

EFFECTS OF DIFFERENT LIGHT SPECTRA ON THE GROWTH PERFORMANCE AND SURVIVAL OF LARGEMOUTH BASS (*MICROPTERUS SALMOIDES*) LARVAE IN RECIRCULATING AQUACULTURE SYSTEMS



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ABSTRACT. *The effects of light-emitting diode (LED) spectra on the survival rate and growth performance of largemouth bass in the nursery stage were explored in this study. By means of recirculating aquaculture systems, largemouth bass larvae (initial body length of 6.5 ± 0.23 mm) were reared under four different light spectra: blue (half-peak bandwidth = 429 to 450 nm), green (half-peak bandwidth = 503 to 535 nm), white ($320 < \lambda < 950$), and full spectrum. After the 16-day experiment, the results showed that the survival rate was highest for the larvae in the full spectrum group ($33.5\% \pm 3.5\%$) and lowest for the larvae in the blue light group ($11.6\% \pm 0.7\%$). The final body lengths of the larvae under different light spectra was as follows: the full spectrum group had the longest average body length (12.4 ± 0.3 mm), followed by the white light group and the green light group, and the larvae in the blue light group had the shortest average body length (12.1 ± 0.4 mm). The dorsal and anal fins of the larvae differentiated fastest in the full spectrum group, and the caudal fins differentiated fastest in the green light group. The maximal and minimal malformation rates of the larvae occurred under white light ($26.7\% \pm 0.2\%$) and full spectrum light ($16.7\% \pm 0.9\%$), respectively. The results demonstrated that a full spectrum light environment can improve the survival of largemouth bass larvae and promote their growth and development.*

Keywords. *Growth performance, Light spectrum, Micropterus salmoides, Recirculating aquaculture system, Survival rate.*

The largemouth bass is native to North America (Zhang and He, 1994). Due to its strong adaptability, fast growth, and tasty meat, the largemouth bass was introduced into China in the late 1970s (Cai et al., 2011). According to the latest data in the China Fishery Statistical Yearbook (China, 2018), the production of largemouth bass in 2017 reached 456,900 tons, an increase of 109,600 tons compared with the production of 347,300 tons in 2016 and the highest increase among freshwater fish, reaching 31.57%. The largemouth bass is praised as the “fifth major Chinese carp” after the four major Chi-

nese carps (black carp, grass carp, silver carp, and bighead carp). Currently, largemouth bass larvae are usually nursed in open-water environments, such as ponds. This farming model makes the larvae susceptible to weather. Due to the difficulty in regulating the water quality and the frequent occurrence of diseases, the survival rate of larvae is generally low. For these reasons, some largemouth bass producers have turned to recirculating aquaculture systems (RAS) (Park et al., 2015).

Artificial illumination is an important environmental factor in industrial RAS. To design a light environment that is suitable for larval survival and development, it is necessary to understand the physical and biological factors affecting larval development (Downing and Litvak, 1999). The relevant reported studies have demonstrated that the light spectrum, light intensity, and photoperiod are crucial for fish farming, and the light needs of fish vary with different growth stages. (Bonvini et al., 2016; Chi et al., 2017; Villamizar et al., 2011).

In the propagation of light in water, the light energy attenuates, and the spectral components are inconsistent at different depths. As the depth increases, long-wavelength (red) light is consumed due to its lower energy, and short-wavelength (blue) light becomes dominant (Myrberg and Fuiman, 2002; Song et al., 2013). The light requirements in fish habitats vary with fish species, and different spectral components affect the growth and development of fish. For exam-

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ple, barfin flounder (*Verasper moseri*) grow faster under short-wavelength blue light (Yamanome et al., 2009). Luchiaro et al. (2009) showed that long wavelengths can improve the growth performance of zander (*Sander lucioperca*) juveniles, and their feeding rate and feed conversion efficiency were higher than with short wavelengths. Head and Malison (2000) pointed out that different spectral components significantly affect the growth performance of yellow perch (*Perca flavescens*). Blue light had a significant negative effect on the growth of rainbow trout (*Oncorhynchus mykiss*), accompanied by a decrease in total lipids and plasma glucose in the liver (Karakatsouli et al., 2007). This finding is consistent with the results of Wang et al. (2003), who reported that Chinese shrimp (*Fenneropenaeus chinensis*) were active in feeding under blue light and had a large feed intake. However, due to the low feed conversion rate, the specific growth rate under blue light was low. The number and sensitivity of visual pigment cells of fish living in different environments may be an evolutionary response to their habitat.

In addition to the light spectrum, the light intensity also plays an important role in fish growth. For fish that rely on vision for hunting, the effects of different light intensities on their feeding and growth may be more important than the effects of different spectral components (Zhou et al., 2000). Some studies pointed out that rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), and orange-spotted grouper (*Epinephelus coioides*) grew faster under high light intensity (Taylor et al., 2005; Oppedal et al., 2003; Wang et al., 2013). The light intensity that supports normal growth and development of fish is far beyond the minimum required light intensity threshold (Boeuf and Le Bail, 1999). However, some studies have found that high light intensity can damage the retinas of fish (Vera and Migaud, 2009), which is harmful to the growth performance of fish. Therefore, under low light intensity, starry flounder (*Platichthys stellatus*) and haddock (*Melanogrammus aeglefinus*) had better growth performance (Bögnér et al., 2018; Downing and Litvak, 1999).

The photoperiod is also an important factor affecting the growth performance of fish. Some fish, such as red sea bream (*Pagrus major*), largemouth bass (*Micropterus salmoides*), and striped trumpeter (*Latris lineata*) grew faster and had higher survival rates under a long photoperiod (Biswas et al., 2005; Petit et al., 2003; Trotter et al., 2003), while green abalone (*Haliotis fulgens*), which is active at night, had the highest growth rate under a short photoperiod (García-Esquivel et al., 2007). Aquatic organisms may have an optimal photoperiod for growth; however, this optimal photoperiod remains to be further explored.

The effects of light on fish are quite different for different fish species (Puvanendran and Brown, 2002; Trotter et al., 2003; Yoseda et al., 2008). However, few studies have reported the effects of light spectra on the growth and development of largemouth bass, especially in controllable RAS. Moreover, in previous studies, the light spectrum and intensity above the water surface were usually measured as the experimental conditions, ignoring the fact that the diffusion and attenuation of light in water are not consistent with light in air. This phenomenon leads to a disparity in the light in-

tensity as well as the light spectrum above and below the water surface. Therefore, with the condition that the underwater intensity of each light source is the same, this experiment was designed to evaluate the effects of light spectra on the survival and growth of largemouth bass in the nursery stage.

MATERIALS AND METHODS

ANIMAL RIGHTS STATEMENT

All experimental protocols in this study were approved by the Committee for the Care and Use of Animals of Zhejiang University. The methods used in this study were carried out in strict accordance with the guidelines of the Association for the Study of Animal Behavior Use of Zhejiang University.

LARVAE REARING

The experiment was performed in the indoor RAS facility of the Aquaculture Experimental Base of Zhejiang University. The largemouth bass used in the experiment were purchased from Sanshui Baijin Aquatic Seedling Co., Ltd. (Foshan City, Guangdong Province, China). The fish were larvae that had just hatched from eggs; 600 larvae were randomly placed in each culture tank. The average body length of the larvae was 6.52 ± 0.23 mm, and the average body weight was 0.56 ± 0.02 mg. Fresh live brine shrimp (*Artemia salina*) were fed into the culture tanks every 3 h from 8:00 to 20:00 every day, and the initial daily feed was set to 5% of the total larvae weight. Five larvae were randomly taken from each culture tank every five days to measure their body weight with a precision electronic balance, and the weight of the feed was adjusted accordingly. The water temperature in the tanks was $23^\circ\text{C} (\pm 1^\circ\text{C})$, the pH was $7.5 (\pm 0.2)$, and the dissolved oxygen was $5.6 (\pm 0.2)$ mg L⁻². A suction pump was used daily to remove the remaining feed and dead larvae at the bottom of each culture tank. The room was kept quiet to avoid stressing the larvae.

EXPERIMENTAL SYSTEM AND DESIGN

The experiment included 12 indoor RAS (fig. 1). Each system consisted of an LED lamp (40 W), circular culture tank (80 cm diameter \times 70 cm height, 300 L volume), physical filter (100 cm diameter \times 60 cm height), buffer tank (50 cm wide \times 30 cm long \times 60 cm height), biological moving-bed filter (60 cm wide \times 150 cm long \times 60 cm height), centrifugal pump (0.5 kW), aeration pump (45 W), aquarium heating rod (500 W), and UV sterilizer (80 W) (fig. 2). Each culture tank had a bottom drain for removing feces and a side drain to prevent overflow. The settleable solid waste was directed to the physical filter through the bottom drain, and the filter was backwashed once a day. The water flowing out from the physical filter and the overflow water from the culture tank entered the biological moving-bed filter, where nitrification and denitrification reactions removed ammonia nitrogen and nitrite from the water. The treated water was sterilized with the UV reactor, replenished with oxygen, and then returned to the culture tank. The turnover rate of the culture tank was 4 h. The water used in the experiment was

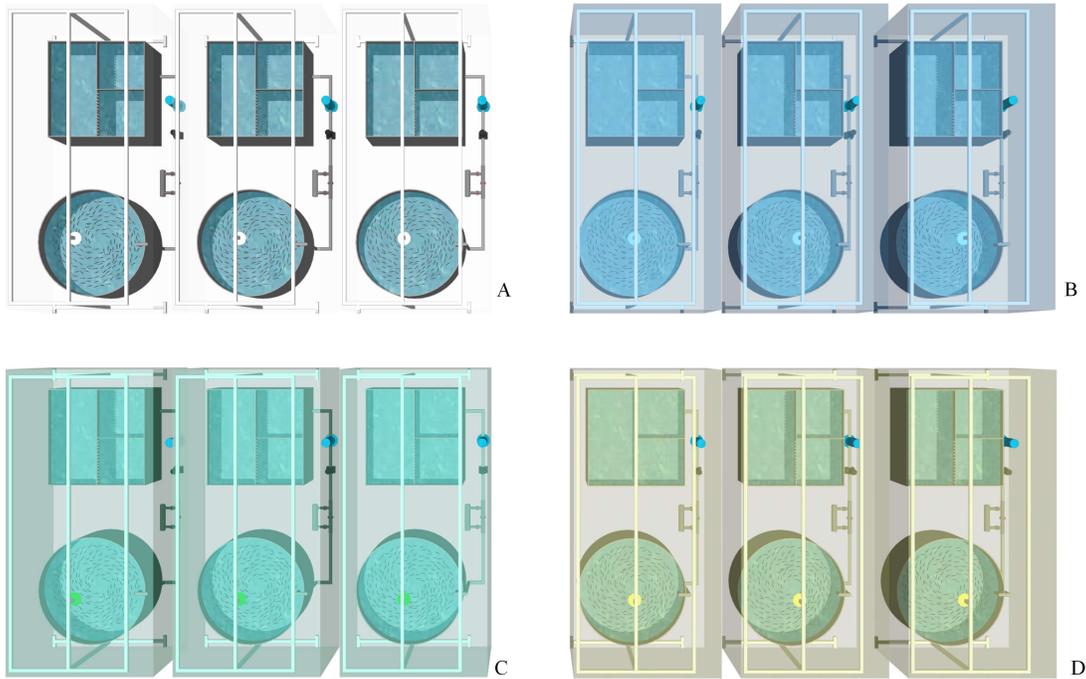


Figure 1. Layout of experiment: A, B, C, and D represent the four colors of the light environment.

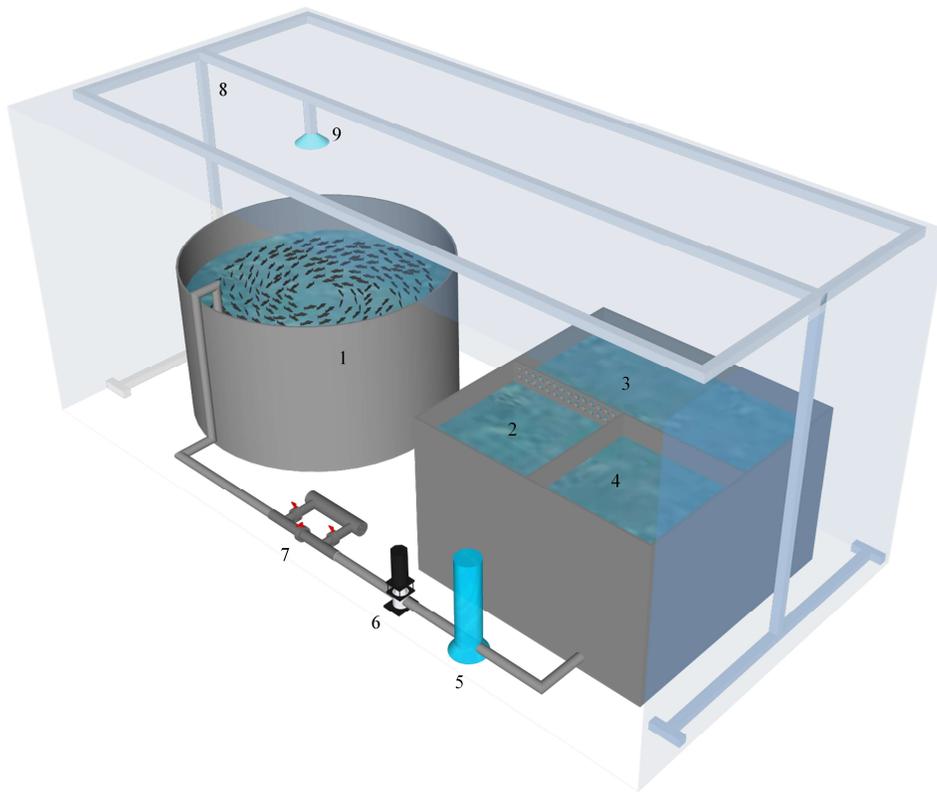


Figure 2. Diagram of recirculating aquaculture system (RAS): 1 = culture tank, 2 = physical filter, 3 = biological moving-bed filter, 4 = buffer tank, 5 = oxygenator, 6 = pump, 7 = UV sterilizer, 8 = stainless steel enclosure, and 9 = LED lamp.

tap water that had been thoroughly aerated and temporarily stored.

The experimental light environment was set up with four light spectra: blue (half-peak bandwidth = 429 to 450 nm), green (half-peak bandwidth = 503 to 535 nm), white ($320 <$

$\lambda < 950$), and full spectrum (fig. 3). Three replicate RAS were used for each light spectrum. The LED lamps were purchased from Shenzhen Fluence Technology (Huizhou, China). For each light source, the underwater light intensity at the center of the culture tank (30 cm above the bottom)

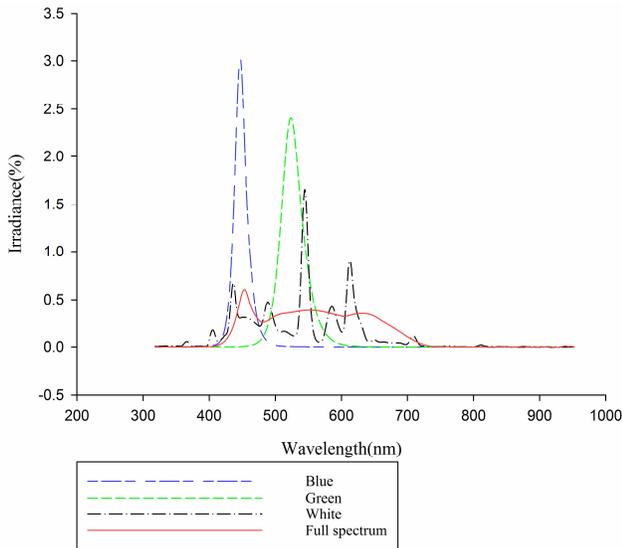


Figure 3. Spectrum of each LED lamp (blue, green, white, and full spectrum) expressed as percentage of irradiance (Villamizar et al., 2009).

was measured with an underwater spectrometer (RAMSES, TriOS GmbH, Rastede, Germany) before the start of the experiment and adjusted to 0.5 W m^{-2} using a rotary switch once a week. To avoid light interference between the experimental groups, each RAS group was separated with light-shielding cloth. The experimental photoperiod was set to 12 h light/12 h dark.

DATA COLLECTION

For each LED lamp, the underwater light intensity and spectral composition were measured with an underwater spectrometer (RAMSES, TriOS GmbH, Rastede, Germany). Dissolved oxygen (DO) and water temperature were measured once a day with a multiplicative DO meter (556 MPS, YSI, Yellow Springs, Ohio). The concentrations of total ammonia nitrogen (TAN) and nitrite nitrogen ($\text{NO}_2\text{-N}$) in the water were measured every three days with a spectrophotometer (721 Series, Jinghua Technology Instrument Co., Ltd., Shanghai, China).

On days 1, 4, 7, 10, 13, and 16 of the experiment, five larvae were randomly selected from each culture tank, killed on ice, and fixed under a microscope with 25% glutaraldehyde. After imaging the larvae with a digital camera (Toup-Cam, Hangzhou ToupTek Photonics, Hangzhou, China), image analysis software (ToupView, Hangzhou ToupTek Photonics) was used to obtain the standard body length of the larvae. On days 7 and 10, six larvae were randomly selected from each culture tank, killed on ice, and fixed under a microscope with 25% glutaraldehyde to observe the development of the anal, caudal, and dorsal fins (Villamizar et al., 2009). On day 13, ten larvae were randomly taken from each culture tank to detect deformities. On day 16, five larvae were randomly selected from each culture tank and rinsed with purified water. The excess water was removed with a lint-free paper towel, and the wet weight of the larvae was measured with a precision electronic balance. The number of remaining larvae in each of the three culture tanks for each light spectrum was counted with XperCount2 software, and

the survival rate was calculated using the following equation (Bergot et al., 1986):

$$S_0 (\%) = \frac{(n_0 - d_1)}{n_0} \times \frac{(n_0 - d_1 - s_1 - d_2)}{(n_0 - d_1 - s_1)} \times \frac{(n_0 - d_1 - s_1 - d_2 - s_2 - d_3)}{(n_0 - d_1 - s_1 - d_2 - s_2)} \times \frac{(n_0 - d_1 - s_1 - d_2 - s_2 - d_3 - s_3 - d_4)}{(n_0 - d_1 - s_1 - d_2 - s_2 - d_3 - s_3)} \times 100 \quad (1)$$

where n_0 is the initial number of larvae, d_1 is the number of larval deaths before the first sampling, s_1 is the number of larvae taken in the first sampling, d_2 is the number of larval deaths during the first and second samplings, s_2 is the number of larvae taken in the second sampling, d_3 is the number of larval deaths during the second and third samplings, s_3 is the number of larvae taken in the third sampling, and d_4 is the number of larval deaths during the third and fourth samplings.

STATISTICAL ANALYSIS

All data are given as means \pm SD. Differences in growth, development, and survival among the treatments were analyzed by one-way ANOVA followed by Duncan's test. Significance was set at $p < 0.05$ in all cases. Statistical analyses were performed using SPSS Statistics (version 22.0) and SigmaPlot (version 14.0).

RESULTS

SURVIVAL

During the entire experiment, the survival rates of the larvae in the green light group, the full spectrum group, and the white light group were significantly higher than in the blue light group ($p < 0.05$) (fig. 4). On day 16 of the experiment, the survival rate of larvae in the full spectrum group was highest ($33.5\% \pm 3.5\%$), followed by the white light group ($20.4\% \pm 4.6\%$). The survival rates of the larvae in the green and blue light groups were less than 20%, i.e., $17.7\% \pm 2.4\%$ in the green light group and $11.6\% \pm 0.7\%$ in the blue light group.

GROWTH

As shown in figure 5, the growth rates of the larvae in all groups was faster during days 1 to 13 of the experiment and then slowed during days 13 to 16. From day 10 onward, the body lengths of the larvae were significantly different, and the growth rate of the larvae in the full spectrum group was fastest, while that in the blue light group was slowest ($p < 0.05$). At the end of the experiment (day 16), the average length of the larvae in the full spectrum group was $12.4 \pm 0.3 \text{ mm}$, followed by the white light group ($12.3 \pm 0.03 \text{ mm}$) and the green light group ($12.2 \pm 0.1 \text{ mm}$). The average body length of the larvae in the blue light group was the shortest ($12.1 \pm 0.4 \text{ mm}$) ($p < 0.05$). The body weight of each group of larvae was measured on day 16 of the experiment, at which time the larvae in the white light group ($18.4 \pm 2.7 \text{ mg}$)

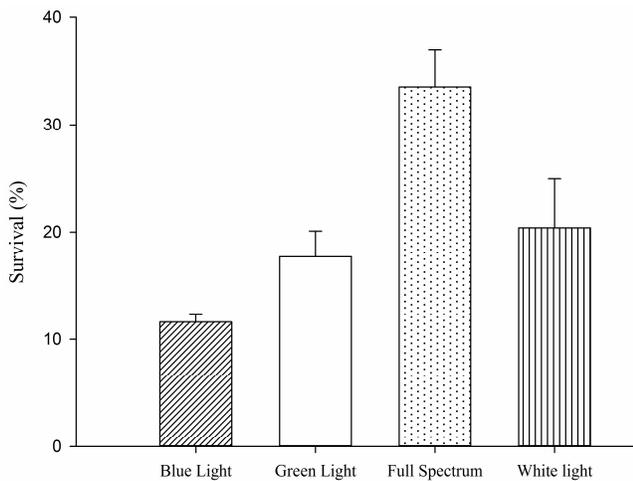


Figure 4. Effects of light spectra on survival of largemouth bass larvae on day 16 of the experiment.

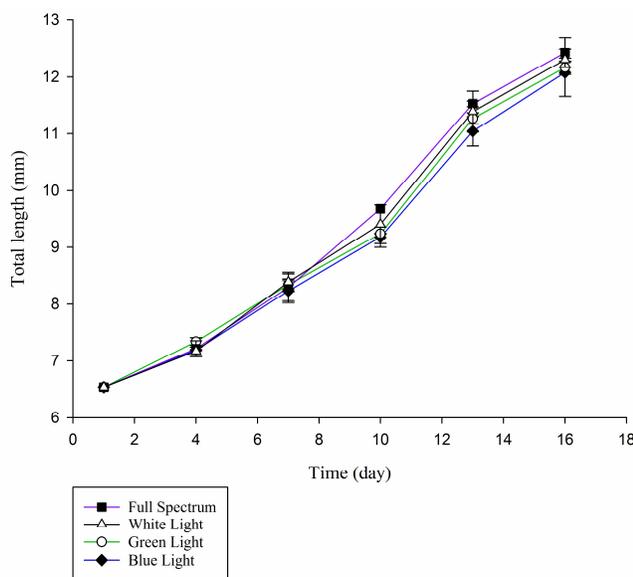


Figure 5. Growth in total body length (mm) of largemouth bass larvae under different light spectra from day 1 to 16.

and the full spectrum group (18.4 ± 0.2 mg) were heaviest, followed by the green light group (16.8 ± 1.5 mg), while the larvae in the blue light group were smallest (16.7 ± 1.0 mg) ($p < 0.05$).

FIN DEVELOPMENT

On day 7 of the experiment, the larvae in all groups exhibited differentiation of fins (fig. 6), but the degree of fin development was different ($p > 0.05$) for each group. In the green light group, the development rate of the caudal fin was 89%, which was significantly higher than that of the blue light group and the white light group, and the development rate of the caudal fin of the full spectrum group (77.8%) was also significantly higher than that of the blue light group and the white light group ($p < 0.05$). The development rate of the caudal fin in the white light group was 61.1%, while that in the blue light group was only 56%. On day 10 of the experiment, 83% of the larval dorsal fins were differentiated in the

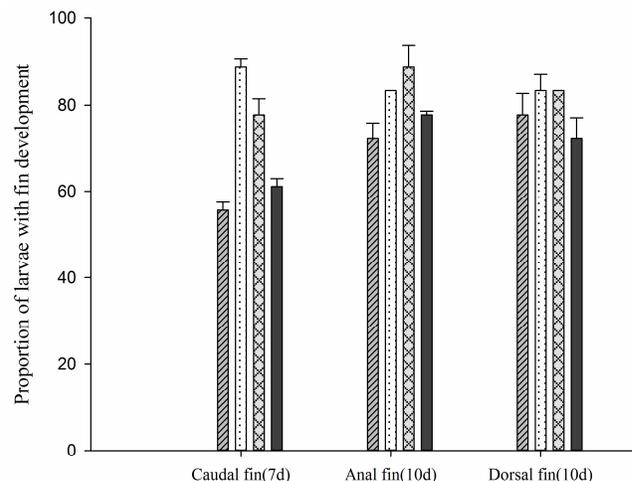


Figure 6. Influence of light spectrum on largemouth bass larvae development, showing proportion of larvae with caudal fins on day 7 and proportion of larvae with anal and dorsal fins on day 10.

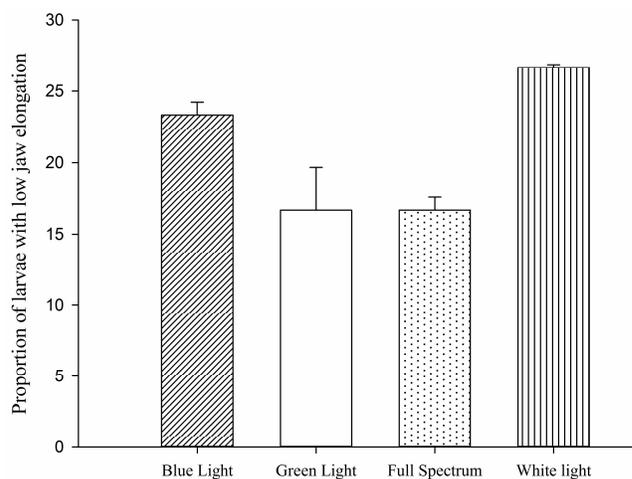


Figure 7. Effect of light spectrum on frequency of lower jaw elongation of largemouth bass larvae on day 13.

full spectrum and green light groups, followed by the blue light group (77.8%), and the white light group had the lowest proportion of dorsal fin differentiation (72.2%) ($p < 0.05$). Similarly, on day 10 of the experiment, 89% of the larvae in the full spectrum group exhibited anal fin differentiation, followed by the green light group (83.3%) and the white light group (77.8%), with the lowest proportion in the blue light group (72%) ($p < 0.05$).

MALFORMATION RATE

In this study, deformity of the larvae was characterized by elongation of the lower jaw, and different light spectra had a significant effects on this deformity ($p < 0.05$) (fig. 7). On day 13 of the experiment, the malformation rates of the larvae in each group showed significant differences ($p < 0.05$) (fig. 7).

The malformation rate of the larvae in the white light group (26.7% \pm 0.2%) was significantly higher than that in the blue light group (23.3% \pm 0.9%), the full spectrum group (16.7% \pm 0.9%), and the green light group (16.7% \pm 3%). The malformation rate of the larvae in the blue light group was also significantly higher than that of the larvae in the full spectrum group and the green light group ($p < 0.05$).

DISCUSSION

The light spectrum is an important exogenous environmental factor that affects the growth, development, and survival of aquatic animals. As light propagates in water, its energy is consumed due to absorption and scattering. Light at various wavelengths can be better transmitted in water with shallow depth and low turbidity. As the depth increases, short wavelengths (blue light) will dominate (Song et al., 2013). In a long-term evolutionary process, organisms respond to changes in the light spectrum through various aspects, such as morphology, behavior, and physiological responses (Villamizar et al., 2009). In their natural environment, largemouth bass prefer sandy or low-turbidity water, especially clear, slow-flowing water (Lan et al., 2014). In this experiment, the largemouth bass larvae were obviously affected by different light spectra, and they grew faster and had a higher survival rate in the full spectrum light environment, which was similar to their natural environment. In artificial conditions, a suitable light spectrum (such as full spectrum) will have a positive impact on the growth and survival of the larvae.

The effects of spectral components on the survival of aquatic animals are species-specific. Petit et al. (2003) reported that the photoperiod is not the main ecological factor affecting the survival of largemouth bass. In this experiment, different light spectra significantly affected the survival rate of largemouth bass larvae. The results showed that the survival rate was low under blue light. In addition, growth and feeding were lower under blue light than under the other light spectra, which indicates that blue light may be a stress factor for largemouth bass larvae. Under blue light, the largemouth bass larvae could not adapt to the lighting conditions through behavioral and physiological mechanisms, and their mortality was higher. These results are consistent with the results of Liu et al. (2019), who reported that tiger puffer (*Takifugu rubripes*) fry had the lowest survival rate under blue light at a light intensity of 0.5 W m⁻². Under white light, the survival rate of the fry increased with an increase in light intensity (0.5 to 1.5 W m⁻²). This suggests that lighting designs for fish culture systems must consider the light spectrum as well as the light intensity. This experiment contrasts with the results reported by Downing (2002) and Villamizar et al. (2009), i.e., the survival rate of haddock (*Melanogrammus aeglefinus*) was 77.5% under blue light but only 13.0% under white light, and the survival rate of sea bass larvae was 21.6% under blue light but only 11.6% under white light. These different results may be due to differences in the natural light environments of haddock, European sea bass, and largemouth bass. Therefore, identification of the fish species is necessary for effective lighting designs in industrial RAS.

Studies of fish growth have found that different light spectra have different effects on fish growth characteristics due to differences in habitat characteristics and the specific visual functions of fish. The light environment dynamics of fish habitats vary widely. According to species ecology, fish photoreceptor cells have the best visual contrast or the maximum visual sensitivity at certain wavelengths (Lythgoe, 1979). Fish should be raised in the most desirable light environment so that they can best respond to feeding and achieve the best growth and development (Downing and Litvak, 2001). In this study, the body length and weight of the larvae were greater for the full spectrum group than for the other treatment groups. While it is not possible to measure the amount of feed eaten by individual larvae and the amount of feed remaining in the culture tank, we speculate that the larvae had a higher feed intake or feed conversion rate under full spectrum light. White light is the most common light environment used for the cultivation of largemouth bass larvae. In this study, the body length and body weight of the larvae were also higher under white light.

These results are supported by the results of Karakatsouli et al. (2007), who found that blue and red wavelengths reduced the growth performance of rainbow trout and gilthead as compared to white light. In the present study, the larvae in the blue light group grew slowest, similar to the results of Yan et al. (2019), who found that the growth rate of sea bass larvae was significantly lower under blue light than under red, green, or white light. Guo et al. (2012) reported that whiteleg shrimp (*Litopenaeus vannamei*) grew slowly under blue light, and the energy used for excretion was relatively high, making the specific growth rate significantly lower than for the other light spectrum groups. In addition, Karakatsouli et al. (2010) showed that red light was more conducive to the growth of carp (*Cyprinus carpio*) when the culture density was lower. In the case of high density, the carp grew faster in the blue light environment. In the present study, the largemouth bass larvae grew faster in the first 12 days of the experiment, and the growth rate in each group slowed during days 13 to 16, as shown in figure 5. This may have occurred because the brine shrimp (*Artemia salina*) based feed did not meet the growth requirements of the larvae, so the larvae should be supplied a fish-based feed in the later stage (i.e., days 13 to 16) to ensure healthy and rapid growth. These results indicate that physical factors, such as feed, density, temperature, and DO, as well as the lighting, affect the growth performance of fish and should be taken into account in the production process.

The effects of light on the deformity of aquatic animals are manifested by spine curvature, fin deformity, lower jaw elongation, etc., and are species-specific. In this study, the larvae had lower malformation rates in the green light and full spectrum groups but higher malformation rates in the white light and blue light groups, which is consistent with the results of Yan et al. (2019) for sea bass. They found that sea bass larvae had the lowest proportion of jaw deformities under red and green light, and the malformation rates were higher in the white and blue groups. Villamizar et al. (2009) also found that, with 12 h of light, the rate of lower jaw deformity of sea bass larvae was higher in the blue and white light groups than in the red and dull groups. With extension

of the lighting duration from 12 h to 24 h, the malformation rate of the larvae in the white light group increased from 10% to 38%. Malformation of the lower jaw may lead to damage of the oral mucosa during eating and bacterial infection, which affects the survival of the larvae due to their low immunity. In addition, jaw malformations can affect feeding activities, leading to hunger and even death (Barahona-Fernandes, 1979). This can explain why the survival rate of the larvae was lower in the white light group than in the full spectrum group. In addition to the effects of light on larvae, several parameters during the processes of spawning and hatching are linked to the deformity of larvae. Hatching temperature and salinity (Ottesen and Bolla, 1998) and inappropriate light intensity and spectrum (Battaglione and Talbot, 1990) may cause fry abnormalities. Cobcroft and Battaglione (2009) found that striped trumpeter larvae in the red pool had the highest deformity rate, while striped trumpeter larvae in the black pool had the lowest deformity rate. In addition to the deformities that occur during the growth of fins, an appropriate stocking density and transport method can greatly reduce damage to the fins (Jones et al., 2010; Santurtun et al., 2018). Therefore, further exploration of the impacts of environmental factors and their interactions on larvae malformation is essential for achieving healthy fish production.

In general, this study pointed out that the spectral composition of the lighting is an important environmental factor affecting the growth and survival of largemouth bass larvae. The survival rate of the larvae was significantly higher under full spectrum light than under white, green, or blue light. In addition, the growth performance was significantly higher in the full spectrum group than in the blue and green light groups, and the malformation rate was significantly lower in the full spectrum group than in the blue and white light groups. The largemouth bass larvae had the slowest growth rate, the lowest survival rate, and a high malformation rate under blue light. As a consequence, avoiding unsuitable illumination (blue light) in the breeding of largemouth bass and constructing a full spectrum light environment is conducive to the survival and growth of larvae. Future research will focus on using lighting to eliminate stress response, regulate gonadal development, and improve growth performance to achieve high-density, large-scale, healthy fish farming.

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