

Effects of Different LED Light Spectra on Growth and Immunity of the Japanese Eel (*Anguilla japonica*) and Giant Mottled Eel (*A. marmorata*)

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Indoor recirculating aquaculture systems make light control possible and enable the usage of specific coloured lights to promote the growth and immunity of aquaculture species. Five different LED wavelengths (white light, red light [622 nm], green light [517 nm], blue light [467 nm], and dark) were used in this study to evaluate growth and immunity in the glass eel stage of two high-valued anguillid species, Japanese eel (*Anguilla japonica*) and giant mottled eel (*A. marmorata*). There were no significant differences in growth of the Japanese eel among the groups after 12 weeks of feeding ($p > 0.05$); the survival rate of each group was over 95%. The giant mottled eel showed better growth in total length and body weight in the red light and dark groups ($p < 0.05$). Expression levels of immune-related genes were not significantly different between each group of the Japanese eel and the giant mottled eel ($p > 0.05$). The growth of the Japanese glass eel was not significantly sensitive to different LED wavelengths, while the giant mottled glass eel showed

better growth under red light and dark environments. Neither eel species showed significant differences in innate immunity under different LED wavelengths.

Key words: *Anguilla* eel, Growth, Immunity, LED, Light spectrum.

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BACKGROUND

Aquaculture has emerged as the most rapidly growing animal food-producing industry in the last two decades (FAO 2014). Anguillid eels are regarded as an important commercial aquaculture species in East Asia because of their high market demand and nutritional value (Ahn et al. 2015; Shahkar et al. 2015). The Japanese eel *Anguilla japonica* is a traditionally reared, highly valued anguillid species. However, the amount of natural resources of *A. japonica* available is currently only 5% that in the 1970s because of the impact of habitat destruction and overfishing (Chen et al. 2014; Dekker 2004). According to the International Union for Conservation of Nature and Natural Resources (IUCN), *A. japonica* has been classified as “Endangered” in the Red list and needs more attention in the eel aquaculture industry (Jacoby and Gollock 2014). On the other hand, the cultivation of the giant mottled eel (*A. marmorata*), which has abundant glass eels and has low fry prices, has increased in Southeast Asia in recent years (Leander et al. 2013; Luo et al. 2013). However, the low growth rate and high mortality rate caused by disease are still considered the biggest obstacles in the development of giant mottled eel aquaculture (Han 2010).

Indoor recirculating aquaculture systems (RAS) have become increasingly popular because of environmental pollution, lack of water resources and land, and frequent extreme weather events (Martins et al. 2011). Indoor RAS can reduce the water exchange rate to 1/30–1/50 that of

traditional outdoor aquaculture ponds, and improve the production capacity by more than ten times (Deviller et al. 2005; Martins et al. 2011). *A. japonica* and *A. marmorata* are usually reared in an enclosed RAS at high density (Hsu 1997; Li 2018). Fish tend to be more easily affected by disease in intensive aquaculture systems due to the stress-induced weakening of their immune systems (Raman et al. 2013). Aquaculturists have traditionally used antibiotics in rearing systems to control various diseases. The overuse of antibiotics causes many negative side effects, such as drug resistance, decline of the immune system, environmental pollution, and food safety issues (Bachere et al. 2003; Pelgrift et al. 2013; Cabello et al. 2013). In addition, previous studies have shown that fish in intensive RAS usually show growth retardation and impaired larval development (Davidson et al. 2009; Martins et al. 2009). Therefore, it is imperative to determine an environmentally sustainable method to improve the immunity and growth rate of the cultured species to ensure efficient usage of indoor RAS.

Many studies have indicated that the use of a specific light wavelength (light colour) under different light intensities and photoperiods can improve the growth performance of fish and fish embryos and crayfish (Boeuf and Le Bail 1999; Ruchin 2004; Han et al. 2005; Marchesan et al. 2005; Toyota et al. 2022). For example, the juvenile stage of *Candidia barbata* tends to have the best body length growth rate under blue light (460–470 nm) and the worst growth rate under red light (620–630 nm) (Chang 2016). The guppy *Poecilia reticulata* grows better under blue light, while the Chinese sleeper *Perccottus glenii* has a higher growth rate under blue and green light. The crucian carp *Carassius carassius*, the Atlantic halibut *Hippoglossus hippoglossus*, the rainbow trout *Oncorhynchus mykiss*, and the silver carp *Hypophthalmichthys molitrix* showed the best growth under green light and the worst growth under red light (Radenko 1991; Boeuf and Pierre-Yves 1999; Ruchin et al. 2002; Ruchin 2004; Luchiari and Pirhonen 2008). The preleptocephalus stage of the European eel *A. anguilla* showed the best survival rate under low luminosity of red light with a normal photoperiod (Politis et al. 2014). Glass eel-stage *A. marmorata* individuals with a small amount of pigmentation on the skin tend to stay in areas without light, while those without pigmentation tend to stay under red light environments (Mo et al. 2019). Moreover, light of

different colours can also affect the innate immune system of the goldfish *Carassius auratus* (Eslamloo et al. 2015). Furthermore, Boeuf and Pierre-Yves (1999) showed that lights of different colours may promote the growth of gonads. For example, the blue damselfish *Chrysiptera cyanea* showed the best gonad development under red light, while *O. niloticus* showed better gonad development under blue light.

Currently, there are no studies comparing the effect of lights of different colours on the growth and immune response of *A. japonica* or *A. marmorata*. Since RAS is mostly an indoor system, it is easy to control light artificially. Therefore, we aimed to determine the effect of light spectrum on the growth and immune response in the glass eel stages of the Japanese eel and the giant mottled eel cultured in an indoor RAS system.

MATERIALS AND METHODS

Experimental animals and feeding

Glass eels of *A. japonica* and *A. marmorata* were caught from eastern Taiwan (*A. japonica* from the Yilan River, 24.7163°N, 121.8348°E, and *A. marmorata* from Xiugulan River, 23.4612°N, 121.5008°E). Eel sampling was approved by the Fishery Agency, Council of Agriculture, Executive Yuan, Taiwan. The specimens were transported at low temperatures through live fish bags filled with oxygen. The health condition of the eels was checked upon arrival at the laboratory located at the Institute of Fisheries Science of National Taiwan University, Taipei. Individuals in good condition were disinfected with 2.5 ppm of potassium permanganate (KMnO₄) solution for 10 min to avoid pathogen contamination of the experimental system. After sterilization, the eels were kept in five sets of indoor RAS systems with five tanks (30 × 30 × 45 cm) for each set and maintained in freshwater for three days before feeding. Photoperiods were set at 12 h light (natural light, 7:00–19:00) and 12 h dark during acclimation.

The initial body weight and total length of *A. japonica* and *A. marmorata* (20 *A. japonica* for each tank; 30 *A. marmorata* for each tank in triplicates) were measured before experiment started (56.7 ± 2.0 mm, 0.14 ± 0.01 g for *A. japonica*; 51.04 ± 2.1 mm, 0.15 ± 0.02 g for *A. marmorata*). An LED (EVERLIGHT Electronics Co., Ltd., Taiwan) was used as the light source to control the background spectra for the experiment. Each set of RAS included five tanks (30 L water/tank), each exposed to either white light, red light (622 nm), green light (517 nm), or blue light (467 nm) under 100 Lux (lx) light intensity with photoperiod 12 hours light and 12 hours dark, or dark (< 5 lx). Each RAS tank was covered by a black board to avoid any light influence from neighbouring tanks or the environment (Fig. 1). The water temperature and pH were controlled between $28 \pm 1^\circ\text{C}$ and 7.5 ± 0.5 , respectively, with a water exchange rate of 20 L/day for each RAS; oxygen was dissolved to near saturation by aeration. Fish were fed with blood worms (*Chironomus dorsalis* larvae) in the size about 10 mm \times 1.5 mm, which was often used as glass eel feed, at an amount of 10% of their body weight twice a day for a total of 12 weeks. The remaining feed was removed from the tanks an hour after feeding. The experiment was performed in accordance with the recommendations from the Institutional Animal Care and Use Committee for the care of animals used for experimental or other scientific purposes (approval number 'NTU-110-EL-00009').

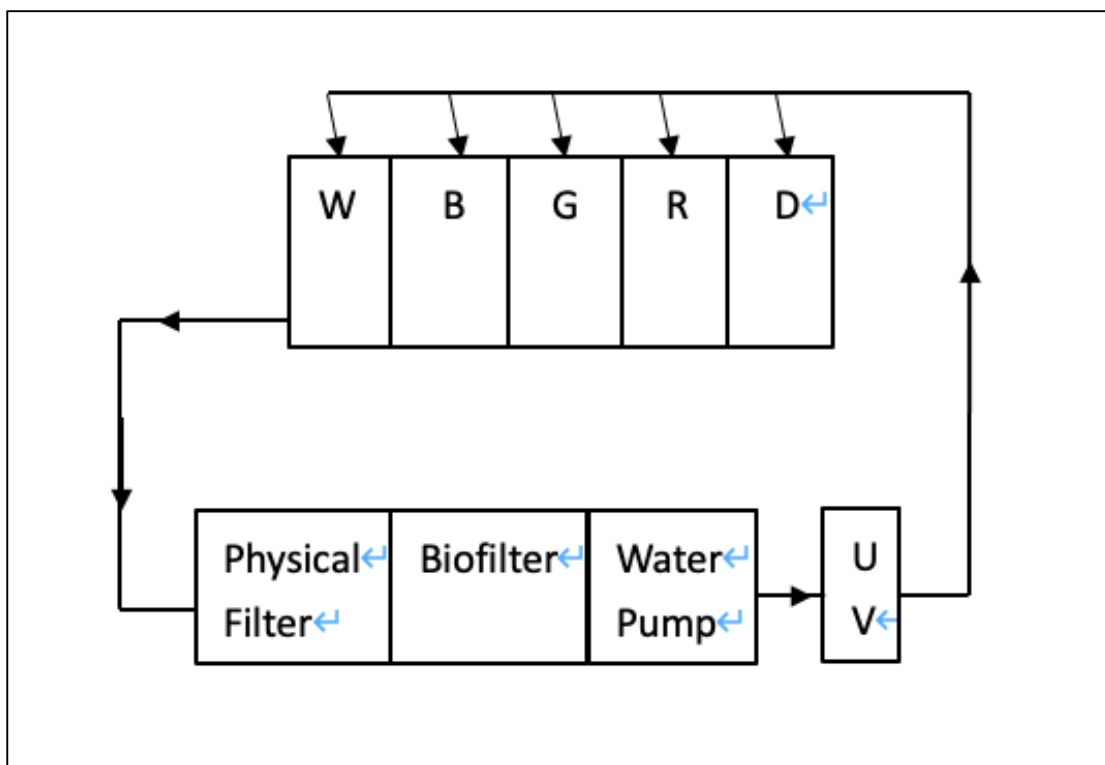


Fig. 1. Graph of a set of recirculating aquaculture systems (RAS) used in this study. The five tanks were each 40 L in volume and covered by a black board. W: white light; R: red light (622 nm); G: green light (517 nm); B: blue light (467 nm).

Sample Collection and Analyses

The total length (to the nearest 0.1 mm) and body weight (to the nearest 1 mg) were measured every two weeks. The percentage weight gain, condition factor, specific growth rate, and the survival rate in each group were calculated as follows:

$$\text{Percentage weight gain (\%)} = \frac{\text{Final body weight (g)} - \text{Initial body weight (g)}}{\text{Initial body weight}} \times 100$$

$$\text{Condition factor (K)} = 1000 \times \frac{\text{Body weight (g)}}{\text{Body length}^3 \text{ (cm)}}$$

$$\text{Specific growth rate} = \frac{\text{Exp}[\ln(\text{Final body weight}) - \ln(\text{Initial body weight})]}{84} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{Final n}}{\text{Initial n}} \times 100$$

Three fish from each tank were randomly selected and sacrificed to obtain head kidney tissues.

Three head kidney tissues from the same tank were pooled together and stored in an RNA protecting reagent at -80°C before extracting total RNA using an RNA kit (Bioman Scientific Co. Ltd., Taiwan) for real-time PCR of immune-related genes.

Real-time PCR

Specific candidate genes were selected for real-time PCR based on previous study about eel immunology (B.T. Birhanu et al. 2016; Lee et al. 2017). Four immune-related genes, namely, superoxide dismutase (*SOD*), lysozyme (*LZM*), peroxidase (*POD*), and interleukin-6 (*IL-6*) were selected as the target genes for real-time PCR, and acidic ribosomal protein (*ARP*) was used as the reference gene. The whole genome of *A. japonica* was successfully assembled in our previous study (<http://molas.iis.sinica.edu.tw/jpeel/>) (Hsu et al. 2015), and was used as a template to annotate the transcriptome data of *A. japonica* and *A. marmorata*. By the website, all the gene we chose can be annotated in both eels. TRIzol reagent (Bioman Scientific Co. Ltd) was used to extract total RNA, and the purity was quantified by spectrophotometry (Medclub Scientific Co. Ltd). Reverse transcription was performed to synthesize complementary DNA (cDNA) for real-time PCR (Bio-Rad). The primers used for real-time PCR are listed in table 1.

Table 1. Primers used for qPCR amplification

Genes	Primer	Sequences
ARP (reference gene)	Forward	5'-GTGCAGCTCATTAAGACCGG-3'
	Reverse	5'-GGCGATATTCCTCACACCCT-3'
SOD	Forward	5'-TAACGTACGACTATGGGGCC-3'
	Reverse	5'-GCCGCCACCATTAAACTTCA-3'
LZM	Forward	5'-TGCTGGAATGGATGGATAACC-3'
	Reverse	5'-GTAATCGCAGTGCTGATGTC-3'
POD	Forward	5'-GACATCACCCGTTTCTGCAA-3'
	Reverse	5'-GTGGATGAAGGAGGGGAACA-3'
IL-6	Forward	5'-CCAGATGTCGCTTCACTTCG-3'
	Reverse	5'-ACTTGGATGTCGTCACCCAT-3'

Statistical analysis

All data were analysed by one-way analysis of variance (IBM SPSS Statistics 24.0) to determine the effects of different spectra. Statistical significance was set at $p < 0.05$. A significant effect was followed up with the least significant difference test to compare the means.

RESULTS

Growth rate

The mean initial total length and body weight of the eels from each tank were not significantly different before the start of the experiment (Tables 2, 3). The growth of *A. japonica* showed no significant difference in the total length (Fig. 2) and body weight (Fig. 3) among the different groups ($P > 0.05$) after 12 weeks of feeding (Table 2). The percentage weight gain, condition factor (K), and survival rate also did not show significant differences among the groups ($P > 0.05$) (Table 3).

Table 2. Growth of *A. japonica* reared in different LED light spectra after 12 weeks

	White	Red	Green	Blue	Dark
Initial TL (mm)	56.8 ± 2.1 ^{a*}	57.0 ± 2.0 ^a	56.8 ± 1.9 ^a	56.7 ± 1.6 ^a	56.3 ± 2.0 ^a
Final TL (mm)	95.9 ± 10.2 ^a	94.0 ± 10.0 ^a	93.9 ± 13.7 ^a	93.7 ± 12.5 ^a	95.7 ± 7.7 ^a
Initial BW (g)	0.14 ± 0.01 ^a	0.15 ± 0.01 ^a	0.15 ± 0.01 ^a	0.14 ± 0.01 ^a	0.14 ± 0.01 ^a
Final BW (g)	0.78 ± 0.25 ^a	0.74 ± 0.28 ^a	0.73 ± 0.22 ^a	0.75 ± 0.32 ^a	0.74 ± 0.28 ^a
SGR (%)	1.95 ± 0.43 ^a	1.90 ± 0.66 ^a	1.88 ± 0.40 ^a	1.99 ± 0.65 ^a	1.98 ± 0.65 ^a
PWG (%)	446.8 ^a	376.4 ^a	397.4 ^a	409.8 ^a	376.9 ^a
Initial number	40	40	40	40	40
Final number	37	39	39	39	39
Survival rate (%)	92.5 ^a	97.5 ^a	97.5 ^a	97.5 ^a	97.5 ^a
Initial K	0.784 ^a	0.804 ^a	0.791 ^a	0.783 ^a	0.802 ^a
Final K	0.852 ^a	0.841 ^a	0.867 ^a	0.842 ^a	0.841 ^a

TL: total length; BW: body weight; SGR: specific growth rate; PWG: percentage weight gain; K: condition factor. Different letters indicate significant differences between groups ($p < 0.05$).

Table 3. Growth performance of *A. marmorata* reared in different LED light spectra after 12 weeks

	White	Red	Green	Blue	Dark
Initial TL (mm)	51.0 ± 2.1 ^{a*}	50.7 ± 2.1 ^a	51.1 ± 2.2 ^a	51.0 ± 1.6 ^a	51.4 ± 2.0 ^a
Final TL (mm)	62.6 ± 6.1 ^a	66.0 ± 6.5 ^b	63.3 ± 7.6 ^a	62.2 ± 6.1 ^a	68.0 ± 7.4 ^b
Initial BW (g)	0.15 ± 0.03 ^a	0.15 ± 0.03 ^a	0.15 ± 0.03 ^a	0.15 ± 0.02 ^a	0.15 ± 0.02 ^a
Final BW (g)	0.32 ± 0.12 ^a	0.39 ± 0.18 ^b	0.33 ± 0.13 ^a	0.31 ± 0.11 ^a	0.43 ± 0.18 ^b
SGR (%)	0.82 ± 0.46 ^{ab}	1.11 ± 0.47 ^{ac}	0.90 ± 0.39 ^{abc}	0.67 ± 0.43 ^b	1.14 ± 0.38 ^c
PWG (%)	112.9 ^{ab}	173.4 ^{ac}	123.7 ^{abc}	86.6 ^b	174.1 ^c
Initial n	90	90	90	90	90
Final n	54	68	56	51	39
Survival rate (%)	60 ^b	76 ^c	62 ^b	57 ^b	44 ^a
Initial K	1.106 ^a	1.080 ^a	1.093 ^a	1.120 ^a	1.078 ^a
Final K	1.245 ^a	1.262 ^a	1.182 ^a	1.206 ^a	1.384 ^a

TL: total length; BW: body weight; SGR: specific growth rate; PWG: percentage weight gain; K: condition factor. Different letters indicate significant differences between groups ($p < 0.05$).

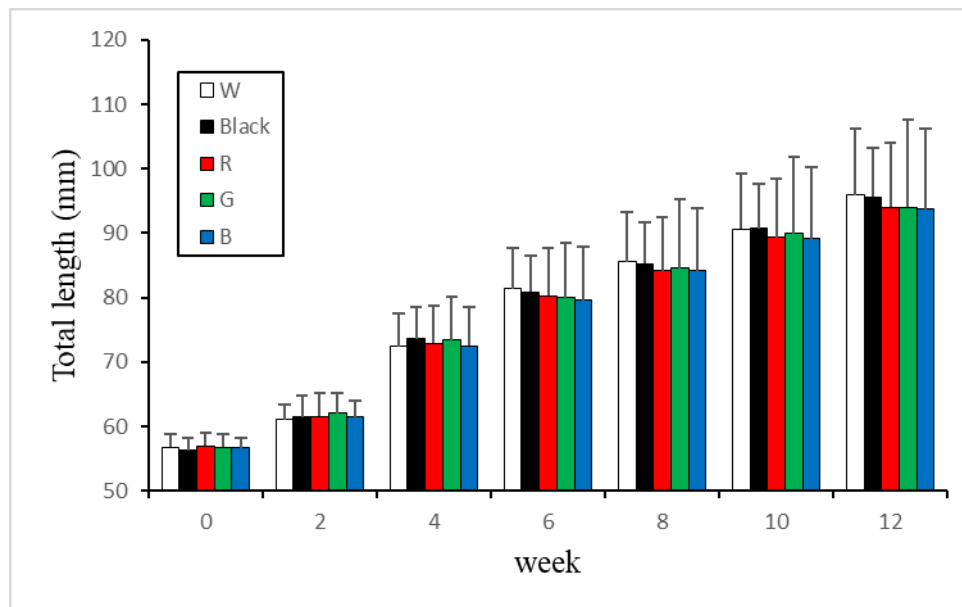


Fig. 2. The total length of Japanese eel reared in different light spectra for 12 weeks. W: white light; Black: dark; B: blue light; G: green light; R: red light.

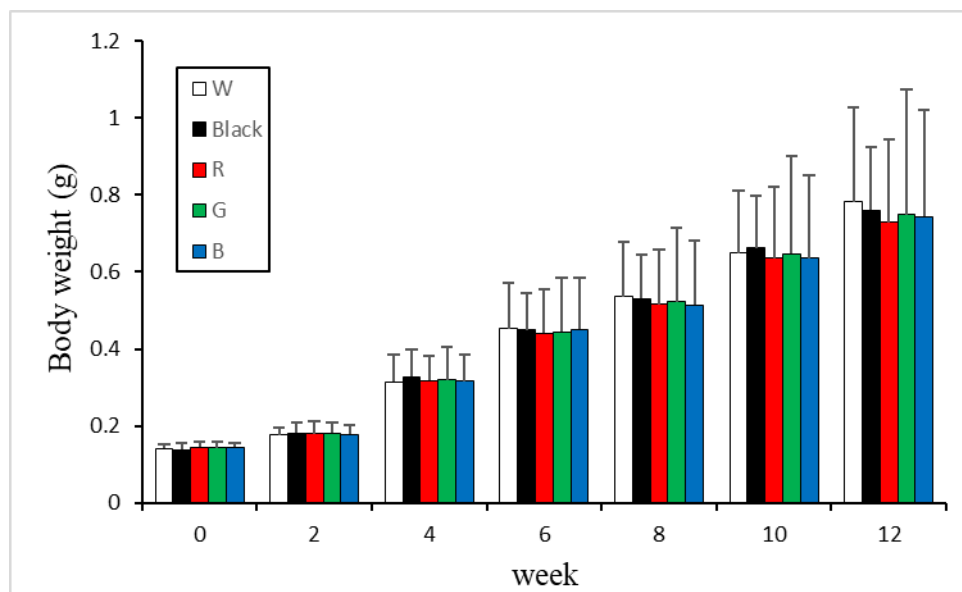


Fig. 3. The body weight of Japanese eel reared in different light spectra for 12 weeks. W: white light; Black: dark; B: blue light; G: green light; R: red light.

Although *A. marmorata* grew much slower than the Japanese eel, its growth rate was significantly different among each of the treatment groups ($p < 0.05$) (Figs. 4, 5). The mean total length and body weight were significantly higher in the dark and red light groups than in the other groups ($p < 0.05$) (Table 3). The specific growth rate and percentage weight gain in these groups were also significantly higher than those in the blue light group. The survival rate was significantly higher in the red light group than in the green light and dark groups. However, there was no significant difference in the condition factor (K) among the groups (Table 3). The fastest growing period of the giant mottled eel occurred from the sixth to the eighth week (Figs. 4, 5). On the other hand, the white, green, and blue light groups showed some growth retardation during the eighth to the tenth week. The red light group showed no decrease in growth during the entire experimental period.

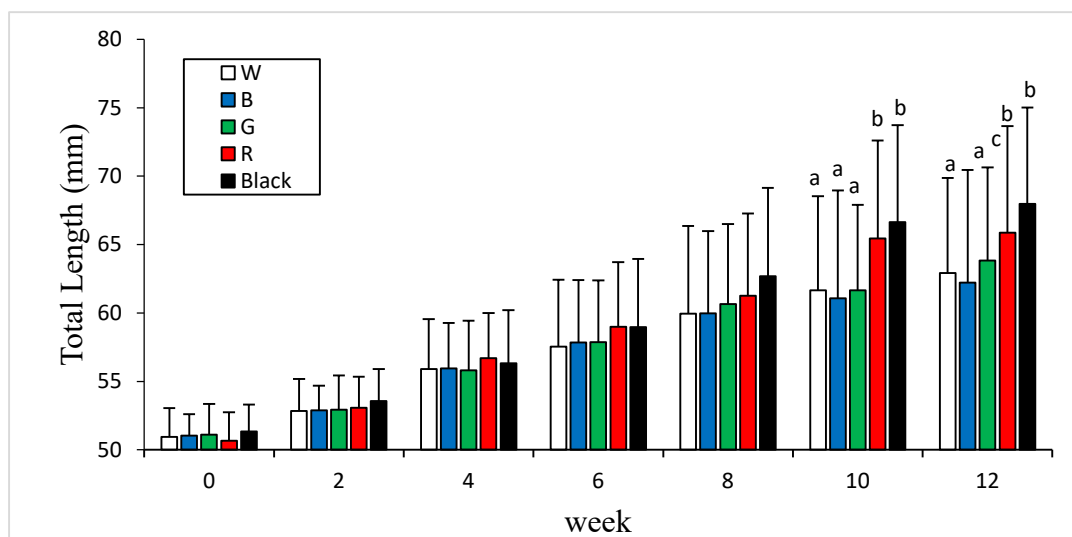


Fig. 4. The total length of giant mottled eel reared in different light spectra for 12 weeks. W: white light; Black: dark; B: blue light; G: green light; R: red light. Different letters indicate significant differences between groups of the same week ($p < 0.05$).

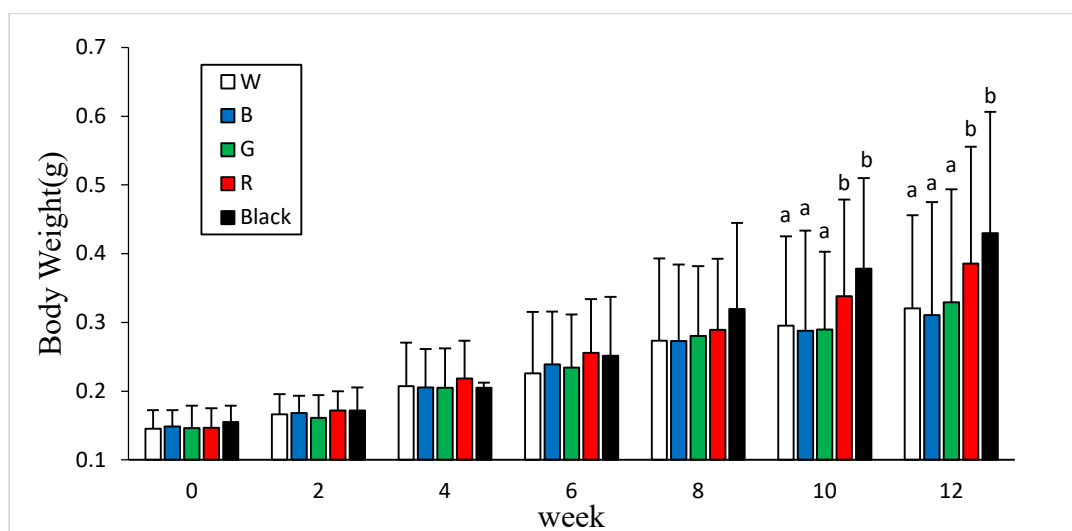


Fig. 5. The body weight of giant mottled eel reared in different light spectra for 12 weeks. W: white light; Black: dark; B: blue light; G: green light; R: red light. Different letters indicate significant differences between groups of the same week ($p < 0.05$).

Real-time PCR

Real-time PCR was conducted for precise quantification to compare whether the innate immunity of both eel species was affected by different light spectra. The target genes of real-time PCR were *SOD*, *LZM*, *POD*, and *IL-6*, which referred to previous research about eel immunology (B.T. Birhanu et al. 2016; Lee et al. 2017) and the *ARP* was used as the reference gene. The results

for *A. japonica* indicated that although the dark group showed higher *SOD* expression, there was no significant difference among the groups ($p > 0.05$) (Fig. 6). The expression levels of *LZM* in the red light and dark treatment groups were higher than in others, but there were no significant differences among groups ($p > 0.05$) (Fig. 7). The white light group showed the highest expression of *IL-6*; however, there was no significant difference among the groups ($p > 0.05$) (Fig. 8). The expression of *POD* was highest in the green light group but was not significantly different from the other groups ($p > 0.05$) (Fig. 9).

The real-time PCR results of the giant mottled eel showed that *SOD* expression was higher in the dark group than in the other groups, but there was no significant difference among the groups ($p > 0.05$) (Fig. 6). The expression level of *LZM* in white light and red light groups was the highest; however, there was no significant difference among the groups ($p > 0.05$) (Fig. 7). The red light and white light groups also showed higher expression levels of *LZM* than the other groups, but without a significant difference ($p > 0.05$) (Fig. 8). The *POD* expression levels in the blue light group were lower than those in others; however, there was no significant difference among the groups ($p < 0.05$) (Fig. 9). Moreover, comparison of the qPCR results between both eel species showed no significant differences ($p > 0.05$) in the expression levels of the four immune-related genes under all light spectra (Figs. 6–9).

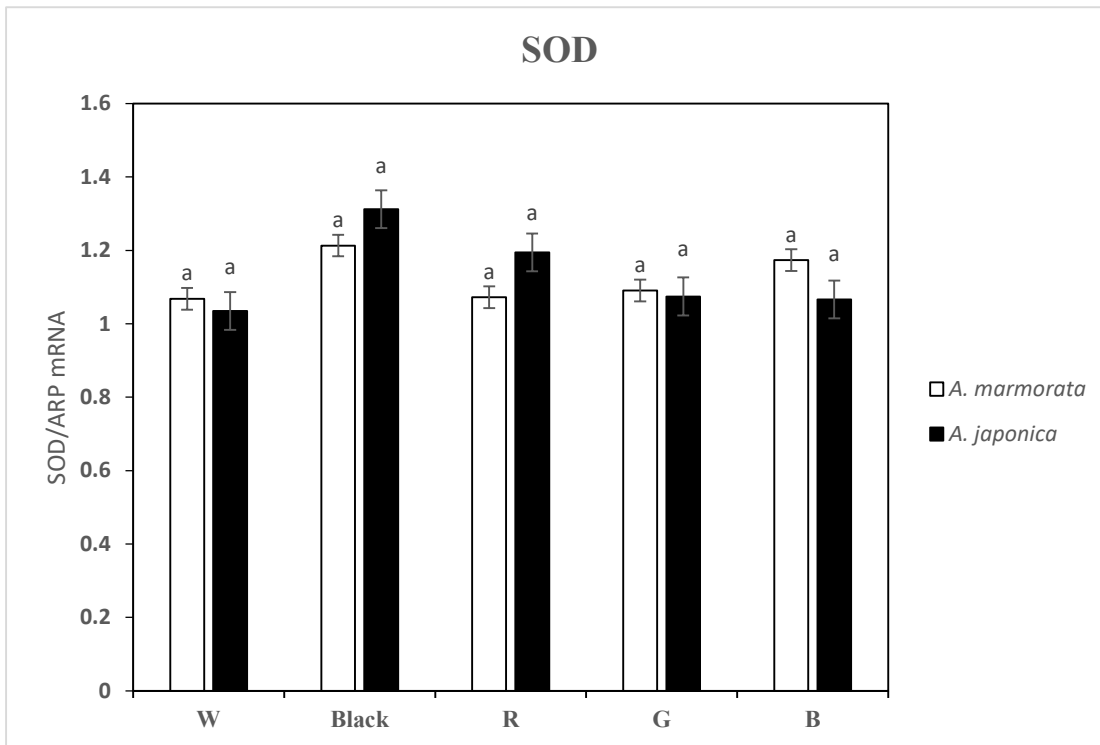


Fig. 6. The *SOD* expression levels of Japanese eel and giant mottled eel reared in different light spectra. W: white light; B: blue light; G: green light; R: red light; black: dark. Different letters indicate significant differences between different spectra groups of the same eel species ($p < 0.05$).

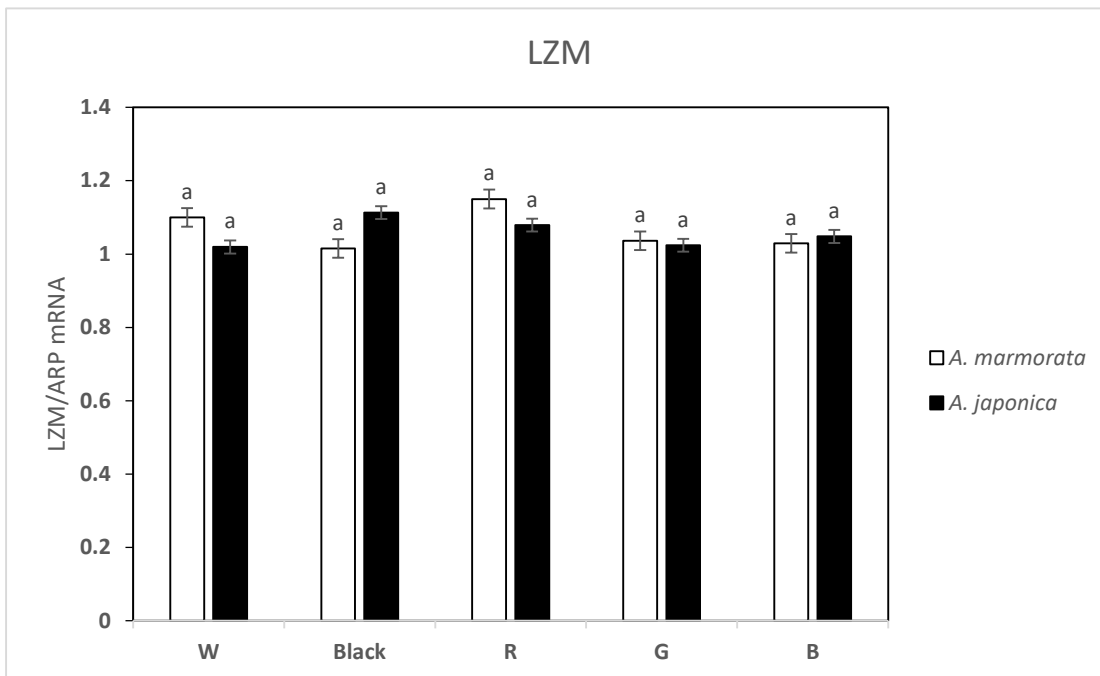


Fig. 7. The *LZM* expression levels of Japanese eel and giant mottled eel reared in different light spectra. W: white light; B: blue light; G: green light; R: red light; black: dark. Different letters indicate significant differences between different spectra groups of the same eel species ($p < 0.05$).

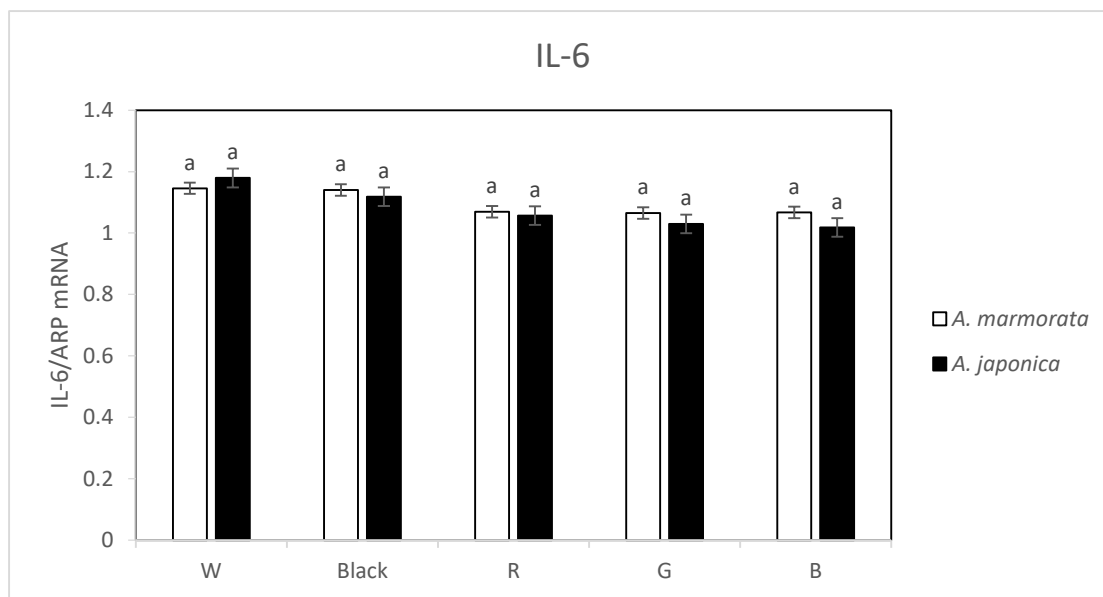


Fig. 8. The *IL-6* expression levels of Japanese eel and giant mottled eel reared in different spectra. W: white light; B: blue light; G: green light; R: red light; black: dark. Different letters indicate significant differences between different spectra groups of the same eel species ($p < 0.05$).

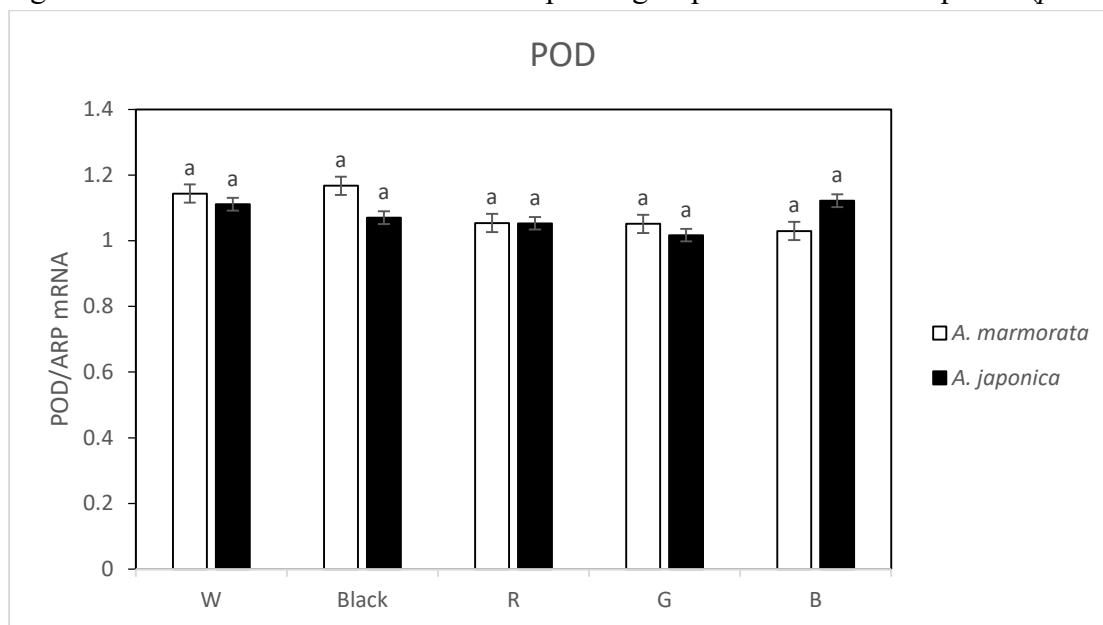


Fig. 9. The *POD* expression levels of Japanese eel and giant mottled eel reared in different spectra. W: white light; B: blue light; G: green light; R: red light; black: dark. Different letters indicate significant differences between different spectra groups of the same eel species ($p < 0.05$).

DISCUSSION

An earlier study has shown that some fish showed different growth rates under a specific

background light spectrum, but the most suitable spectrum differed among species. For example, the pikeperch *Sander lucioperca* exhibits the highest growth rates and cortisol levels under white light and the lowest under blue light due to the enhancement of cone cells for visual sensitivity under longer-wavelength light, thus improving their growth (Luchiari et al. 2009). Others, such as the barramundi *Lates calcarifer*, show the best growth rate under red light environments but the worst under green light, because the increase in spectral sensitivity under longer wavelength conditions enhances their feeding behaviour (Jeremy et al. 2011). Different spectra may affect fish visual systems and further influence their physiological functions, such as growth, immune response, endocrine system, etc.

There were no significant differences among the groups in the growth experiment of the glass eel stage of the Japanese eel. Interestingly, Japanese eels in the blue light group showed better feeding motivation than the other groups. Red light could stimulate the feeding motivation in Nile tilapia *Oreochromis niloticus* but did not improve its growth (Volpato et al. 2013). McLean et al. (2018) indicated that tank colour did not affect the growth performance of juvenile flounder or tilapia, although fish maintained in red-light tanks showed better percent increases in body weight and lower plasma cortisol levels. Some studies have also pointed out that rearing under different light spectra may not change the growth rate of juvenile fish but may have different effects on other behaviours (Villamizar et al. 2011). Such behavioural effects in Japanese eels cultured under different spectra need to be studied further.

On the other hand, the giant mottled eel clearly showed significantly better growth in the dark and red light groups ($p < 0.05$). Aquatic creatures use photoreceptor cells with the highest photosensitivity at a specific wavelength (λ_{\max}) to detect underwater objects. λ_{\max} can maximize visual acuity, such as in deep-sea fish (Bowmaker 1990), or maximize visual contrast, such as in fish inhabiting shallow water or coastal areas (Lythgoe 1979). Therefore, fish tend to live in environments with the best spectral conditions (Downing and Litvak 2001). Light of specific colours may enhance their growth potential by facilitating food capture or detection (Pérez et al. 2019). In addition, there may be some potential non-visual effects of light on endocrine secretion in

non-mammalian vertebrate brains, such as that of growth hormone or thyroid hormone (Jonathan et al. 2019). The giant mottled eel shows a blue-shifted rod photoreceptor during its upstream migration stage (Wang et al. 2014), which is insensitive to red light. Moreover, eels are nocturnal animals that prefer to stay away from light. Therefore, it is likely that red light or dark surroundings may reduce stress for the giant mottled eel, resulting in better overall growth. The Japanese eel seems to be more insensitive to the environmental spectrum, and this may have resulted in the lack of significant difference in growth among the treatment groups.

Most of the mortality of the two anguillid species in this study, especially that of the giant mottled eel, resulted from their escape from the tank (Tables 2, 3). This might be because the eels are less adapted to a specific wavelength, increasing their stress levels and eliciting an escape response. The Japanese eel may be more tolerant to lights of different colour, and is thus well-adapted to the environment, resulting in a high survival rate. The escape rate was generally high for the giant mottled eel, especially in the dark group. However, it also had the largest growth rate, and stress did not seem to be an important factor. Alternatively, an earlier study suggested that the escape behaviour may be a natural instinct for the giant mottled eel (Matsuda et al. 2016), considering that it prefers to migrate in its early life stage.

Interestingly, the body colour of the giant mottled eel in the red light and dark groups was slightly lighter than those of the others, which is similar to the results of an earlier study (Shin and Choi 2014). It has been shown that the pigments in fish can respond to the wavelength of the colour of their environmental background (Bayarri et al. 2002). Biofilm attachments were found on the tank wall in some groups in our study. There were attachments with a dark brown muddy biofilm on the bottom of the blue, green, and white light tanks, while the red and dark tanks had no attachments. This may have caused the lighter body colour in red and dark groups to adapt the environment without dark attachment.

Lysozyme (*LZM*) is an important enzyme that shows antiviral, antibacterial, and anti-inflammatory activities (Saurabh and Sahoo 2010). *LZM* also combines and metabolizes advanced glycosylation end products produced from reactive oxygen species that would otherwise accumulate

and cause harm to organisms. Similar to the antioxidant system, *LZM* may also be affected by stress responses (Eslamloo et al. 2015; Zheng et al. 2016; Gao et al. 2017; Li et al. 2018). *LZM* expression levels increase under a red light environment in the pikeperch *Sander lucioperca* (Baekelandt et al. 2019). The results of real-time PCR of *LZM* in both the Japanese eel and giant mottled eel showed no significant difference among groups (Fig. 7), suggesting that different light spectra may not have any significant effect on *LZM* expression.

SOD and *POD* are both important components of the antioxidant system, and catalyse the conversion of superoxide into hydrogen peroxide and oxygen to remove reactive oxygen species; they are also key components of the Nrf2 pathway (Fattman et al. 2003; Lin et al. 2008; Shao et al. 2010; Li 2012; Liu et al. 2015; Deyashi and Chakraborty 2016). The real-time PCR results of both eel species revealed that although the dark group showed the highest *SOD* value, there were no significant differences among the groups ($p > 0.05$) (Fig. 6; Fig. 9). *SOD* and *POD* have been used as biomarkers of stress in previous studies (Abele and Puntarulo 2004; Oliva et al. 2012) due to a dramatic change in the mRNA content and activity of *SOD* and *POD* in response to stress (Shin et al. 2011; Choi et al. 2016; Osman et al. 2019). The stress level of each eel species in each background spectrum may not have differed significantly from each other in our study.

IL-6 is a chemical secreted by the immune system (Tanaka et al. 2014). It can stimulate the body tissues to activate immune mechanisms, help the growth of cells, promote the activation of immune cells of the acquired immune system, and direct blood cells to help macrophages to destroy the source of infection (Stefan et al. 2017). An increase in its concentration can lead to a cytokine storm (Ana et al. 2020). The results of real-time PCR for both eel species showed no significant differences among the groups (Fig. 8). This suggested that the expressions of the innate immune genes were not affected by different light spectra in either eel species. The results also showed no significant difference in expression levels between both eel species, which indicates that different spectra only affect the growth of giant mottled eel.

CONCLUSIONS

Growth is not affected by the wavelength of light in Japanese eels. However, it is recommended to avoid rearing giant mottled eels in a short-wavelength environment, and rearing in the red light environment is more suitable for their growth and survival. Neither of the eel species showed any significant difference in the expression of four innate immune-related genes (*LZM*, *SOD*, *POD*, and *IL-6*). The regulatory effects of different light spectra on the immune system and endocrine mechanism of fish require further study.

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