($P < 0.05$ indicates a significant difference), and Duncan's test was used for multiple comparisons. All statistical analyses were conducted using IBM SPSS Statistics 26, and graphs were created using Origin 2021.

3. Results

3.1. Growth

We found that the final total length and final weight of *M. Pellegrini* increased with the increasing light intensity (Table 2). The final weight and total length were significantly higher at 2500 Lx than in other groups ($P < 0.05$). The final weight was considerably higher at 500 Lx than in the 0 Lx group ($P < 0.05$).

All the growth performance indices were maximum at 2500 Lx, followed by 500 Lx, and similarly lower at 100 Lx and 0 Lx. At 2500 Lx, the WGR, DWG, SGRW, and DLG were significantly higher than the other groups ($P < 0.05$), and the LGR and SGRL were considerably higher than 100 Lx and 0 Lx ($P < 0.05$). At 0 Lx, the DWG was significantly lower than 500 Lx ($P < 0.05$), and the SGRW was markedly lower than 100 Lx and 500 Lx ($P < 0.05$).

Table 2
Effects of different light intensities on the growth of *M. Pellegrini*.

Light intensity (Lx)	0 Lx	100 Lx	500 Lx	2500 Lx
Initial weight (W1, mg)	0.987 $\pm$ 0.009	1.013 $\pm$ 0.007	0.955 $\pm$ 0.016	1.029 $\pm$ 0.049
Final weight (W2, mg)	131.807 $\pm$ 3.432 ^c	178.757 $\pm$ 15.926 ^{bc}	196.228 $\pm$ 15.224 ^b	399.914 $\pm$ 29.765 ^a
Weight gain rate (WGR, %)	132.484 $\pm$ 2.363 ^b	175.248 $\pm$ 14.8 ^b	204.506 $\pm$ 15.104 ^b	390.94 $\pm$ 42.427 ^a
Daily weight gain (DWG, mg/d)	2.379 $\pm$ 0.062 ^c	3.232 $\pm$ 0.289 ^{bc}	3.55 $\pm$ 0.277 ^b	7.252 $\pm$ 0.542 ^a
Weight specific growth rate (SGRW, %/d)	8.898 $\pm$ 0.032 ^c	9.391 $\pm$ 0.152 ^b	9.673 $\pm$ 0.129 ^b	10.836 $\pm$ 0.195 ^a
Initial total length (L1, mm)	6.771 $\pm$ 0.07	6.798 $\pm$ 0.014	6.797 $\pm$ 0.064	6.760 $\pm$ 0.027
Final Total Length (L2, mm)	21.659 $\pm$ 0.963 ^b	21.301 $\pm$ 0.832 ^b	23.779 $\pm$ 0.146 ^b	26.641 $\pm$ 1.18 ^a
Length gain rate (LGR, %)	2.202 $\pm$ 0.173 ^b	2.134 $\pm$ 0.127 ^b	2.499 $\pm$ 0.012 ^{ab}	2.941 $\pm$ 0.173 ^a
Daily length gain (DLG, mm/d)	0.271 $\pm$ 0.019 ^b	0.264 $\pm$ 0.015 ^b	0.309 $\pm$ 0.002 ^b	0.361 $\pm$ 0.021 ^a
Length specific growth rate (SGRL, %/d)	2.111 $\pm$ 0.098 ^b	2.074 $\pm$ 0.073 ^b	2.277 $\pm$ 0.006 ^{ab}	2.490 $\pm$ 0.081 ^a

Note: Different letters in the same row indicate significant differences between groups ($P < 0.05$), and data in the same column are only compared with the same index under different light intensities.

Table 1
Primers used for RT-qPCR amplification.

Gene	GenBank	Forward (5'-3')	Reverse (3'-5')	Purpose
GH	AY170124.1	GCATTAGTGCTGTTGTCGGT	GGCAACAGGTTGTCCTCAAA	RT-qPCR
GHRa	JN896373.1	ATTGAGCATCTGACCCACC	ATCTGCACATCTGCTGACGG	
IGF-1	JQ398496.1	AGCGGTCAATTCTTCCAGGG	CAGTGTGCGGGAGTCAAC	
IGF-2	JQ398497.1	CTGGTCCATACGGATGCCAA	CCGCCACATAACGTTTCAGC	
β -actin	AY170122.2	CACCTTCTACAACGAGCTGC	GACACCATCACCAGAGTCCA	Reference gene

We performed quadratic regression analyses (Wei et al., 2019) for the SGRW and SGRL within light intensity, respectively (Fig. 2). Both models demonstrated good fitting performance and achieved high goodness-of-fit. Then, the quadratic regression equations were subjected to peak analysis, both yielded one peak: the optimal light intensity for SGRW was 2139.6 Lx, and the optimal light intensity for SGRL was 1911.9 Lx.

3.2. Expression of growth-related genes

We found that different light intensities significantly influenced the expression levels of the *GH* gene, *GHRa* gene, and *IGF-1* gene ($P < 0.05$, Fig. 3). Notably, the expression of the *GH* gene at 2500 Lx was markedly higher than that observed in other groups ($P < 0.05$, Fig. 3-A). Similarly, the expression of the *GHRa* gene at 2500 Lx exceeded that at both 100 Lx and 500 Lx ($P < 0.05$, Fig. 3-B). The *IGF-1* gene exhibited a significant increase in expression at 500 Lx compared to all other conditions, and the expression level of the *IGF-1* gene was significantly higher at 100 Lx and 2500 Lx than at 0 Lx ($P < 0.05$, Fig. 3-C). In contrast, no significant differences were detected in *IGF-2* gene expression among the experimental groups.

3.3. Analysis of enzyme activity

3.3.1. Activity of digestive enzymes

The trend of the AMS activity was similar under different light intensities (Fig. 4-A): it decreased at 12 DAH and 40 DAH, then increased to peak at 60 DAH. The AMS activity at 2500 Lx was generally high and significantly greater ($P < 0.05$) than in the other three groups at 15 DAH. In contrast, the AMS activity at 0 Lx was lower than the other groups for most periods, particularly at 18, 21, 40, and 55 DAH ($P < 0.05$), and also significantly lower than that in the 2500 Lx group at 35 DAH ($P < 0.05$).

The TRY activity among the groups remained relatively low, with minimal fluctuations before 25 DAH. It showed an increasing and then decreasing trend at 30–60 DAH (Fig. 4-B). At 2500 Lx, the TRY activity remained high: it was significantly greater than that of the 100 Lx and 500 Lx groups at 15 and 18 DAH ($P < 0.05$), markedly higher than other

groups at 35 and 40 DAH ($P < 0.05$), and significantly above the levels of the 0 Lx and 100 Lx groups at 60 DAH ($P < 0.05$). At 500 Lx, the TRY activity consistently exceeded that of the 0 Lx and 100 Lx groups (except 50 DAH) and was significantly higher at 35 and 60 DAH ($P < 0.05$). At 100 Lx, the TRY activity remained low after 35 DAH, however, it increased sharply and peaked at 50 DAH, significantly higher than the 0 Lx and 500 Lx groups ($P < 0.05$).

The LPS activity exhibited multiple fluctuations during the experimental period (Fig. 4-C). The differences in the LPS activity at different light intensities were not concentrated over time. The LPS activity at 100 Lx was consistently higher and was significantly higher than the 0 Lx group at 12 DAH ($P < 0.05$), the 500 Lx group at 21 DAH ($P < 0.05$), and the other three groups at 35 DAH ($P < 0.05$). The LPS activity was lower at 0 Lx, significantly lower than the 100 Lx and 500 Lx groups at 35 DAH ($P < 0.05$), and considerably lower than the 2500 Lx group at 55 DAH ($P < 0.05$).

3.3.2. Activity of metabolizing enzymes

The AST activity at different light intensities increased rapidly at 6–12 DAH, decreased at 15 DAH and 40 DAH, and remained dynamically stable at other times. The differences in the AST activity among different light intensities were minor and not concentrated: the 500 Lx group was significantly higher than the other groups at 7 DAH ($P < 0.05$), the 100 Lx group was markedly higher than the 2500 Lx group at 15 DAH ($P < 0.05$), the 0 Lx group was considerably higher than the other groups at 25 DAH ($P < 0.05$), the 0 Lx group was significantly higher than the 2500 Lx and 2500 Lx groups at 35 DAH ($P < 0.05$).

Before 25 DAH, the ALT activity in all groups exhibited two cycles of increase followed by decrease (Fig. 5-B). After 25 DAH, the ALT activity of the 0 Lx and 500 Lx groups remained dynamically stable, whereas the ALT activity of the 100 Lx group and 2500 Lx group initially increased and subsequently decreased. The differences in the ALT activity at different light intensities were concentrated at 12–15 DAH and 30–60 DAH. The ALT activity of the 500 Lx group was significantly lower than that of other groups at 6 and 60 DAH ($P < 0.05$). The ALT activity at 0 Lx was significantly higher than the 2500 Lx group at 12 and 15 DAH ($P < 0.05$), substantially lower than the 100 Lx group at 30 DAH

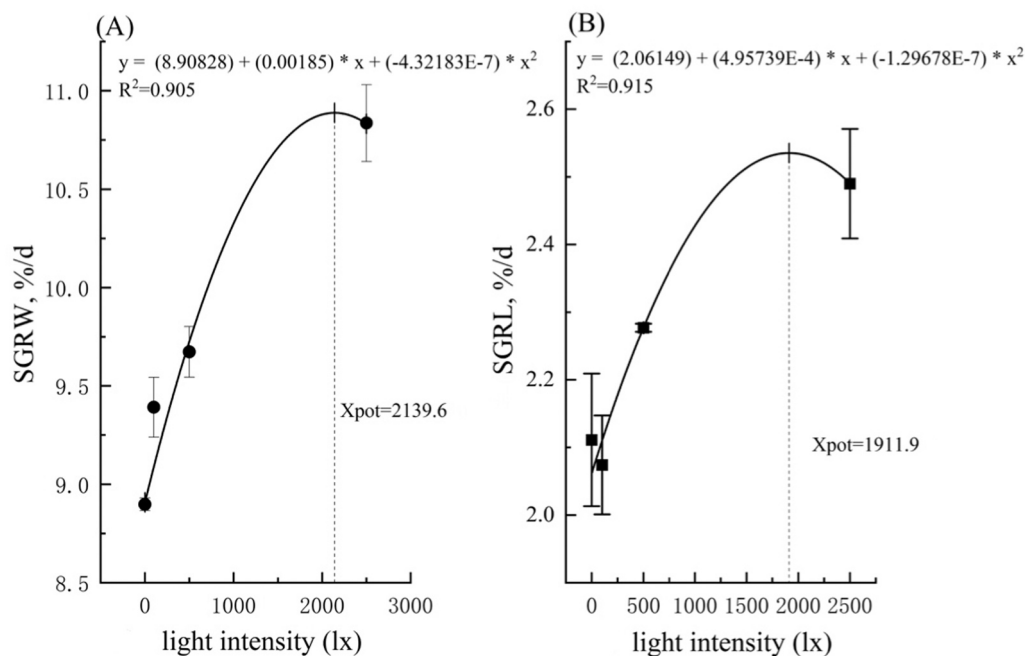


Fig. 2. Quadratic regression analysis between SGRW (A), SGRL (B), and light intensity of *M. Pellegriini* larvae, where Xpot represents the light intensity at the time of maximum SGRW, SGRL.

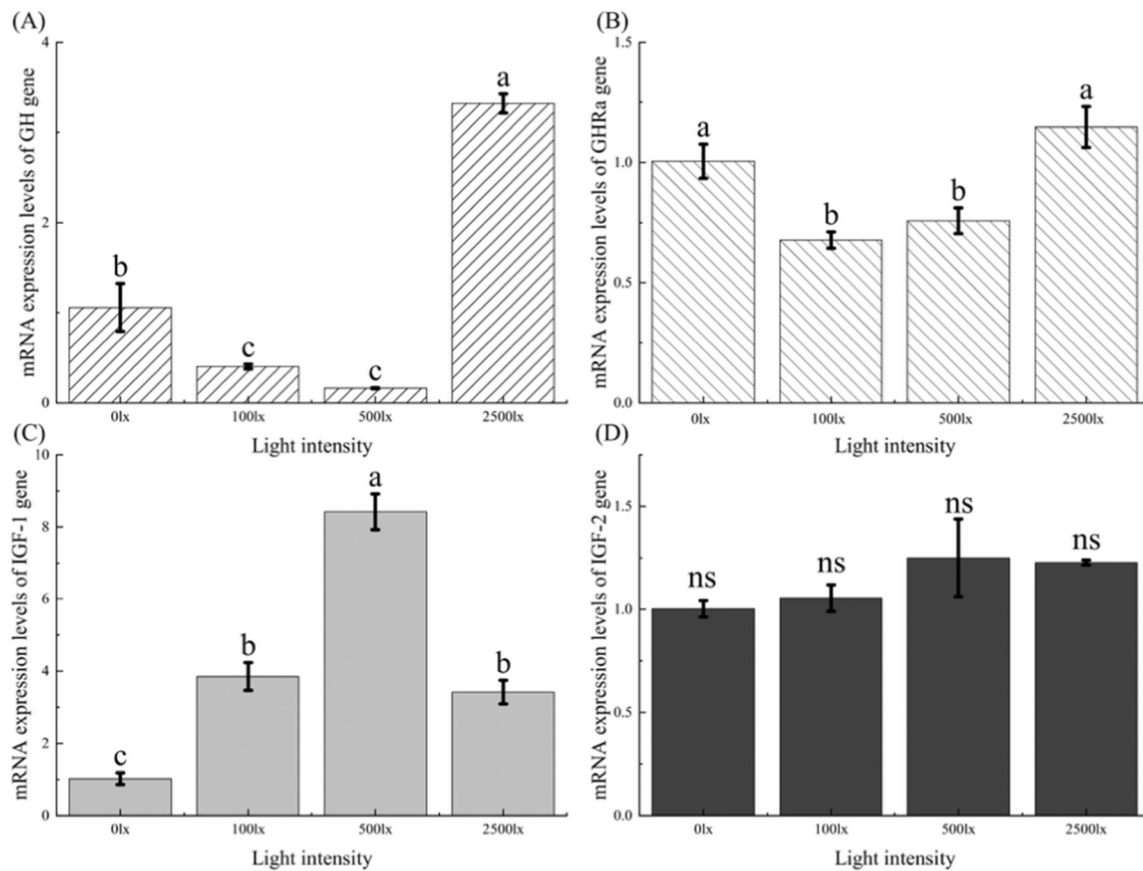


Fig. 3. The expression analysis of growth-related genes under different light intensities of *M. Pellegrini* larvae. A: relative expression of *GH* gene, B: relative expression of *GHRa* gene, C: relative expression of *IGF-1* gene, D: relative expression of *IGF-2* gene. Different letters indicate significant differences ($P < 0.05$) under different light intensities, while bars sharing the same “ns” designation indicate no statistically significant difference ($P > 0.05$).

($P < 0.05$), and the 2500 Lx group at 35 DAH ($P < 0.05$). And at 0 Lx, the AST activity remained consistently lower at 30–50 DAH.

The LDH activity of different light intensities increased gradually at 9–12 DAH, decreased at 15 and 40 DAH, and maintained a dynamic balance at other times. There was less overall difference in the LDH activity between the groups. The 100 Lx group showed significantly higher AST activity than the 2500 Lx group at 15 DAH ($P < 0.05$) and higher than the 0 Lx group at 21 and 50 DAH ($P < 0.05$). The 0 Lx group was significantly higher than the 100 Lx and 500 Lx groups at 25 DAH ($P < 0.05$).

Under different light intensities, the ACP activity increased gradually in general before 35 DAH and decreased at 40 DAH; it rose slowly at 40–55 DAH and declined at 60 DAH (Fig. 5-D). The disparities in the ACP activity among the groups were relatively minor. The 500 Lx group was conspicuously lower than the other groups at 6 DAH and 25 DAH ($P < 0.05$). The 2500 Lx group was significantly lower than the 500 Lx group at 7 DAH ($P < 0.05$) and lower than the 100 Lx and 500 Lx groups at 15 DAH ($P < 0.05$), but it was markedly higher than the 0 Lx group at 15 DAH ($P < 0.05$).

The AKP activity of different light intensities showed similar fluctuations (Fig. 5-E): it increased at 6–12 DAH, decreased at 15 DAH, remained stable afterward, and gradually declined at 25–40 DAH before stabilizing. Differences in the AKP activity were most pronounced between 12 DAH and 35 DAH: during this time, the 2500 Lx group exhibited higher activity while the 0 Lx group had lower levels, and the latter was significantly lower than the former at 12, 15, 18, 25, 30, and 35 DAH ($P < 0.05$).

3.3.3. Activity of antioxidant enzymes

The SOD activity trends across the groups exhibited a similar pattern

(Fig. 6-A): it increased at 6–25 DAH, stabilized at 25–35 DAH, decreased at 40 DAH, then rose again until peaking around 45 DAH before gradually declining. Notably, differences in the SOD activity among various light-intensity groups were mainly observed at 25–35 DAH. The SOD activity at 0 Lx was significantly lower than that of other groups at 15 DAH ($P < 0.05$), as well as at 30 and 35 DAH ($P < 0.05$). Additionally, the SOD activity at 2500 Lx was significantly lower than that of the 500 Lx group at 7 DAH ($P < 0.05$). It was also significantly reduced compared to the 100 Lx and 500 Lx groups at 30 DAH ($P < 0.05$).

Under varying light intensities, the CAT activity initially increased, subsequently decreased, and then increased again before stabilizing dynamically and gradually reducing (Fig. 6-B). Differences in the CAT activity among the groups were minimal: the 100 Lx group exhibited higher activity; the CAT activity at 0 Lx was lower than other groups, and it was remarkably lower than that in the 100 Lx group at 6, 18, 21, 35, 50 and 60 DAH ($P < 0.05$). The difference between the 2500 Lx and 500 Lx groups was minor, with only the former showing significantly higher levels at 50 DAH ($P < 0.05$).

The T-AOC content initially decreased and then increased at 6–12 DAH, stabilized dynamically after 15 DAH, and sharply declined to the lowest level at 60 DAH (Fig. 6-C). The T-AOC content remained consistently high at 2500 Lx and was only marginally lower at 500 Lx. There were slight differences in the T-AOC content among the groups. At 2500 Lx, the T-AOC content was significantly higher than the other groups at 7 DAH and 35 DAH ($P < 0.05$), the 100 Lx and 500 Lx groups at 21 DAH ($P < 0.05$), and the 0 Lx and 100 Lx groups at 60 DAH ($P < 0.05$). At 500 Lx, the T-AOC content was significantly higher than the 100 Lx group at 35 and 60 DAH ($P < 0.05$).

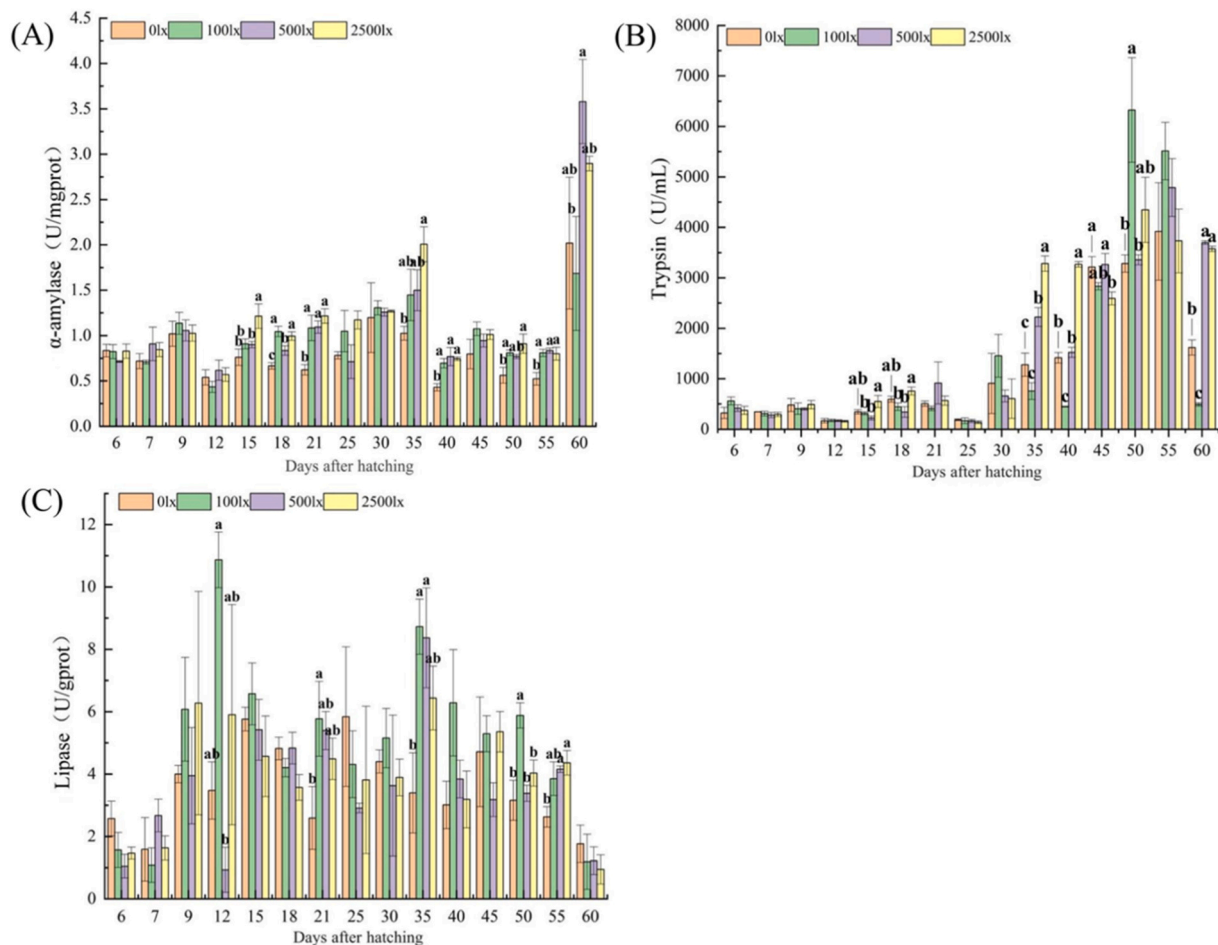


Fig. 4. Changes in metabolizing enzyme activity of *M. Pellegrini* larvae under different light intensities. A: α -amylase (AMS), B: trypsin (TPS), C: lipase (LPS). Note: Different letters indicate significant differences between groups ($P < 0.05$), the same as below.

4. Discussion

In the early life stages of fish, light serves as a crucial environmental factor that significantly influences growth (Ruchin, 2021). Insufficient light intensity can inhibit digestive enzyme activity in fish (Ye et al., 2014), and below a certain threshold, fish may be unable to feed (Qin et al., 2009). Conversely, excessive light intensity can damage their visual organs (Bejarano-Escobar et al., 2014), impairing feeding behavior and triggering emergency responses that elevate activity levels (Qin et al., 2017), ultimately hindering growth. Under the current experimental conditions, we observed that the growth indices of *M. Pellegrini* at 2500 Lx were significantly higher than those of other groups. This phenomenon may be because feeding intensity, digestibility (Lou et al., 2008; Xie, 2002), and feed conversion (Di et al., 2023) are increased under suitable light-intensity conditions, thus promoting growth. Notably, this finding contrasts with previous results indicating optimal growth at 400 Lx for juveniles of the same genus, *Megalobrama amblycephala* (Tian et al., 2015). Such discrepancies may arise from differences in sample stages as well as species variations. The significant differences in the body weight and total length were not detected until 15 DAH and 18 DAH, respectively, suggesting that the effects of light intensity on the growth require time to manifest, with the weight responding more rapidly than the total length. Jinghui Fang (Fang et al., 2013) and Hui Wei (Wei et al., 2019) established regression equations for SGR and light intensity, and the light intensity data corresponding to the peak of their SGR was taken as the optimum light intensity for growth performance. Consequently, we conducted quadratic regression analyses for light intensity and SGRW and light intensity and SGRL,

respectively. We found that the former showed an optimal light intensity of 2139.6 Lx and the latter an optimal light intensity of 1911.9 Lx, which were both within the experimental design of the maximum light intensity of 2500 Lx, and thus presumed that the optimal growth performance interval was in the range of 1911.9–2139.6 Lx.

The growth status of fish can be reflected by the expression levels of genes associated with the GH/IGF-1 axis in vivo (Kaneko et al., 2019a). Many genes within this axis are capable of stimulating cell growth and differentiation, thereby influencing overall growth (Lan and Liang, 2023). Physiological effects from environmental and physical stressors have been shown to impact both fish growth and the GH/IGF axis (Greene et al., 1999). Light, as a significant environmental factor, affects growth by modulating mRNA levels of GH and IGF-1 (Fu et al., 2022a,b). IGF-2, a homologous peptide hormone to IGF-1, plays a crucial role in cell proliferation, growth, migration, differentiation, and survival during embryonic development (Chu et al., 2022). The mRNA levels of GH, GHRA, and IGF-2 in the experiment were upregulated under high light intensity (2500 Lx), consistent with observed trends in the body weight and total length under similar conditions. This phenomenon suggested that the high light intensity enhanced the synthesis of GH, GHRA, and IGF-2, increased the binding capacity of GH/GHRA, and promoted growth. Conversely, at 500 Lx, the mRNA levels of IGF-1 were significantly higher than those in other groups; however, the mRNA levels of GH were lowest. This phenomenon may result from the overproduction of IGF-1, which produces a negative feedback regulation inhibiting the release of GH (Kaneko et al., 2019b), ultimately leading to impaired growth of *M. Pellegrini* at 500 Lx.

The impact of light on fish growth is well documented, but its effects

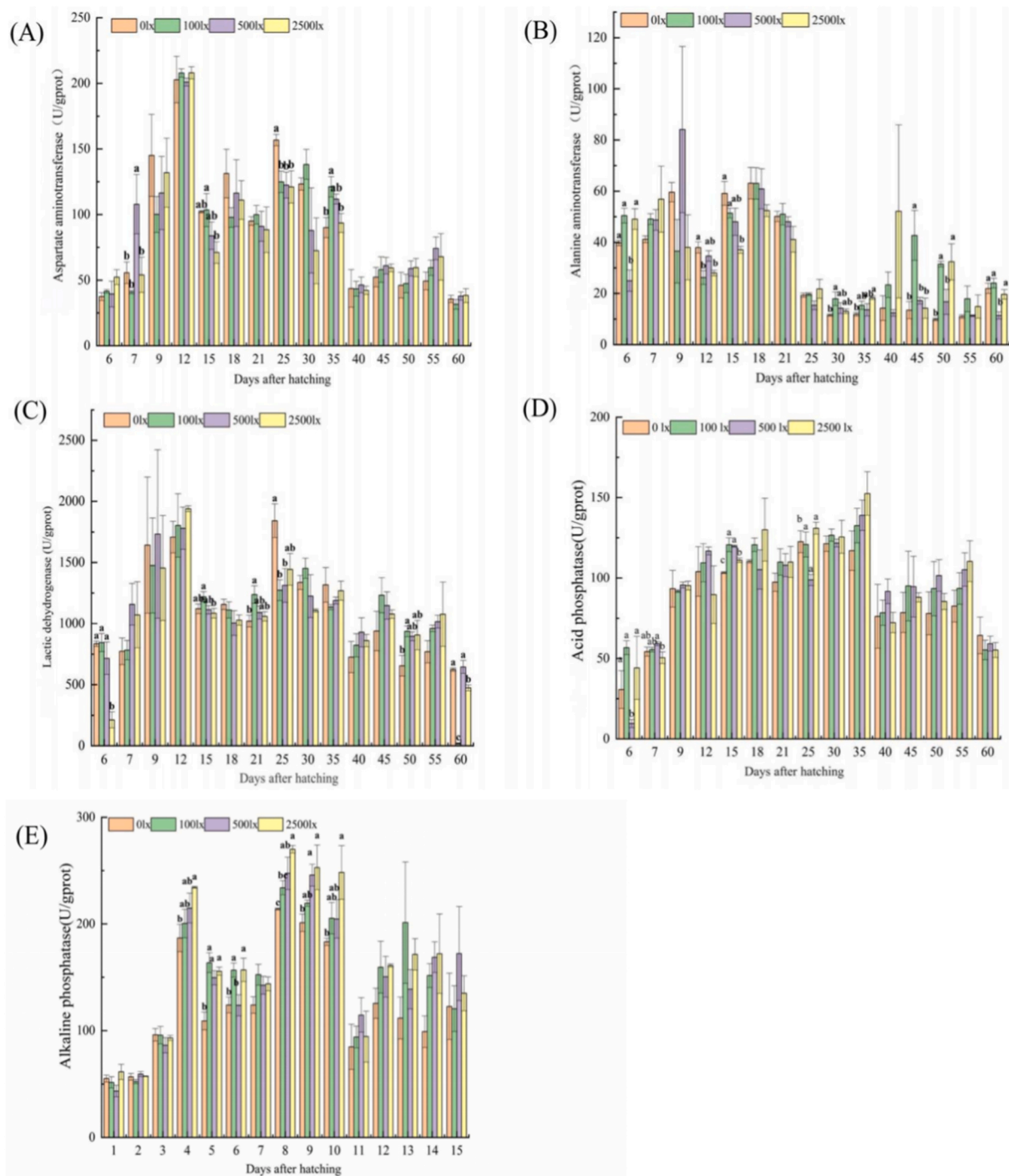


Fig. 5. Changes in metabolizing enzyme activity of *M. Pellegrini* larvae under different light intensities. A: aspartate aminotransferase (AST), B: alanine aminotransferase (ALT), C: lactate dehydrogenase (LDH), D: acid phosphatase (ACP), E: alkaline phosphatase (AKP).

go beyond this aspect of fish biology (Villamizar et al., 2011). Light plays a crucial role in regulating many physiological functions in fish. Changes in light intensity can induce physiological and biochemical alterations within the fish's body. These alterations can affect the development of digestive organs and the activity of digestive enzymes, influence metabolic rates, modify the activity of various enzymes involved in metabolism, trigger stress responses and oxidative stress, and alter the activity of antioxidant enzymes in fish, among other effects.

The findings of the experiment showed that digestive enzyme activity fluctuations in the days after hatching were similar across different light intensities. However, the variations observed among the various

digestive enzymes differed significantly. The overall AMS activity remained stable with increasing age. It decreased only at 12 DAH and 40 DAH, which is similar to the results of Qin Li on AMS activity in the early developmental stages of *M. Pellegrini* (Li et al., 2013; Li and Tang, 2012). This may be attributed to the fact that the larvae tend to be carnivorous at this stage and do not have a high demand for starch. The decline in the TRY activity at 12, 25, and 60 DAH is consistent with the "four-phase model" of TRY variation in fish during the early developmental stage. The initial phase is the period of mixed nutrition, which is distinguished by an increase in TRY activity. The second phase is a critical phase of decreasing TRY activity, which may result in poor growth and high

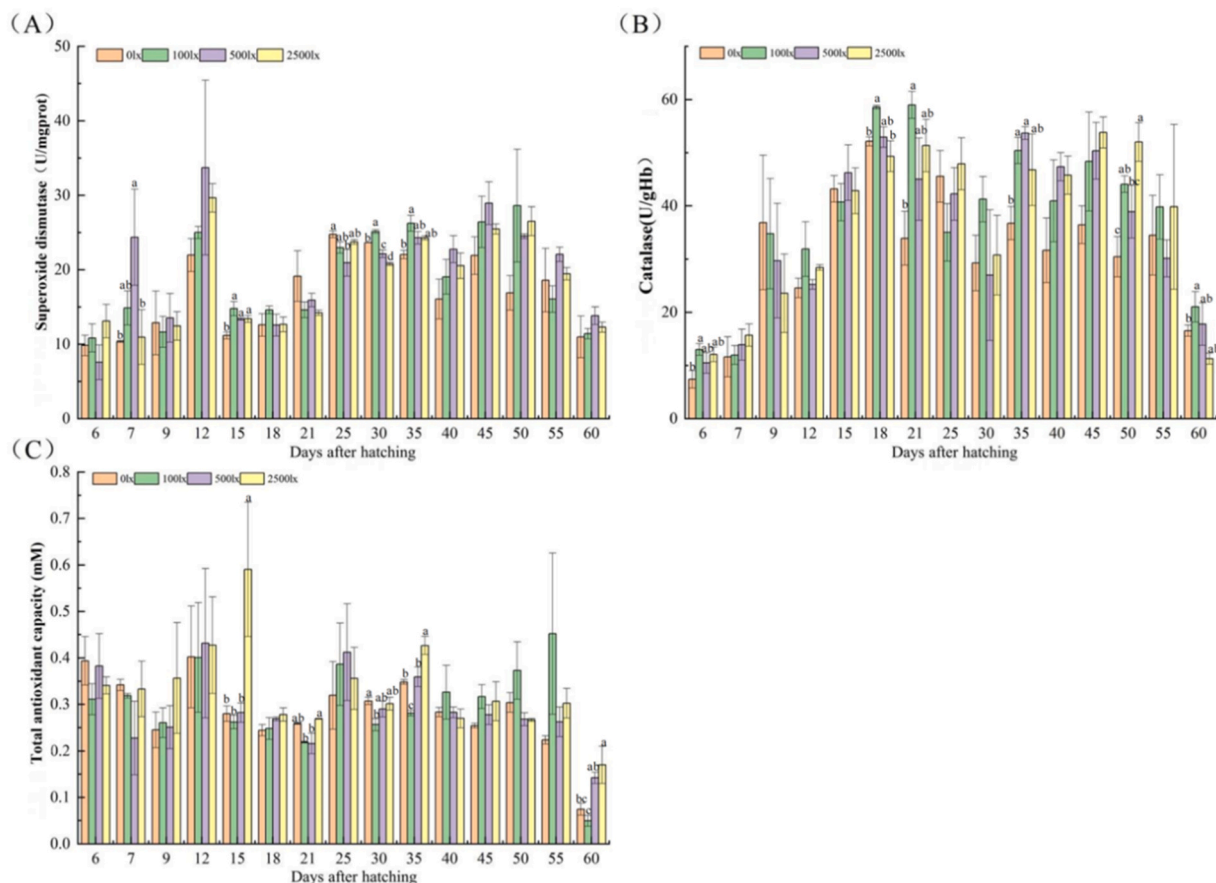


Fig. 6. Changes in antioxidant enzyme activity of *M. Pellegrini* larvae under different light intensities. A: superoxide dismutase (SOD), B: catalase (CAT), C: total antioxidant capacity (T-AOC).

mortality. An increase in TRY activity distinguishes the third phase. In the fourth phase, TRY activity declines due to the rise of other enzyme activities. The LPS activity, on the other hand, had a zigzag pattern with several increases followed by decreases. The decrease in the AMS and TRY activities at 12 DAH may be due to the reduction of fresh *Artemia salina* larvae in the diet from 10 DAH onwards. The increase in the LPS activity at 35 DAH may be due to a change in the feed composition at this time: the percentage of protein decreased, and the rate of fat increased. This is consistent with the fact that the LPS activity in *Spinibarbus caldwelli* juveniles increases with increasing fat content in the diet (Qin et al., 2023). Under different light intensities, the LPS activity differed significantly only after 9 DAH. In comparison, the AMS and TRY activities didn't vary considerably until 12 DAH, earlier than the time of the significant differences in the weight and total length. This suggests that the body weight differences may result from the changes in digestive enzyme activities. After 12 DAH, the variations in the AMS and TRY activities across different light intensities aligned with the trends observed in the SGRW and SGRL. At 2500 Lx, the AMS and TRY activities consistently remained elevated, significantly surpassing those of other groups during specific periods. Conversely, LPS activities were lower in the 0 Lx group compared to others. Therefore, we conclude that adequate light intensity is beneficial for enhancing AMS and TRY activities and promoting growth in black bream. In contrast, insufficient light may lead to a reduction in LPS activities.

The activities of AST and ALT can serve as indicators of the intensity of amino acid metabolism and the normalization of liver function (Ma et al., 2021). The AST and ALT activities demonstrated an elevation before 9 DAH, which may be attributed to the development of the liver organs. Additionally, food intake appeared to stimulate the elevation of enzyme activities. At 12 DAH and 15 DAH, the ALT and AST activities

decreased, and the differences between different light intensities began to emerge. This may be due to the cessation of *artemia* feed and the introduction of powdered feed. In this experiment, the different light intensities had a greater effect on the ALT activity and a lesser impact on the AST activity. One of the pivotal regulatory points in glycolysis and carbohydrate metabolism is LDH, and alterations in its activity directly impact the organism's energy metabolism (Zakhartsev et al., 2004). The pattern of change in LDH under different light intensities in this experiment was not statistically significant. Both ACP and AKP play a critical role in regulating immune and metabolic functions, serving as essential detoxification systems within the organism (He and Sun, 1992). The ACP and AKP activities fluctuated before 21 DAH, indicating that the larvae were undergoing continuous adaptation to varying light intensities. In this experiment, the activities of ACP and AKP were higher at 2500 Lx, whereas lower light intensities resulted in reduced activities.

Reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\cdot}$) are naturally produced by the organism during oxidative metabolism. Excessive ROS can damage cell structure and function, accelerate cell senescence and apoptosis, and cause oxidative stress in fish. SOD and CAT are important oxidative enzymes for fish to remove excess ROS from the organism. T-AOC is a comprehensive indicator of the body's antioxidant capacity (Ma et al., 2021), and an increase in its activity indicates that the organism is under a certain level of oxidative stress (Zhang et al., 2019). In this experiment, the activities of SOD and CAT at varying light intensities exhibited a similar trend characterized by 'increase-decrease-stable-decrease,' which aligns with the findings reported by Guocong Li (Liu, 2019). This phenomenon suggests that in the artificially engineered light environment, the ROS levels in *M. Pellegrini* increased during initial adaptation to light intensity, leading to elevated activities of the SOD and CAT.

After a gradual adjustment to light intensity, the SOD and CAT activities stabilized. And after 50 DAH, excess ROS was cleared, resulting in a gradual reduction of the SOD and CAT activities back to baseline levels observed at the start of the experiment. We observed reduced activities of the SOD and CAT, accompanied by elevated T-AOC at 2500 Lx, suggesting that the ROS content in samples at this light intensity was lower than in other groups. The elevated T-AOC activity effectively scavenges excess ROS in fish (Zhang et al., 2019), facilitating a more advantageous response to environmental changes. This result was consistent with the optimal growing conditions. While the SOD and CAT activities were lower at 0 Lx, the T-AOC activity was also lower in the body, indicating a compromised ability to respond to environmental changes. At 100 Lx, the activities of SOD and CAT were elevated, but the T-AOC activity was lower. This indicates that the organism has a stress response with increased ROS content in the body. Furthermore, the accumulation of ROS exceeded a certain threshold, thereby damaging the biological membrane and enzyme system and consequently resulting in a decline in the T-AOC activity.

5. Conclusion

This study examined the impact of four light intensities in RAS on growth performance and physiology during the early developmental stages of *M. Pellegrini*. Based on our results, high light intensity (2500 Lx) was found to enhance growth performance, promote digestive and metabolic capabilities, and effectively resist the impact of oxidative stress. Through quadratic regression analysis, we estimated that the optimal light intensity range for the seedling cultivation stage of *M. Pellegrini* was 1911.9–2139.6 Lx. Further experiments are recommended to determine the most appropriate light intensity for this developmental phase.

CRedit authorship contribution statement

Wenyu Li: Investigation, Formal analysis, Data curation. **Yonghua Zheng:** Funding acquisition, Conceptualization. **Qin Li:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Feifan Ma:** Writing – review & editing, Writing – original draft, Visualization, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Xiaogang Lin:** Writing – original draft, Investigation, Data curation. **Hongyu Tang:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was funded through Chongqing's Key Aquatic Science and Technology Innovation Project (No. CQFTIU202502-3), Chongqing Modern Mountain Characteristic and Efficient Agriculture Industrial Technology System Ecological Fishing Innovation Team Special Project (No. 4322500369), and Scientific Research Project on the Effects of Jiangjin Degan Flood Control and Bank Protection Comprehensive Remediation Project on Aquatic Organisms and Their Habitats in the National Nature Reserve for Rare and Endemic Fishes in the Upper Reaches of the Yangtze River (No. 2020-zh-251).

Data Availability

Data will be made available on request.

References

- Bejarano-Escobar, R., Blasco, M., Martín-Partido, G., Francisco-Morcillo, J., 2014. Molecular characterization of cell types in the developing, mature, and regenerating fish retina. *Rev. Fish. Biol. Fish.* 24, 127–158. <https://doi.org/10.1007/s11160-013-9320-z>.
- Chu, M., Jia, Y., Wu, Z., Huan, H., Guo, X., Yin, S., Zhang, K., 2022. Genome-wide characterization of three IGFs in hybrid yellow catfish (*Pseudobagrus fulvidraco* ♀ × *Pseudobagrus vachellii* ♂) and the association of IGF2 allelic variants with growth traits. *Aquac. Rep.* 26, 101315. <https://doi.org/10.1016/j.aqrep.2022.101315>.
- Di, Z., Li, K., Li, T., Yan, L., Jiang, H., Liu, L., 2023. Effects of light intensity and photoperiod on the growth performance of juvenile Murray cods (*Maccullochella peelii*) in recirculating aquaculture system (RAS). *Aquac. Fish.* 8, 274–279. <https://doi.org/10.1016/j.aaf.2021.12.009>.
- Falcón, J., Migaud, H., Muñoz-Cueto, J.A., Carrillo, M., 2010. Current knowledge on the melatonin system in teleost fish. *Gen. Comp. Endocrinol. Fish. Reprod.* 165, 469–482. <https://doi.org/10.1016/j.yggen.2009.04.026>.
- Fang, Jinghui, Wei, X., Wang, Z., Liu, X., Song, X., Zhou, Q., Jianguang, Fang, Jiang, H., 2013. Effects of light intensity on the growth, biochemical composition and energy budget of Juvenile Schlegel's Rockfish (*Sebastes schlegelii*). *Period. Ocean Univ. China* 43, 47–53. <https://doi.org/10.16441/j.cnki.hdx.2013.09.007>.
- Fei, F., Cao, S., Li, Wensheng, Zhu, Z., Li, Wenyang, Gao, X., Zhang, X., Sun, Y., Zhang, C., Liu, B., 2024. Effects of spectral on growth, physiology, and growth axis gene expression in *Plectropomus leopardus*. *Aquaculture* 593, 741290. <https://doi.org/10.1016/j.aquaculture.2024.741290>.
- Fu, X., Zou, Z., Zhu, J., Xiao, W., Li, D., Yu, J., Chen, B., Yang, H., 2022a. Effects of different photoperiods on growth performance, daily rhythm of growth axis-related genes, and hormones in Nile tilapia (*Oreochromis niloticus*). *Aquaculture* 553, 738071. <https://doi.org/10.1016/j.aquaculture.2022.738071>.
- Fu, X., Zou, Z., Zhu, J., Xiao, W., Li, D., Yu, J., Chen, B., Yang, H., 2022b. Effects of different photoperiods on growth performance, daily rhythm of growth axis-related genes, and hormones in Nile tilapia (*Oreochromis niloticus*). *Aquaculture* 553, 738071. <https://doi.org/10.1016/j.aquaculture.2022.738071>.
- Greene, M.W., Shambloot, M.J., Chen, T.T., 1999. Presence of GH-dependent IGF-II mRNA in the diffuse pancreatic tissue of a teleost. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 122, 287–292. [https://doi.org/10.1016/S0305-0491\(99\)00010-3](https://doi.org/10.1016/S0305-0491(99)00010-3).
- He, H., Sun, F., 1992. Characterization of acid and alkaline phosphatases in the Chinese shrimp, *Fenneropenaeus chinensis*. *Oceanol. Limnol. Sin.* 555, 560.
- Hu, J., Sun, J., Zhang, M., Yan, K., Zhang, Y., Li, Yaya, Wang, G., Wang, X., Jiang, H., Li, Yunbo, Zheng, R., Wang, D., Wang, Y., Xu, S., Yan, X., 2023. Effects of different light intensity on growth, feeding, retinal structure and expression of vision-related genes of silver pomfret. *Aquac. Rep.* 30, 101631. <https://doi.org/10.1016/j.aqrep.2023.101631>.
- Kaneko, N., Torao, M., Koshino, Y., Fujiwara, M., Miyakoshi, Y., Shimizu, M., 2019a. Evaluation of growth status using endocrine growth indices, insulin-like growth factor (IGF)-I and IGF-binding protein-1b, in out-migrating juvenile chum salmon. *Gen. Comp. Endocrinol.* 274, 50–59. <https://doi.org/10.1016/j.yggen.2019.01.001>.
- Kaneko, N., Torao, M., Koshino, Y., Fujiwara, M., Miyakoshi, Y., Shimizu, M., 2019b. Evaluation of growth status using endocrine growth indices, insulin-like growth factor (IGF)-I and IGF-binding protein-1b, in out-migrating juvenile chum salmon. *Gen. Comp. Endocrinol.* 274, 50–59. <https://doi.org/10.1016/j.yggen.2019.01.001>.
- Lan, J., Liang, X., 2023. Effects of light intensity and spectrum on feeding, growth, and related gene expression of Chinese perch, *Siniperca chuatsi*. *J. Fish. Sci. China* 30, 975–988.
- Lee, J.S.F., Britt, L.L., Cook, M.A., Wade, T.H., Berejikian, B.A., Goetz, F.W., 2017. Effect of light intensity and feed density on feeding behaviour, growth and survival of larval sablefish *Anoplopoma fimbria*. *Aquac. Res.* 48, 4438–4448. <https://doi.org/10.1111/are.13269>.
- Li, X., Liu, S., Fan, K., Zhang, J., Wei, P., Liu, Y., Tian, Y., Ma, H., 2022. Effects of illumination intensities on growth, digestive and metabolic enzyme activities and antioxidant capacities of juvenile *Takifugu rubripes*. *Aquaculture* 548, 737594. <https://doi.org/10.1016/j.aquaculture.2021.737594>.
- Li, Q., Tang, H., 2012. Assessment of digestive enzymes activities during larval development of *Megalobrama pellegrini*. *Freshw. Fish.* 42, 9–13. <https://doi.org/10.3969/j.issn.1000-6907.2012.04.002>.
- Li, Q., Tang, H., Zheng, Y., Zhang, W., 2013. Effects of starvation and re-feeding on the growth and digestive enzyme activities of juvenile *Megalobrama pellegrini*. *J. Southwest Univ. Sci.* 35, 39–44. <https://doi.org/10.13718/j.cnki.xdzk.2013.07.029>.
- Liu, G., 2019. Effects of Light Intensity and Wavelength on Characteristics of Activity, Physiology and Growth of Juvenile Hybridized Grouper (*Epinephelus fuscoguttatus* × *E. lanceolatus*) (bachelor's degree). Guangdong Ocean University.
- Lou, B., Shi, H., Mao, G., Luo, J., Xin, J., Zheng, D., 2008. Effects on feeding and growth of Juvenile Zhou Shan paralicthys olivaceus under different illuminances times and intensities. *J. Zhejiang Ocean Univ. Sci.* 140, 143.
- Ma, H., Wei, P., Li, X., Liu, S., Tian, Y., Zhang, Q., Liu, Y., 2021. Effects of photoperiod on growth, digestive, metabolic and non-special immunity enzymes of *Takifugu rubripes* larvae. *Aquaculture* 542, 736840. <https://doi.org/10.1016/j.aquaculture.2021.736840>.
- Qin, Z., Liang, P., Lin, J., Gao, S., Qiu, M., Li, X., Liu, Y., Lai, M., Wu, B., 2023. Effects of dietary protein levels on growth performance, digestive enzyme activities, protein metabolism and intestinal morphology of juvenile *Spinibarbus caldwelli*. *Chin. J. Anim. Nutr.* 35, 1134–1146.

- Qin, Z., Lin, Y., Zhang, Y., Huang, R., He, W., 2009. Effects of light on feeding, growth and survival of larval *Paralichthys lethostigma*. *J. Jimei Univ. Nat. Sci. Web Version Prepr.* 14, 14–18.
- Qin, X., Liu, G., Wu, Y., Shi, X., Wang, X., 2017. Effects of light intensity on the hatching rate of fertilized *Schizothorax chongi* eggs and on the growth and feeding of larvae. *J. Hydroecology* 38, 97–102. <https://doi.org/10.15928/j.1674-3075.2017.05.014>.
- Ruchin, A.B., 2021. Effect of illumination on fish and amphibian: development, growth, physiological and biochemical processes. *Rev. Aquac.* 13, 567–600. <https://doi.org/10.1111/raq.12487>.
- Stuart, K.R., Drawbridge, M., 2011. The effect of light intensity and green water on survival and growth of cultured larval California yellowtail (*Seriola lalandi*). *Aquaculture* 321, 152–156. <https://doi.org/10.1016/j.aquaculture.2011.08.023>.
- Tian, H.-Y., Zhang, D.-D., Xu, C., Wang, F., Liu, W.-B., 2015. Effects of light intensity on growth, immune responses, antioxidant capability and disease resistance of juvenile blunt snout bream *Megalobrama amblycephala*. *Fish. Shellfish Immunol.* 47, 674–680. <https://doi.org/10.1016/j.fsi.2015.08.022>.
- Vera, L.M., Migaud, H., 2009. Continuous high light intensity can induce retinal degeneration in Atlantic salmon, Atlantic cod and European sea bass. *Aquaculture* 296, 150–158. <https://doi.org/10.1016/j.aquaculture.2009.08.010>.
- Villamizar, N., Blanco-Vives, B., Migaud, H., Davie, A., Carboni, S., Sánchez-Vázquez, F. J., 2011. Effects of light during early larval development of some aquacultured teleosts: a review. *Aquac. Larvi* 2009 (315), 86–94. <https://doi.org/10.1016/j.aquaculture.2010.10.036>.
- Wang, K., Li, K., Liu, L., Tanase, C., Mols, R., van der Meer, M., 2023. Effects of light intensity and photoperiod on the growth and stress response of juvenile Nile tilapia (*Oreochromis niloticus*) in a recirculating aquaculture system. *Aquac. Fish.* 8, 85–90. <https://doi.org/10.1016/j.aaf.2020.03.001>.
- Wei, H., Li, H.-D., Xia, Y., Liu, H.-K., Han, D., Zhu, X.-M., Yang, Y.-X., Jin, J.-Y., Xie, S.-Q., 2019. Effects of light intensity on phototaxis, growth, antioxidant and stress of juvenile gibel carp (*Carassius auratus gibelio*). *Aquaculture* 501, 39–47. <https://doi.org/10.1016/j.aquaculture.2018.10.055>.
- Xie, C., 2002. Feeding intensity and dynamics of juvenile southern Sheatfish (*Silurus Meridionalls*) under different illuminances. *Chin. J. Appl. Environ. Biol. Chin. J. Appl. Env. Biol.* 267, 269.
- Ye, L., Hu, J., Wang, Y., Wu, K., 2014. Effects of Light on Survival, Development and Growth of Larvae of *Amphiprion clarkii*. *J. Hainan Trop. Ocean Univ.* 21, 78–86. <https://doi.org/10.13307/j.issn.1008-6722.2014.05.16>.
- Zakhartsev, M., Johansen, T., Pörtner, H.O., Blust, R., 2004. Effects of temperature acclimation on lactate dehydrogenase of cod (*Gadus morhua*): genetic, kinetic and thermodynamic aspects. *J. Exp. Biol.* 207, 95–112. <https://doi.org/10.1242/jeb.00708>.
- Zhang, W., Jia, H., Niu, C., Chen, X., Storey, K.B., 2019. Effect of exogenous hydrogen peroxide on ROS balance and antioxidant response in Chinese soft-shelled turtle *Pelodiscus sinensis*. *Aquaculture* 501, 293–303. <https://doi.org/10.1016/j.aquaculture.2018.11.040>.
- Zheng, J.-L., Gao, L., Zhang, H.-T., Chen, X., Zhu, Q.-L., Han, T., 2024. LED light spectra differently affected growth, antioxidant capacity, stress response, GH/IGF and HPI axis in largemouth bass (*Micropterus salmoides*) larvae. *Aquaculture* 578, 740019. <https://doi.org/10.1016/j.aquaculture.2023.740019>.