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EDITED BY
Xiaolong Gao,
Xiamen University, China

REVIEWED BY
Baoquan Gao,
Yellow Sea Fisheries Research Institute (CAFS), China
Bin Wen,
Shanghai Ocean University, China

*CORRESPONDENCE
Zhiming Ren
renzhiming@nbu.edu.cn
Ce Shi
shice3210@126.com

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Light spectrum impacts on development respiratory metabolism and antioxidant capacity of larval swimming crab *Portunus trituberculatus*

Feifei Zhang[1,2,3,4], Shuai Zhang[1,3,4], Zhiming Ren[5]*, Changbin Song[6], Yangfang Ye[1,3,4], Changkao Mu[1,3,4], Chunlin Wang[1,3,4] and Ce Shi[1,2,3,4]*

[1]Key Laboratory of Aquacultural Biotechnology, Ningbo University, Chinese Ministry of Education, Ningbo, China, [2]Marine Economic Research Center, Dong Hai Strategic Research Institute, Ningbo University, Ningbo University, Ningbo, China, [3]Collaborative Innovation Center for Zhejiang Marine High-efficiency and Healthy Aquaculture, Ningbo University, Ningbo, China, [4]Key Laboratory of Green Mariculture (Co-construction by Ministry and Province), Ministry of Agriculture and Rural, Ningbo, China, [5]Zhejiang Provincial Engineering Research Center for the Safety of Pressure Vessel and Pipeline,Ningbo University, Ningbo, China, [6]Institute of Semiconductors, Chinese Academy of Sciences, Beijing, China

The effects of the spectrum on the development, respiratory metabolism, and antioxidant capacity of the larval swimming crab *Portunus trituberculatus* were studied. Seven light spectra, *i.e.*, purple (400 nm), blue (425 nm), cyan (510 nm), green (525 nm), yellow (598 nm), red (638 nm), and white (full spectrum), were estimated. The larvae had the optimum survival rate and development under cyan light. On the contrary, larvae in red and yellow lights had poor growth performance. The oxygen consumption rate (OCR) dropped while the ammonia excretion rate (AER) rose as the larvae developed. Early larvae's oxygen-nitrogen ratio (O: N) fell when exposed to red light, suggesting more protein was utilized in the respiratory process. Regarding the antioxidant system, crab had the lowest malondialdehyde (MDA) under green, cyan and yellow light, and the highest total antioxidant capacity (T-AOC) in cyan light. Taken together, the current results suggest that cyan was the optimum spectrum for the development of *P. trituberculatus* larvae.

KEYWORDS

swimming crab, light spectrum, respiratory metabolism, antioxidant capacity, development

Introduction

The swimming crab, *Portunus trituberculatus*, is an important commercial marine crab species in China (Dou et al., 2021), with the yield increased to 100,895 tons in 2020. (Fisheries Bureau of Agriculture Ministry of China, 2021). Currently, *P. trituberculatus* larvae are mainly bred in ponds with natural live food, resulting in inconsistent production because of unpredictable circumstances (Shi et al., 2019). Consequently, the indoor nursery of swimming crabs, which could supply the larvae stability, has expanded quickly in recent years. In the indoor nursery, light is an indispensable environmental factor.

Three components of light, including intensity, spectrum, and photoperiod, are among the most crucial environmental parameters in indoor farming (Boeuf and Le Bail, 1999; Hoang et al., 2002; Chen et al., 2021; Chen et al., 2022). Spectrum directly impacts early larvae's physiology, development, growth, and behavior (Pierce et al., 2008; Wu et al., 2020).The effects of the light spectrum on aquatic creatures are species-specific (Cheng and Flamarique, 2007; Migaud et al., 2010). For example, the giant freshwater prawn (*Macrobrachium rosenbergii*) had better development under green and white light (Wei et al., 2021). Blue or cyan light can enhance the growth performance of mud crabs (*Scylla paramamosain*) (Chen et al., 2022). For larval and juvenile *P. trituberculatus*, the optimal light intensity (Dou et al., 2021; Xu et al., 2022a) and photoperiod (Xu et al., 2022b) have been determined. However, the ideal light spectrum for *P. trituberculatus* larvae was poorly understood.

The respiratory system metabolizes most energy in the anmials (Leiva et al., 2015). Light modulates the respiratory metabolism of crustaceans by affecting endogenous motor rhythms and hormones (Hoang et al., 2003). As crucial markers of respiratory metabolism in aquatic organisms, oxygen consumption rate (OCR), ammonia excretion rate (AER), and oxygen-nitrogen ratio (O:N) can be used to evaluate crustacean energy consumption patterns (Zhang et al., 2014).

Reactive oxygen species (ROS) are oxygen radicals continuously produced in the immune system (Aguirre et al., 2005; Wu et al., 2016). Hydroxyl radicals (OH), superoxide anions (O^2-), and hydrogen peroxide (H_2O_2) make up the majority of ROS (Jacob, 1995). A suboptimal environment could produce excess ROS, causing oxidative damage to cells and tissues (Lushchak, 2011). Antioxidase, including Superoxide dismutase (SOD), and catalase (CAT) could efficiently remove the excessive ROS and maintain homeostasis. (Craig et al., 2007; Oliva et al., 2012; Malandrakis et al., 2014; Makrinos and Bowden, 2016).

In this study, light-emitting diodes (LEDs) were used as light sources to investigate the effects of different light spectra on the survival, development, respiratory metabolism, and antioxidant capacity of *P. trituberculatus* larvae to explore the optimum light spectrum for *P. trituberculatus* larvae development.

Material and methods

Experimental design, management, and sampling

The experiment was carried out at the Donghai nursery in Xiangshan County, Ningbo City, Zhejiang Province, from May to June 2021. Experimental Zoea I was collected and evenly mixed from four female parent *P. trituberculatus*. In this experiment, the larvae were reared in plastic culture tanks (19.0 cm*12.2 cm*10.9 cm) with 2000mL seawater (Wu et al., 2010). One hunderd Zoea I larvae were placed in each tank in sextuplicate. Larvae were fed newly hatched Artemia nauplii at 10 individuals mL^{-1} at 6:00, 12:00, 18:00, and 24:00 (Shi et al., 2019). Seven treatments were set up with seven distinct spectral LED strips (Yamingjie Intelligent Technology Co., Ltd.), *i.e.*, purple (400 nm), blue (425 nm), cyan (510 nm), green (525 nm), yellow (598 nm), red (638 nm), and white (634 nm, full spectrum). The light intensity was kept at 1W m^{-2} (Fei et al., 2020; Chen et al., 2022) by adjusting dimmers and the distance between lamps and the water surface, and a light cycle of 12L:12D was used throughout the experiment (6:00-18:00 light). During the experiment, the water temperature was maintained at 25 ± 1 °C (Dou et al., 2021; Xu et al., 2022b).

Morality and molting were observed daily until all the larvae died or developed into C1 juvenile crabs. At the end of the experiment, the alive C1 were quickly snap-frozen in liquid nitrogen. The level of SOD, CAT, total antioxidant capacity (T-AOC), hydrogen peroxide (H_2O_2), and malondialdehyde (MDA) were measured with commercial kits (Nanjing Jiancheng Institute of Biological Engineering) (Chen et al., 2022).

The same batch of larvae was used to test respiratory metabolism under different spectrums. The larvae were kept in a cement tank to develop to the targeted stage. After two hours in darkness, 100 larvae of the same developmental stage were put in polypropylene containers (19.0 cm 12.2 cm 10.9 cm) and treated with a specific light spectrum in quadruplicate. The OCR, AER and O: N ratio were measured and calculated as described by (Liu et al., 2022).

Data processing and statistical analysis

Statistical calculations were made using the SPSS 22.0 program. All the data is expressed as Mean ± SD. The homogeneity and normality of the survival, development, respiratory metabolism and antioxidant capability were examined with Levene's and Kolmogorov-Smirnov tests. One-way ANOVA and Duncan's test were performed to determine whether there were significant differences among treatments.

Results

Effect of light spectrum on the survival and development

The survival rate of larvae under red light was significantly lower than that of all other groups ($P<0.05$). On the contrary, the larvae of all the developmental stages had a higher survival rate in cyan light (Figure 1). The larval development under cyan light was shortened from Zoea I to Zoea III (Figures 2A-C). The molt interval of Zoea IV to megalopa was much shorter under purple, blue, cyan, and white light than it was under green, yellow, and red light (Figure 2D). The shortest molt interval was observed under green light from megalopa to C1 (Figure 2E). Overall, the cumulative developmental duration of the larvae under cyan light was the shortest, whereas a retarded development was found in yellow and red light (Figure 2F).

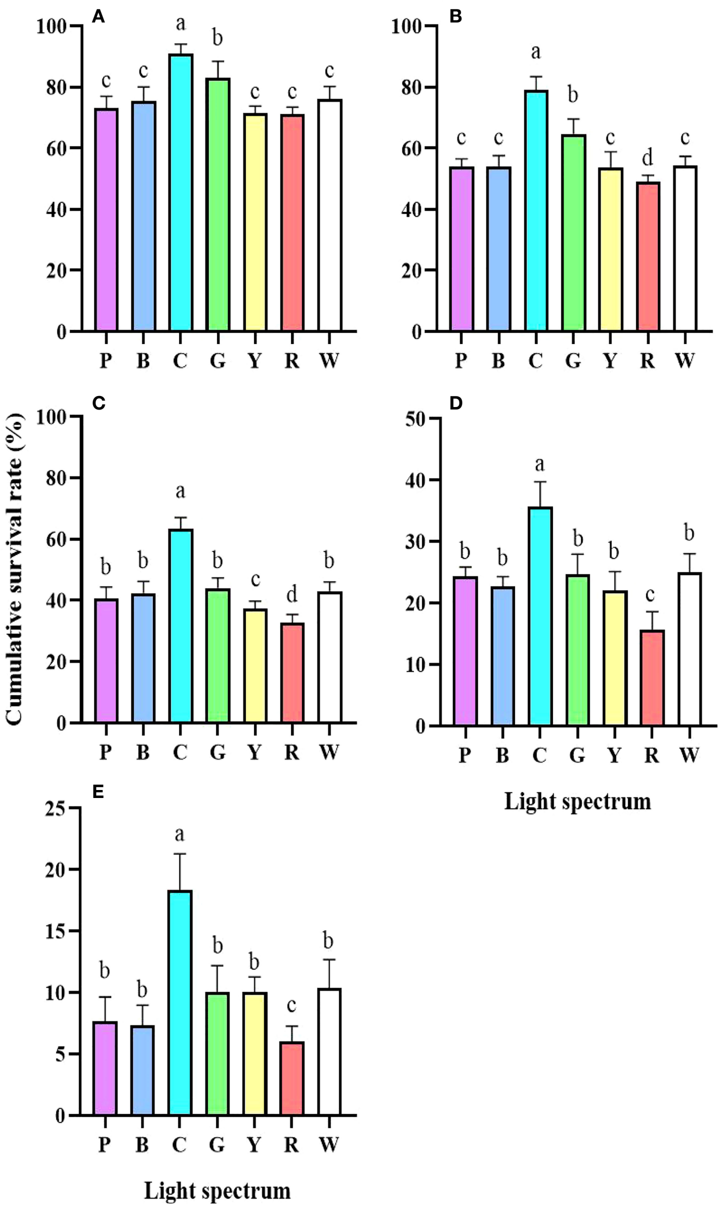

FIGURE 1
Cumulative survival rate of *P. trituberculatus* larvae under different light spectrum (mean ± SD) (n=6). **(A)** Zoea I, **(B)** Zoea II, **(C)** Zoea III, **(D)** Zoea IV, **(E)** Megalopa. P, Purple; B, Blue; C, Cyan; G, Green; Y, Yellow; R, Red; W, White. Different letters indicate significant differences between treatment groups ($P<0.05$).

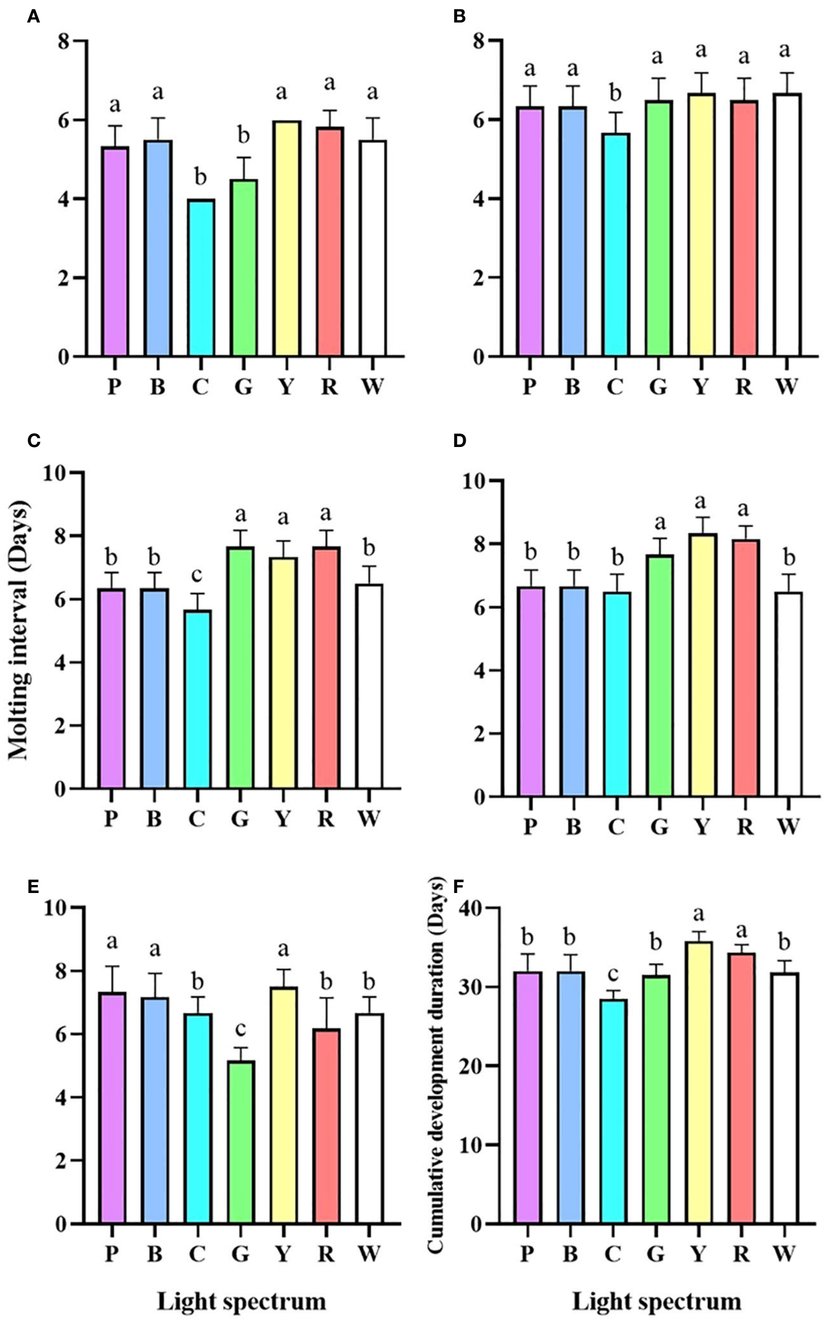

FIGURE 2
Molting interval (Zoea I - Zoea II) **(A)**, Molting interval (Zoea II - Zoea III) **(B)**, Molting interval (Zoea III - Zoea IV) **(C)**, Molting interval (Zoea IV - Megalopa) **(D)**, Molting interval (Megalopa - C1) **(E)** and Cumulative development duration **(F)** of *P. trituberculatus* larvae under different light spectrum (mean ± SD) (n=6). P, Purple; B, Blue; C, Cyan; G, Green; Y, Yellow; R, Red; W, White. Different letters indicate significant differences between treatment groups (*P*<0.05).

Effect of light spectrum on respiratory metabolism and antioxidant stress

As larvae developed, OCR significantly decreased (Figure S1). Zoea I to Zoea III had a considerably higher AER under red light compared to the other treatment (P<0.05) (Figures S2A-C). As a result, the early larvae had the lowest O:N ratio when exposed to red light (Figures 3A-C). Zoea IV has the highest O:N ratio under yellow light (Figure 3D). However, at the end of the experiment, the lowest O:N ratio of megalopa was found in the green light, which was significantly lower than those in the purple light (Figure 3E).

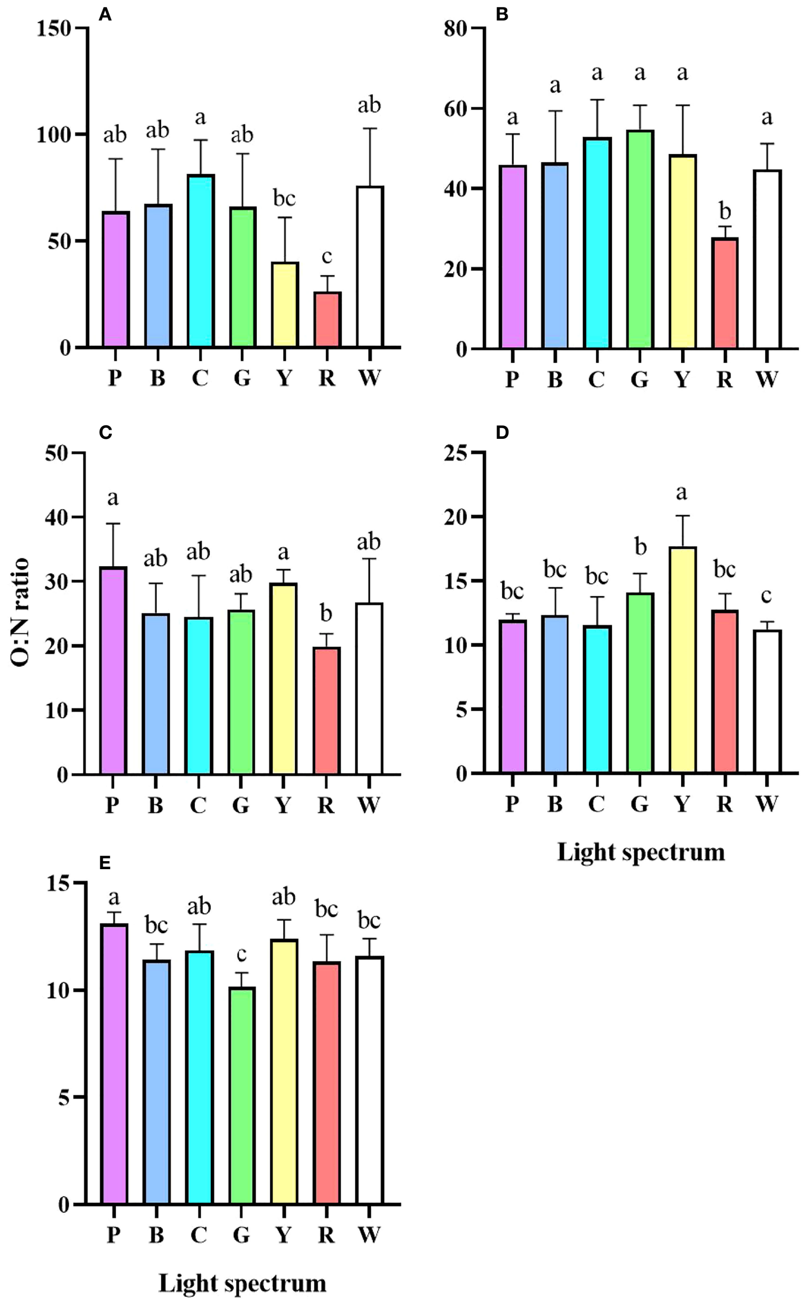

FIGURE 3
Oxygen-nitrogen ratio (O:N ratio) of *P. trituberculatus* larvae under different light spectrum (mean ± SD) (n=4). **(A)** Zoea I, **(B)** Zoea II, **(C)** Zoea III, **(D)** Zoea IV, **(E)** Megalopa. P, Purple; B, Blue; C, Cyan; G, Green; Y, Yellow; R, Red; W, White. Different letters indicate significant differences between treatment groups (P<0.05).

For the antioxidant capacity, under cyan light, the SOD and CAT activity of the crab was considerably reduced (P<0.05) (Figures 4A, B), while the T-AOC was elevated (Figure 4C). Crab under purple light had the lowest T-AOC activity (Figure 4C) and the group also had high H_2O_2 accumulation (P<0.05) (Figure 4D). In comparison to the cyan, green, and yellow light groups, the larvae exposed to purple and red light had higher levels of MDA (P<0.05) (Figure 4E).

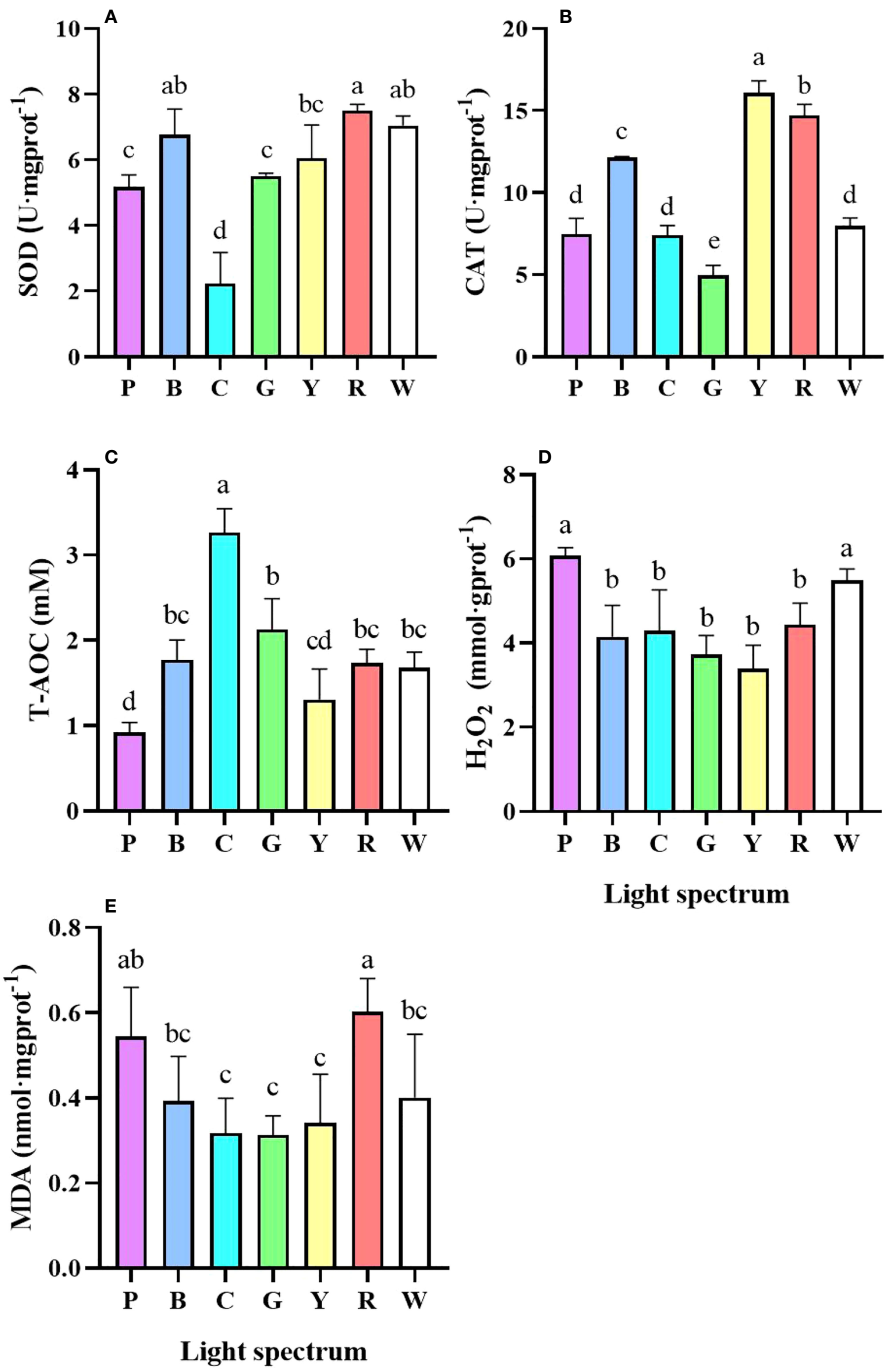

FIGURE 4
The effect of light spectrum on the antioxidant capacity of *P. trituberculatus* larvae (n = 6). **(A)** SOD, **(B)** CAT, **(C)** T-AOC, **(D)** H_2O_2, **(E)** MDA. P, Purple; B, Blue; C, Cyan; G, Green; Y, Yellow; R, Red; W, White. Different letters indicate significant differences (P<0.05).

Discussion

Light spectrum substantially influences the growth and molting of crustaceans (Wang et al., 2003; Guo et al., 2012; Fei et al., 2020). In the present study, the survival rate of larvae treated with cyan light in the current study was much greater than that of the other groups. The wavelength of cyan light was between blue and green light. Similarly, shortwave light as cyan and blue light also positively affected *S. paramamosain* growth performance (Chen et al., 2022). In contrast, the survival rate of larvae treated with red light was significantly lower. However, a previous study suggested that though the *P. trituberculatus* fed more frequently under blue light, those under yellow and red light had a better growth performance (Wang et al., 2014). This could be attributed to two reasons, i) in Wang's study, the crab used were subadult *P. trituberculatus*, and ii) the light source used Wang's study was fluorescent lamps, which has a wider light spectrum compared with LED (Kim et al., 2015).

The O: N ratio has been frequently used to identify the oxidative metabolic substrates (Dall and Smith, 1986). The catabolism of pure protein yields 3–16 O: N. High O:N denotes enhanced catabolism of lipids and carbonhydrate, 50–60 O: N denotes similar amounts of protein and lipids were used as metabolic substrates (Mayzaud and Conover, 1988). As the larvae developed, the OCR and the O: N continued to fall, indicates that the larvae's metabolic substrate shifted to protein. In the present study, a significant low O: N ratio of early larvae was observed under red light. The result suggests that the larvae mobilized more proteins during respiratory metabolism in under red light, leading to suboptimal survival rate and development.

Unsuitable lighting conditions have been reported to cause xidative stress in crustaceans (Chen et al., 2021; Dou et al., 2021; Xu et al., 2022a; Chen et al., 2022). In this study, crab under white, purple and red light showed not only high levels of SOD and CAT, but also higher H_2O_2 and MDA, indicating substantial oxidative stress over activated antioxidase. On the contrary, for cyan and green light, T-AOC activity of the crab was significantly increased, while MDA and H_2O_2 decreased. These results suggest the crab had a better adaptability to these spectrum.

Conclusion

In this research, we looked into how the light spectrum affected the growth, respiratory metabolism, and antioxidant capability of *P. trituberculatus* larvae. Larvae showed the best growth performance and lower oxidative stress damage under cyan light. On the contrary, yellow and red light resulted in a low survival rate by altering respiratory metabolism and antioxidant pathways. Overall, these findings demonstrated that cyan light was the optimal spectrum for larval *P. trituberculatus*.

Data availability statement

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

Author contributions

FZ, SZ and ZR: Investigation, Data curation, Visualization, Writing - original draft. CM, ChS, CW and YY: Review & editing, Funding acquisition. CeS: Conceptualization, Writing - review & editing, Project administration, Funding acquisition. All authors contributed to the article and approved the submitted version.

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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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.1071469/full#supplementary-material

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