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Effects of Light Intensity and Wavelength on the Phototaxis of the *Crassostrea gigas* (♂) and *Crassostrea sikamea* (♀) Hybrid Larvae

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Light sensitivity is important for marine benthic invertebrates, and it plays a vital role in the oysters settling. Generally, the emerging of eyespot is a signal of oyster larvae settling, while like most of the other coastal species, the oysters are threatened by artificial light pollution. *Crassostrea gigas* and *Crassostrea sikamea* are two oyster species naturally distributed in China, and their hybrids are potential material for oyster cross-breeding. Therefore, we investigated the phototaxis of hybrid eyespot larvae and eyeless larvae under different light intensities and wavelengths to uncover how light affects their behaviors. The results indicated that hybrid oyster larvae had positive phototaxis to specific light intensity and wavelength. We further concluded that 5 lx was the positive phototaxis light intensity for the eyeless hybrid larvae, and that the acceptable light intensity range of the eyespot hybrid larvae expanded to 5–10 lx, but no higher than 15 lx; besides, the hybrid larvae behaved negatively to the light over 25 lx. The present study also suggested the positive effects of green light on larvae gathering and the induction of red light on eyespot larvae settling. In conclusion, our study may contribute to the understanding of phototaxis of hybrid oyster larvae, as well as the further perspective of light pollution on benthic communities and coastal system restoration.

Keywords: phototaxis, light intensity and wavelength, eyespot larvae, RGB color model, hybrid oyster

INTRODUCTION

The environment has an attractive impact on the development of behavioral patterns, evolution, and morphology (Brown and Braithwaite, 2005). Light, as one of the most basic environmental factors, is the compelling one that has massive ecological functions. Generally, light is an energy signal that directly or indirectly affects the growth and metabolism of organisms. In the process of biological evolution, many animals have gradually formed a biological clock to the rhythmic changes of light that are largely mediated by the occurrence of the photosensitive organ (Naylor, 1999; Gaston et al., 2017; Hobbs et al., 2021).

In nature, animals have evolved various delicate light sensors to adapt to the environment. The pediveliger larva is the last pelagic phase before oysters settling, during which the oyster larvae develop foot and also a pigmented eyespot. Eyespot is the simplest animal eye that is composed of sensory cells and shading pigment cells (Jékely et al., 2008). Ultrastructural studies on the epidermal eyespots of *Microstomum lineare* and *Oncholaimus vesicarius* show that pigment cells envelop the processes of the sensory cells, which indicates that the pigmented eyespots have dual functions of photoreceptors and chemoreceptors (Burr and Burr, 1975; Palmberg et al., 1980). Eyespot structure is characteristic of the pelagic larvae for invertebrates in the ocean, though eyespots cannot form images but enable animals to sense the direction of light that mediates the phototaxis of larvae (Thorson, 1964; Jékely et al., 2008).

Light is an electromagnetic radiation that occurs over an extremely wide spectrum, ranging from 10^{-2} nanometers to meters. Visible light narrowly ranges from approximately 700 nm for red light to 400 nm for violet light in the broad spectrum. Light intensity represents the amplitude of light with the same wavelength, and light wavelength is a property of light that determines the colors of the light (Sliney, 2016). For most marine invertebrates, light can influence the development of their pelagic larvae. A previous study on the response of 141 different oceanic larvae to light suggested that 82% of these larvae respond positively to light, but too much light caused photonegative behaviors (Thorson, 1964). In terms of light wavelength, invertebrate larvae also have notable features of wavelength-dependent phototaxis (Kim et al., 2021). Baker and Mann (1998) suggested that planktonic invertebrates are usually unresponsive, or only weakly responsive, to long-wavelength lights, but the *Crassostrea virginica* larvae can respond to most of the visible lights.

As a mixture of visible light, ultraviolet, and infrared, sunlight is susceptible to plankton and dissolved organic matters in water, which results in a variety of light intensities and colors on different water layers (Blaxter, 1968). Previous studies indicated that the phototactic behaviors of plankton are highly related to the absorption pattern of different wavelengths by eyespot (Forward and Cronin, 1979; Marsden, 1988; Kim et al., 2018). It was reported that light wavelength ranging from 500 to 650 nm can be efficiently absorbed by the eyespot of *Crassostrea gigas* larvae, especially in the pigmented area (Kim et al., 2021). The RGB color is an additive color model of which the three primary colors (TPCs; red, green, and blue) are added together in various ways to reproduce a broad array of colors (Ibraheem et al., 2012). By this color model, computers can visualize what the human does in the hue and lightness of colors. According to the principle of liquid crystal display (LCD) processing, displays can emit 700 (red, R), 546.1 (green, G), and 435.8 nm wavelength (blue, B) (Trussell et al., 2005) that offers a reliable artificial light source for the zooplankton phototaxis research.

Recently, light pollution has sparked scientific interests in many ways, one of which is the negative effect on the biological processes of the marine environment. Although the negative impacts of artificial light have been reported in the marine environment, it remains largely unknown how marine organisms in coastal areas had been impacted (Davies et al., 2014; Bolton et al., 2017; O'Connor et al., 2019). As the dominant species in the intertidal zone, the oysters occupy a precise niche in the coastal ecosystem that is also a potential indicator of light pollution. Besides, the oyster is a worldwide aquaculture shellfish with high commodity value as a protein source. The wild hybrid of *C. gigas* and *Crassostrea sikamea* has been identified in Suncheon Bay, Korea (Hong et al., 2012) and the northern Ariake Sea, Japan (Hedgecock et al., 1999), where *C. gigas* and *C. sikamea* natively live together. Unsurprisingly, both *C. gigas* and *C. sikamea* are native species in China, and they are complementary to each other in ecological habits (Wang et al., 2013). As a potential aquaculture variety, the biological characteristics of *C. gigas* and *C. sikamea* hybrids need to be further explored (Gaffney and Allen, 1993; Xu et al., 2019).

In this study, we firstly investigated the phototaxis of the *C. sikamea* and *C. gigas* hybrid larvae in terms of visible light intensity and light wavelength. Then, we analyzed the relationship between computerized TPCs and the phototaxis of hybrid larvae by the pathway analysis.

MATERIALS AND METHODS

Eyespot Larvae Recruitment

The *C. gigas* and *C. sikamea* were collected from oyster farms located in Rushan, Shandong (36.41°N, 121.36°E) and Beihai, Guangxi (21.46°N, 109.39°E), respectively, in China. Oyster broodstocks were collected before March 2019, then identified by restriction fragment length polymorphism (RFLP)–PCR, and spawned in May 2019. Indoor maturation was deployed to ensure the synchronization of gametes. The hybridization can only be complete between *C. sikamea* eggs and *C. gigas* sperms, but not the opposite way (Banks et al., 1994; Xu et al., 2019).

Daily management of the hybrid larvae referenced to the procedure described by Xu et al. (2019). In brief, the fertilized eggs were hatched for 20 h, and hybrid larvae were reared in sand-filtered seawater and initially fed with *Isochrysis galbana*; when the mean shell height of hybrid larvae exceeded 120 μm, a mixture of *I. galbana* and *Platymonas subcordiformis* was added. In the experiment, the eyespots of hybrid larvae emerged around day 24.

Light Source Calibration

Calibrating of Light Intensity and GMTPC

The light source images were built by Adobe Photoshop (PS; Adobe Inc., Delaware, United States; **Figure 1A**) based on geometric mixed TPCs (GMTPC). The default quantized value of R/G/B (255/255/255) was defined as 100%, then graded the GMTPC image by 10%, and measured the light intensity of each gradient. After that, the fitting formula was calculated statistically (**Table 1**).

Calibrating of Light Intensity With TPCs and GMSCs

The light source images based on TPCs and geometric mixed secondary colors (GMSCs) were also built by PS. For the TPCs, we defined the default quantized values of R = 255, G = 255, and B = 255 as 100%; similarly, the GMSCs quantized in

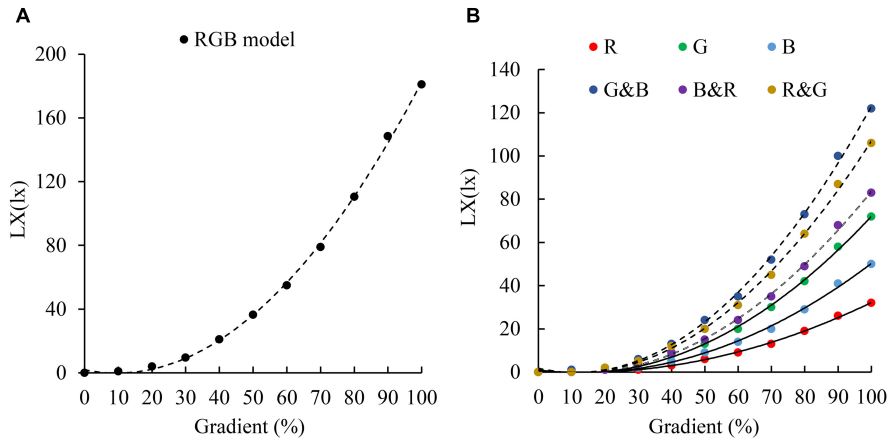

FIGURE 1 | Light intensity adjustment for RGB color model on TFT-LCD display. **(A)** The quadratic fitting line of light intensity and GMTPC gradients. **(B)** The quadratic fitting lines of light intensity with TPCs and GMSCs gradients.

TABLE 1 | The fitting formula of light intensity with the different gradients of GMTPC, TPC, and GMSC.

Color index		Quadratic fitting formula	R^2
GMTPC		$Y_{GMTPC} = 0.0223X^2 - 0.4280X + 1.9091$	$R^2 = 0.9991$
TPCs	Red	$Y_{(R)} = 0.0042X^2 - 0.1079X + 0.5455$	$R^2 = 0.9979$
	Green	$Y_{(G)} = 0.0094X^2 - 0.2282X + 1.0280$	$R^2 = 0.9987$
	Blue	$Y_{(B)} = 0.0067X^2 - 0.1742X + 0.8811$	$R^2 = 0.9975$
GMSCs	Green and blue	$Y_{(G\ and\ B)} = 0.0135X^2 - 0.2935X + 1.2168$	$R^2 = 0.9986$
	Blue and red	$Y_{(B\ and\ R)} = 0.0107X^2 - 0.2491X + 1.0280$	$R^2 = 0.9986$
	Red and green	$Y_{(R\ and\ G)} = 0.0135X^2 - 0.2935X + 1.2168$	$R^2 = 0.9987$

R^2 represents the coefficient of determination, which reflects the proportion of all the variation that can be explained by the regression relationship, similarly hereinafter.

R/G = 255/255, G/B = 255/255, and B/R = 255/255 were defined as 100%, then graded the TPC and GMSC images by 10%, and measured the light intensity. After that, the fitting formulas were calculated statistically (**Figure 1B** and **Table 1**).

Experimental Design

This study was deployed in a dark room, and each experiment was repeated three times. The hybrid larvae were collected at day 26 with a 200-micrometer sieve with a mean shell height of 307.73 ± 29.63 μm. In this condition, we obtained a mixture of hybrid eyespot larvae and eyeless larvae, of which the eyespot can be easily distinguished under a microscope. Hybrid oyster larvae were placed and aerated in a 20-liter bucket as a larvae pool, and the larvae density was approximately 57.5 ± 8.0 ind/ml. The first and second phases were light intensity experiments. The first phase was set to determine the lighting time and narrow the range of light intensity. The second phase was set to find the specific intensity light that hybrid larvae positively respond to. Based on the above results, the third phase explored the phototaxis of hybrid larvae to various wavelengths at a certain intensity.

The Photosensitivity Experiment of Larvae to Light Intensity

In terms of the first phase, light intensities were graded at 25/50/75/100 lx, and the quantized RGB values were calculated by the fitting formula "Y_{GMTPC}" in **Table 1**, then converting parameters into images by PS (**Figure 2B** and **Table 2**). After that, 300 ml of larvae was transferred into a Petri dish from the larvae pool, then stirred, and left for 5–10 min to ensure that the hybrid larvae were evenly distributed in the Petri dish. Turning on the display, hybrid larvae were sampled after 30 and 60 min, respectively.

The range of light intensity was narrowed to 0–25 lx in the first experiment. Thus, the light intensities in the second phase were graded at 0/10/15/20/25 lx, and the quantized RGB values are calculated by the fitting formula "Y_{GMTPC}" in **Table 1**, then converting parameters into images by PS (**Figure 2C** and **Table 2**). The hybrid oyster larvae were only sampled after 60 min of lighting in this section. All larvae were distinguished and counted under a microscope.

The Photosensitivity Experiment of Larvae to Different Wavelengths of Light

The second experiment finalized the optimum light intensity as 5 lx. In terms of the third phase, the TPCs and GMSCs were fixed, and the quantized RGB values were calculated by the fitting formulas in **Table 1**. The fixed parameters were converted into images by PS (**Figure 2D** and **Table 3**). The hybrid larvae were deployed and transferred in the same way as that of section "The

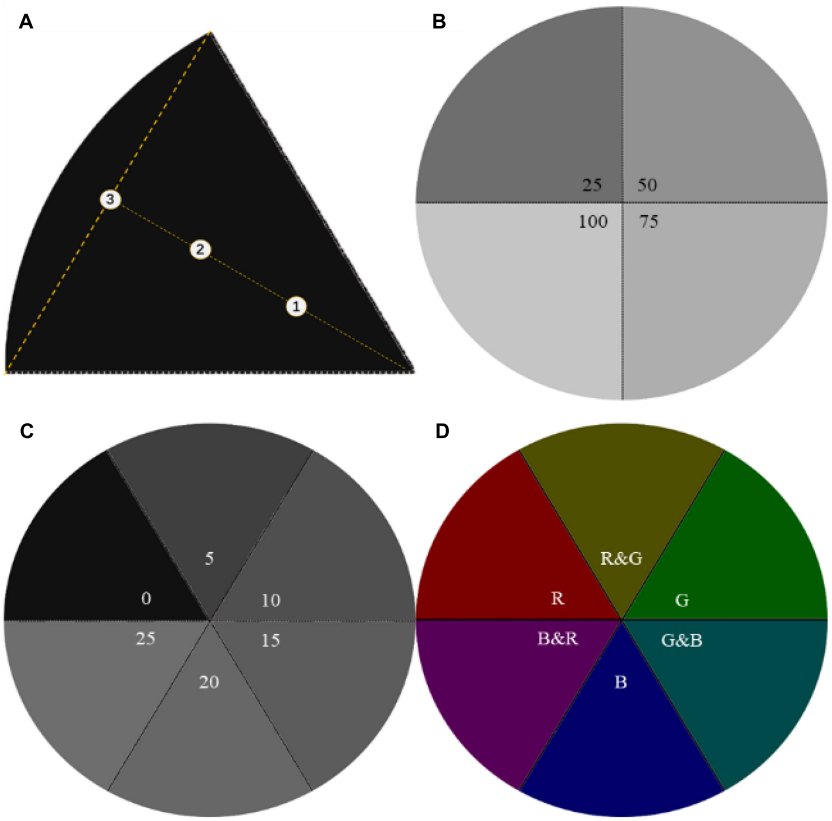

FIGURE 2 | Sampling points and light source images. **(A)** The markers ①②③ are the sampling points on the centerline in a sector, and ①② is the trisection of the vertical. **(B)** Light intensity of GMTC at 25/50/75/100 lx in the first phase. **(C)** Light intensity of GMTC at 0/5/10/15/20/25 lx in the second phase. **(D)** The three primary colors (TPCs) and the geometric mixed secondary colors (GMSCs) at 5 lx in the third phase.

Photosensitivity Experiment of Larvae to Light Intensity." Then, the display was turned on and the larvae were sampled after 60-minute lighting. All larvae were distinguished and counted under a microscope.

Data Collection

The lights were emitted by light-emitting diodes (LEDs) inside of the thin-film transistor-liquid crystal display (TFT-LCD, B156HAN02.1; AU Optronics Co., China), RGB (vertical strip), maximum brightness (250 cd/m^2), contrast (800:1), and gamma value (2.2); light intensity was measured by a smart sensor photometer (AS813; Arco Electronics Ltd., China) in lux, with an accuracy of ± 5%rdg; the size of the Petri dish is φ1,500 mm, with a depth of 31 mm, and made of borosilicate; larvae were sampled with 1,000 μl Eppendorf pipettes in the middle layer of water. Three sampling points were set in each sector, where points were located on the centerline of equal intervals (**Figure 2A**).

TABLE 2 | The gradient and quantized RGB values of fixed light intensity for the first phase and second phase.

Color index	Phase	LX (lx)	Gradient (%)	Quantized value of R/G/B
GMTPC	First	25	43	110/110/110
		50	57	145/145/145
		75	68	174/174/174
		100	77	197/197/197
	Second	0	7	18/18/18
		5	25	64/64/64
		10	31	79/79/79
		15	36	92/92/92
		20	40	103/103/103
		25	43	110/110/110

Statistical Analysis

Normality and homogeneity of variance assumptions for statistical data were tested using the Shapiro–Wilk and Bartlett tests, respectively (with R package "car" version 3.0-8). A: Permutational multivariate analysis of variance (PERMANOVA) with 1,000 permutations per analysis based on Euclidean distance was performed to evaluate the changes in larvae distribution and proportion after light stimulation, and PERMANOVA pairwise comparison tests were run if necessary (Anderson, 2014). PERMANOVA was performed with R package "vegan 2.5-7" version 4.0.3. B: One-way analysis of variance (ANOVA) was carried out to test the difference in larvae counts between sectors. Where significant effects were found, Duncan's new multiple

TABLE 3 | The gradient and quantized RGB values of the TPCs and the GMSCs at 5 lx for the third phase.

Phase	Color index	Color/wavelength	LX (lx)	Gradient (%)	Quantized value of R/G/B
Third	TPCs	Red/700 nm	5	48	122/0/0
		Green/546.1 nm	5	36	0/92/0
		Blue/435.8 nm	5	41	0/0/105
	GMSCs	Red and green/700 and 546.1 nm	5	34	79/79/0
		Green and blue/546.1 and 435.8 nm	5	31	0/74/74
		Blue and red/435.8 and 700 nm	5	29	87/0/87

range comparison was performed to compare the difference between groups with R package "agricolae" version 1.3-3. C: Path analysis (Gauss–Doolittle algorithm) was run to analyze the coefficient of light wavelength to larvae phototaxis with R package "agricolae" version 1.3-3. D: Pearson concordance index analysis was performed to analyze the correlation between larvae distribution and TPCs with R package "agricolae" version 1.3-3. Computations were running in R version 4.0.3 (64 bit) for Windows, and all statistical analyses were carried out at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

How Does Light Intensity Affect the Phototaxis of Hybrid Oyster Larvae?

The results showed that both distributions of eyespot and eyeless hybrid oyster larvae were significantly affected by light at 25/50/75/100 lx with time ($p < 0.001$). The distribution change of total larvae and eyespot larvae could be explained at a relatively high level by time ($R^2 = 0.968$ and $R^2 = 0.922$, respectively). The subsequent PERMANOVA pairwise comparisons test showed that the *F*-value for the comparison of 0 and 60 min was greater than that of the other two comparisons, indicating that the polarized level of total larvae and eyespot larvae distribution was increased at 60 min; on the contrary, the level of polarized distribution for eyeless larvae was reduced (**Table 4**). Similar to the first phase, the distribution of total larvae, eyespot larvae, and eyeless larvae changed significantly under 0/5/10/15/20/25 lx in the second experiment ($p < 0.01$). The *F*-value showed that the distribution of eyespot larvae changed visibly, and *F*-values were ordered as $F_{(totallarvae)} > F_{(eyespotlarvae)} > F_{(eyelesslarvae)}$ (**Table 5**). The changes in larvae distribution suggested that hybrid larvae were sensitive to light, and that the response could be traced within only 30 min and intensified later.

For the first phase, massive larvae with eyespot moved to the area at 50 lx light after 30 min where eyespot larvae density was significantly higher than the other sectors. However, there was no significant difference in the distribution of eyeless larvae compared between the 25 lx sector, 50 lx sector, and 75 lx sector. In terms of total larvae, when the lighting time extended to 60 min, the density of total larvae amounted to 205.0 ± 17.5 and 224.0 ± 10.0 (per 1.5 ml) in the 25 lx sector and 50 lx sector, respectively, resulting in no significant difference between the two sectors (**Table 6** and **Figure 3A**). As the extension of lighting time, there was no significant difference in the distribution of eyespot larvae between the 25 lx sector and 50 lx sector ($p > 0.05$; **Table 6**). The results showed that the density of eyeless larvae was positively correlated with light intensity in the 30-min group (except for the 100 lx sector); interestingly, the correlation was reversed in the 60-minute group (**Figure 3A**).

In general, zooplankton will migrate to the depth layer during daylight to avoid the threats of visual predation and surfacing at night to feed (Medcof, 1955; Forward, 1988; Hobbs et al., 2021); thus, many of them evolved to adapt to low light environments and cannot endure high-intensity light. Kim et al. (2014) found that reproduction of *Brachionus plicatilis* will be negatively affected by light over 0.5 W/m^2, and Tielmann et al. (2017) reported that larval *Sander lucioperca* had significantly higher natural mortality when reared under a light intensity of 2,500 lx. Similarly, in the present study, in terms of total larvae when the light intensity was over 25 lx and lighting time exceeded 30 min, oyster larvae would move to the weakest light area that was the evidence of negative phototaxis.

In the second phase, the 5 lx light sector gathered the most larvae of 192.0 ind (±23.5 ind per 1.5 ml), which was significantly higher than the other light intensities ($p < 0.01$); the highest density of eyespot larvae was sampled in the 10 lx sector (118.0, ± 4.0 ind per 1.5 ml), and there was no significant difference from the 5 lx sector; eyeless larvae density under the 5 lx light (87.5, ± 32.5 ind per 1.5 ml) was also significantly higher than the other intensities ($p < 0.01$; **Table 7** and **Figure 3B**). Based on the above results, we gave the following conjecture and interpretation that hybrid oyster larvae had positive phototaxis to a certain range of light intensity, but it could reverse to be negative if the light intensity exceeded a critical intensity.

TABLE 4 | PERMANOVA pairwise comparison analysis for the distribution difference in eyespot larvae, eyeless larvae, and total larvae of the hybrid oyster with time.

Metric	Pairwise	*F*-value	R^2	*p*-value	*p*-Adjusted A
Eyespot larvae	0 vs. 30 min	75.270	0.883	0.002	0.006
	30 vs. 60 min	23.628	0.703	0.003	0.009
	0 vs. 60 min	189.146	0.950	0.003	0.009
Eyeless larvae	0 vs. 30 min	26.087	0.723	0.001	0.003
	30 vs. 60 min	17.703	0.639	0.002	0.006
	0 vs. 60 min	16.428	0.622	0.002	0.006
Total larvae	0 vs. 30 min	167.915	0.944	0.001	0.003
	30 vs. 60 min	56.417	0.849	0.002	0.006
	0 vs. 60 min	1439.290	0.993	0.004	0.012

TABLE 5 | PERMANOVA analysis for the distribution difference in eyespot larvae, eyeless larvae, and total larvae of the hybrid oyster with time.

Phase	Pairwise	Metric	Sum of sq.	Mean sq.	*F*-value	R^2	*p*-value A
First	0 vs. 30 vs. 60 min	Eyespot larvae	0.397	0.198	88.299	0.922	0.001***
		Eyeless larvae	0.637	0.318	19.816	0.725	0.001***
		Total larvae	0.391	0.195	229.530	0.968	0.001***
Second	0 vs. 60 min	Eyespot larvae	0.355	0.355	231.490	0.959	0.002**
		Eyeless larvae	0.319	0.319	9.441	0.486	0.003**
		Total larvae	0.286	0.286	181.020	0.948	0.003**

*R^2 represents the degree to explain the differences among groups. Metrics marked with asterisks significantly differed (Signif. codes: 0, ***; 0.001, **; 0.01), similarly hereinafter.*

A previous study inferred that the invertebrate larvae had positive phototaxis to light at a low intensity and negative phototaxis to light at a high intensity (Forward, 1976), which was in conjunction with our findings. On this basis, we had a further conclusion that 5 lx intensity light was the positive phototaxis light for eyeless hybrid larvae, and that the optimal intensity for eyespot larvae was between 5 and 10 lx, but no higher than 15 lx. In a nutshell, the presumed optimal light intensity ranged from 0 to 15 lx for hybrid oyster larvae, and the light over 25 lx might cause negative phototaxis.

How Do Light Wavelengths (TPCs) Affect the Phototaxis of Hybrid Oyster Larvae?

Previous studies suggested that the sensitivity wavelength of zooplankton could be predicted by the efficient absorbance of light wavelengths by eyespot (Kim et al., 2013, 2014, 2018). The eyespot of *C. gigas* had a higher absorbance in the range of visible light, from 500 to 650 nm, and the highest absorbance of wavelength was at 620 nm (Kim et al., 2021), which meant that oyster larvae were more sensitive to green light (wavelength at 546.1 nm) among TPCs. In the present study, the PERMANOVA analysis of the third experiment showed that light wavelength could significantly affect the distribution of hybrid oyster larvae under the same light intensity ($p < 0.01$). Differences in the distribution of eyespot larvae, eyeless larvae, and total larvae could be highly explained by time changes ($R^2 = 0.986$, $R^2 = 0.989$, and $R^2 = 0.995$, respectively). Besides, the PERMANOVA analysis also showed that the number of attached larvae was significantly related to light wavelength ($p < 0.01$; **Table 8**). The above results further proved that hybrid oyster larvae were sensitive to light wavelength.

TABLE 6 | The density distribution of eyespot larvae, eyeless larvae, and total larvae of the hybrid oyster with time and light intensity (25/50/75/100 lx).

Metric	LX (lx)	Time		Δ
		30 min	60 min	
Eyespot larvae	25	105.0 ± 9.5^{b}	141.5 ± 6.5^{a}	36.5
	50	131.0 ± 11.0^{a}	167.0 ± 28.5^{a}	36.0
	75	81.0 ± 18.0^{c}	52.0 ± 6.0^{b}	−28.5
	100	8.5 ± 2.0^{d}	2.5 ± 1.5^{c}	−6.0
	p-Value B	$7.74e^{-06}$	$3.03e^{-06}$	
Eyeless larvae	25	57.0 ± 43.5^{a}	63.5 ± 7.0^{a}	6.0
	50	74.0 ± 10.0^{a}	57.0 ± 39.0^{a}	−17.0
	75	93.0 ± 14.5^{a}	25.0 ± 7.5^{ab}	−68.0
	100	1.0 ± 1.5^{b}	3.0 ± 2.0^{b}	1.5
	p-Value B	0.00724	0.0201	
Total larvae	25	162.0 ± 38.0^{b}	205.0 ± 17.5^{a}	43.0
	50	205.0 ± 11.0^{a}	224.0 ± 10.0^{a}	19.0
	75	174.0 ± 6.0^{ab}	77.0 ± 3.5^{b}	−97.0
	100	10.0 ± 1.0^{c}	5.5 ± 0.5^{c}	−4.0
	p-Value B	$9.87e^{-06}$	$3.74e^{-10}$	

The density data show the number of eyespot larvae, eyeless larvae, and total larvae per 1.5 ml. "Δ" represents the difference in the number of larvae between the 30-min group and the 60-min group.

The absorption of visible light in aquatic environments led to the common assumption that aquatic organisms sensed and adapted to penetrative blue/green light but little or no response to red light (Fortunato et al., 2016). Coincidentally, the green light patch and the green-blue light patch gathered the most eyespot larvae and eyeless larvae in the study, which was significantly higher than the other patches ($p < 0.01$). The eyespot larvae were significantly gathered by red-green light but less than that of green light and green-blue light ($p < 0.01$), and eyeless larvae were also relatively highly gathered in the red-green patch. These results indicated that green light caused positive phototaxis of hybrid oyster larvae. Surprisingly, the settling data suggested that though the red-green patch did not gather the most larvae, it collected a massive amount of settled larvae (20.0, ± 3.5 ind), which were significantly higher than the other patches (**Table 9**). Thus, we gave a bold assumption that red light had the function of settling induction to eyespot larvae, and that green light had a certain attraction to both eyespot larvae and eyeless larvae resulting in the largest number of hybrid larvae settled onto the red-green patch. The promotion of red light on eyespot larvae settling was also proposed in the study of *C. gigas* (Kim et al., 2021). Recently, four rhodopsin-like superfamily genes were identified in the genomic study of *C. gigas* (Wu et al., 2018), which provided us with another perspective on it.

The relationship between TPCs (red, green, and blue) and hybrid larvae distribution was deployed by path analysis (**Table 10**). The result showed that green light and red light could be two key points to the distribution of eyespot larvae and eyeless larvae ($p < 0.01$). Besides, red light and blue light were significantly correlated with the settling of oyster larvae ($p < 0.05$).

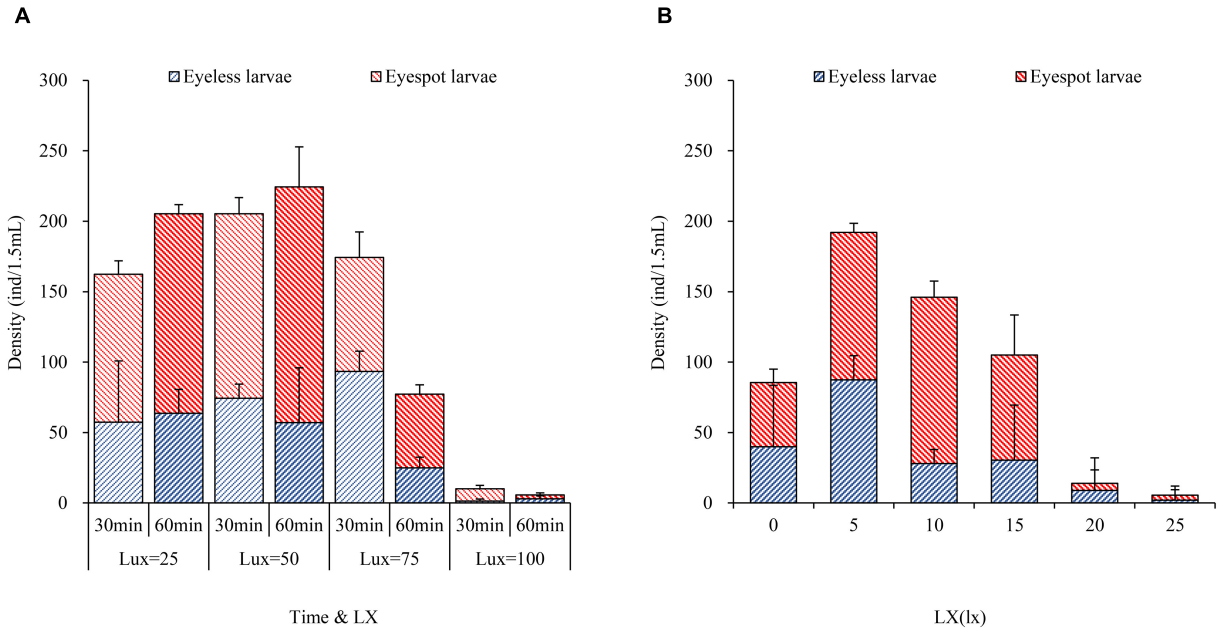

FIGURE 3 | The bar plots of hybrid oyster larvae distribution to light intensity. **(A)** The density of eyespot larvae and eyeless larvae with time and light intensity. **(B)** The density of eyespot larvae and eyeless larvae with light intensity.

The direct-acting and indirect-acting coefficients of red light on total larvae distribution were negative ($P_{0.j} = -0.50$, $P_{i.j} = -0.17$), but positive for green light ($P_{0.j} = 0.48$, $P_{i.j} = 0.29$), and total larvae distribution was poorly determined by blue light. In terms of eyespot larvae, the distribution was positively related to green light ($P_{0.j} = 0.51$), and red light had a negative attraction on them ($P_{0.j} = -0.66$). Similar to eyespot larvae, red light and green light had opposite effects on eyeless larvae ($P_{0.j} = -0.68$, $P_{0.j} = 0.74$; $p < 0.01$; **Table 10**).

In terms of larvae settling, green light had no significant effect on hybrid larvae settling, but red light was positively related to it, which meant that red light might be a factor in inducing hybrid oyster settling. Besides, blue light was negatively related to larvae settling, and the direct-acting coefficient on larvae settling was −0.37, which suggested that blue light could be an inhibiting factor to eyespot larvae settling ($p < 0.01$, **Table 10**). Oyster spat collection is an important process of oyster seedling (Taylor et al., 1998; Funo et al., 2019; Poirier et al., 2019), while oyster spat collection is unstable in the wild, as it is largely dependent on environmental situation (Absher, 2016). Many studies on oyster aquaculture had tried to enhance spat collection by developing tools (Holliday et al., 1993; Devakie and Ali, 2002; Buitrago and Alvarado, 2005; Arini and Jaya, 2011). Our findings demonstrated that red light had the function of inducing oyster larvae settling and green light had a certain attraction to oyster larvae that could be a guideline in oyster spat collection.

TABLE 7 | The density distribution of eyespot larvae, eyeless larvae, and total larvae of hybrid oysters under 0, 5, 10, 15, 20, and 25 lx light.

LX (lx)	Eyespot larvae	Eyeless larvae	Total larvae
0	45.5 ± 6.5^{c}	40.0 ± 14.5^{b}	85.5 ± 10.5^{c}
5	104.5 ± 9.0^{a}	87.5 ± 32.5^{a}	192.0 ± 23.5^{a}
10	118.0 ± 4.0^{a}	28.0 ± 14.5^{b}	146.5 ± 11.0^{b}
15	74.5 ± 16.5^{b}	30.5 ± 29.0^{b}	105.0 ± 12.5^{c}
20	5.0 ± 2.5^{d}	9.0 ± 4.0^{b}	14.0 ± 1.5^{d}
25	3.5 ± 2.0^{d}	2.0 ± 0.0^{b}	5.5 ± 2.0^{d}
p-Value B	$2.16e^{-09}$	0.00275	$2.27e^{-09}$

On a global scale, the oyster is distributed widely in tropical and temperate waters, predominantly coastal, and occupying the intertidal estuaries, marshes, and bays (Bayne, 2017). Oyster reefs provide important habitat for a marine animal assemblage that is both ecologically interesting and important to the estuarine food web (Luckenbach et al., 1999; Baggett et al., 2015)9). In the past century, the extent and intensity of nighttime illumination have dramatically increased such that it has substantial effects on the biology and ecology of species in the wild (Cinzano et al., 2001; Longcore and Rich, 2004), resulting in coastal habitats adjacent to populated areas becoming particularly vulnerable to light pollution (Gaston et al., 2013; Tamir et al., 2017). Considering the conclusion of the present study, light intensity and wavelength could be a potential tool for coastal habitat restoration, due to the function of specific light on oyster larvae gathering and settling induction. Surely, more studies need to be done for the comprehensive assessment of artificial light and coastal ecosystems.

CONCLUSION

To our knowledge, the present research firstly reported the impacts of light intensity and artificial TPCs on the phototaxis

TABLE 8 | PERMANOVA analysis for the distribution difference in eyespot larvae, eyeless larvae, and total larvae and the number of attached larvae of hybrid oysters with time.

Phase	Pairwise	Metric	Sum of sq.	Mean sq.	*F*-value	R^2	*p*-Value A
Third	0 vs. 60 min	Eyespot larvae	0.898	0.898	711.510	0.986	0.001
		Eyeless larvae	0.845	0.845	912.850	0.989	0.002
		Attached larvae	0.158	0.158	23.416	0.701	0.004
		Total larvae	0.878	0.878	2121.300	0.995	0.007

TABLE 9 | The density distribution of eyespot larvae, eyeless larvae, and total larvae and the number of attached larvae of the hybrid oyster to each TPC and the GMSCs patch (LX = 5 lx).

RGB color	Eyespot larvae	Eyeless larvae	Attached larvae (per patch)	Larvae
Red	6.0 ± 2.0^{c}	6.5 ± 0.5^{b}	13.0 ± 3.5^{b}	12.5 ± 2.5^{c}
Red and green	16.0 ± 2.5^{b}	7.5 ± 5.0^{b}	20.0 ± 3.5^{a}	23.5 ± 7.0^{b}
Green	112.0 ± 4.5^{a}	74.0 ± 2.5^{a}	7.0 ± 1.0^{c}	186.0 ± 4.0^{a}
Green and blue	108.0 ± 11.5^{a}	72.5 ± 4.0^{a}	2.0 ± 1.5^{d}	181.0 ± 7.5^{a}
Blue	2.5 ± 1.0^{c}	6.0 ± 1.5^{b}	10.0 ± 3.0^{bc}	8.5 ± 2.5^{c}
Blue and red	5.0 ± 1.0^{c}	2.5 ± 2.5^{b}	7.0 ± 1.5^{c}	7.5 ± 1.5^{c}
p-Value B	$4.63e^{-12}$	$9.35e^{-13}$	$3.49e^{-05}$	$4.41e^{-15}$

TABLE 10 | The path analysis for the distribution difference in eyespot larvae, eyeless larvae, and total larvae and the number of attached larvae of the hybrid oyster to the TPCs.

Metric C		Correlation coefficient ($r_{0.j}$) D	Direct-acting ($P_{0.j}$)	$r_{0.j} = P_{0.j}$	Indirect-acting ($P_{i.j}$)			
					Red	Green	Blue	Σ
Eyespot larvae	Red	−0.66**	−0.47	0.3113		−0.23	0.04	−0.19
	Green	0.78***	0.51	0.4007	0.21		0.05	0.27
	Blue	−0.18	−0.11	0.0203	0.18	−0.25		−0.07
Eyeless larvae	Red	−0.68**	−0.56	0.3807		−0.18	0.06	−0.12
	Green	0.74**	0.41	0.3019	0.25		0.08	0.33
	Blue	−0.15	−0.17	0.0250	0.21	−0.20		0.02
Attached larvae	Red	0.54*	0.38	0.2060		0.02	0.14	0.16
	Green	−0.03	−0.04	0.0011	−0.17		0.18	0.01
	Blue	−0.50*	−0.37	0.1865	−0.14	0.02		−0.13
Total larvae	Red	−0.67**	−0.50	0.3369		−0.22	0.05	−0.17
	Green	0.77***	0.48	0.3706	0.23		0.06	0.29
	Blue	−0.17	−0.13	0.0221	0.19	−0.23		−0.04

"$r_{0.j} \times P_{0.j}$" represents the contribution of x_j to R^2. Metrics marked with asterisks "" indicates a significant correlation between color and larvae distribution, and "*" represents $p < 0.05$; "**" represents $p < 0.01$; and "***" represents $p < 0.001$.*

of the *C. gigas* (♂) and *C. sikamea* (♀) hybrid larvae. The result showed that the hybrid oyster larvae had positive phototaxis to specific light intensity and wavelength, and that this ability was not a unique characteristic of eyespot larvae, which was also observed in eyeless larvae. When the eyespot appeared, the acceptable light intensity range of the hybrid larvae was expanded. The present study also suggested the potential positive effects of the green light on oyster larvae gathering and settling induction of the red light. In conclusion, our study may contribute to the understanding of the phototaxis of hybrid oyster larvae, as well as the further perspective of light pollution on the benthic communities and coastal system.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

XuZ, CF, and ZW gathered, analyzed and interpreted data, discussed the results and co-wrote the manuscript. XiZ, QL, and YL contributed constructively ideas for the work. All authors contributed to the article and approved the submitted version.

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