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## The effect of green and red light spectra and their intensity on the oxidative stress and non-specific immune responses in gold-striped amberjack, *Seriola lalandi*

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### ABSTRACT

The present study examined the effects of different light spectra (red and green light-emitting diodes [LEDs]) and light intensities (0.3 and 0.5 W m<sup>-2</sup>) on antioxidant systems (superoxide dismutase, H<sub>2</sub>O<sub>2</sub> and lipid peroxidation) and immunity (lysozyme, melatonin and endorphin) in gold-striped amberjack, *Seriola lalandi*. In groups exposed to green LED, the levels of antioxidant enzyme were significantly lower than in the control, whereas the levels of immune-related parameters were significantly higher compared to other groups. The levels of antioxidant enzyme in individuals exposed to red light increased with increasing light intensity, whereas these levels were not correlated to the intensity of green light and were increased even at low light intensity. These results indicate that exposure to low-intensity green light accelerates the antioxidant system and immune system.

### ARTICLE HISTORY

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### KEYWORDS

Gold-striped amberjack; *Seriola lalandi*; antioxidant enzymes; green LED; immunity; melatonin

## Introduction

Environmental factors such as culture density, salinity, water temperature, photoperiod and light induce physiological stress in fish. Among these, light is the most important factor for controlling the circadian rhythm and endocrine signals by modifying many of the physiological and behavioural changes in organisms such as maturation, reproduction and stress response (Beckmann et al. 1990; Pierce et al. 2008).

Under environmental stress conditions, these effects are generally attributed to reactive oxygen species (ROS) such as superoxide (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), peroxy radicals and hydroxyl radicals (OH<sup>·</sup>). These are generated by an oxidative stress response (Yang & Yeo 2004). ROS overproduction in fish by environmental stress factors can increase lipid peroxidation (LPO) and cause oxidation of nucleic acids and proteins, along with DNA damage. ROS overproduction can also affect cell viability by causing membrane damage and enzyme inactivity, accelerating cell senescence and apoptosis (Roch 1999; Nordberg

& Arnér 2001). ROS may furthermore seriously affect immune function by decreasing lysozyme activity (Wang et al. 2008).

Complex antioxidant defence systems therefore maintain homeostasis and protect aerobic organisms against ROS and subsequent oxidative stress-induced damage (Pandey et al. 2003; George et al. 2004). Antioxidants include enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), and substances with low molecular weight such as glutathione (GSH) and proteins (e.g. metallothionein) (Basha & Rani 2003; Hansen et al. 2006). SOD catalyses dismutation of the superoxide anion radical to  $H_2O_2$  and molecular oxygen ( $O_2$ ), and CAT and GPX are oxidoreductases that break down two molecules of  $H_2O_2$  into two molecules of  $H_2O$  and  $O_2$ , thereby counteracting the toxicity of  $H_2O_2$  (Shull et al. 1991).

Melatonin, produced in the pineal gland, is known to regulate physiological, circadian and seasonal rhythms and furthermore to control reproduction and stress response (Ekström & Meissl 1997; Falcón et al. 2010). Recent studies have reported that melatonin is a broad-spectrum antioxidant more powerful than glutathione in neutralising free radicals and more effective than other antioxidants in protecting cell membranes and enhancing immunity (Gülçin et al. 2009; Carrillo-Vico et al. 2013).

Endorphin, a hormone released by immune cells under the control of the hypothalamic–pituitary–interrenal axis, is furthermore activated by stress and induces an immune reaction in organisms (Blalock 2005).

Several recent studies reported that fish physiological responses are regulated by irradiation from specific wavelength light-emitting diodes (LEDs) (Villamizar et al. 2009; Shin et al. 2011; Choi et al. 2012). LEDs are currently preferred in the fish-farming industry because of their low energy consumption and high efficiency, which in turn improves the efficiency of lighting systems compared with common metal halide light bulbs (Migaud et al. 2007; Villamizar et al. 2009; Choi, Shin et al. 2015).

In their previous studies on LED effects on teleosts, Shin et al. (2011) and Choi et al. (2012) reported that red LEDs light negatively affects physiological processes and induces oxidative stress. However, green and blue LEDs, both of which have short wavelengths, increase the level of antioxidants as a response to oxidative stress.

Research related to the effect of light conditions on target fish generally studies the physiological aspects of different types of light source and different wavelengths in ornamental fish. Investigations on the stress response in fish to light wavelengths and light intensity nevertheless remain very limited.

In this study, we therefore used gold-striped amberjack, *Seriola lalandi*, a large carnivorous fish found in tropical, warm temperate and subtropical waters worldwide (Moran et al. 2007). Additionally, the worldwide demand for gold-striped amberjack is increasing as a high-value species with high meat quality and rapid growth. Aquaculture (breeding) of this species is, however, not easy because it is a large migratory species that is more likely to feel stressed during in-house breeding. Long-term studies conducted on the indoor-bred gold-striped amberjack are thus rarely performed.

We therefore examined the possibility of reducing oxidative stress and enhancing immunity during aquaculture of gold-striped amberjack in breeding tanks. We investigated the possible effects of LED with specific wavelengths and intensity on the control of oxidative stress, including changes in antioxidant genes/protein expression and plasma  $H_2O_2$  and LPO levels in gold-striped amberjack under fluorescent bulb (control) and green and red LEDs at

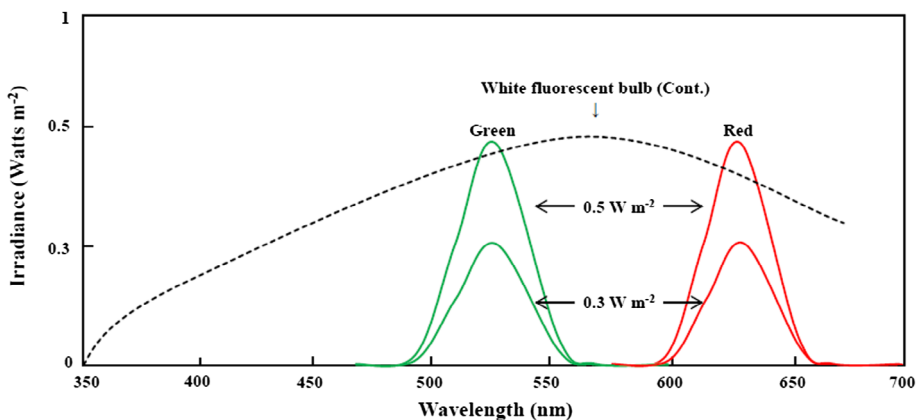
two different intensities ( $0.3$  and  $0.5 \text{ W m}^{-2}$ ) over a period of 4 months. We determined, in addition, the changes in immunity levels in gold-striped amberjack by measuring lysozyme, melatonin and endorphin under the same conditions.

## Materials and methods

### Experimental fish

Gold-striped amberjack, *Seriola lalandi*, (length,  $35.3 \pm 3.3$  cm; mass,  $594 \pm 59$  g) were collected from the Future Aquaculture Research Center, National Fisheries Research and Development Institute (Jeju, Korea) and kept in five 8-ton circulation filter tanks up to 1.5 meter deep prior to the experiments, which were conducted in this same centre on 10 January 2015. Each tank (corresponding to one experimental group) included 30 fish. The experiment was conducted in duplicate. Fish were maintained in outdoor tanks supplied with a continuous flow of seawater, at an ambient temperature, and natural 12-h light:12-h dark photoperiod (lights on at 0700 h and lights off at 1900 h). The fish of the treatment and control groups were fed a commercial feed twice daily (at 0900–1700 h).

The fish in the control group were exposed to light from a white fluorescent bulb, the commonly used light for interior aquaculture (27 W; wavelength range 350–650 nm). The light intensity at the water surface was approximately  $0.96 \text{ W m}^{-2}$ . The fish in the experimental groups were exposed to either green (peak at 530 nm) or red (peak at 630 nm) LEDs (S-tech LED Co., Kyunggi, Korea) for 4 months. These experimental groups are referred to hereafter as green LED and red LED groups, respectively. The LEDs were set 1.5 meters above the water surface, and irradiance at the water surface was maintained at approximately  $0.3$  or  $0.5 \text{ W m}^{-2}$  (Figure 1). The spectral analysis of the lights was performed using a spectroradiometre (FieldSpec®, ASD Inc., Boulder, CO, USA).



**Figure 1.** (Colour online) Spectral profiles of green and red light-emitting diodes (LEDs) and white fluorescent bulb (Cont.) used in this study. Different light intensities ( $0.3$  and  $0.5 \text{ W m}^{-2}$ ) were used for each type of LED. Reprinted from Shin et al. (2011), with permission from Comparative Biochemistry and Physiology, Part-A.

### Tissue sampling

Fish were sampled after 2 and 4 months and were anaesthetised with 2-phenoxyethanol (Sigma, St. Louis, MO, USA) prior to collection. We collected the liver from fish in each group at 1400 h. Immediately after collection, the tissues were frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until total RNA extraction was performed. In addition, blood was collected from the caudal vasculature using a 10-mL syringe coated with heparin. Plasma was separated from the blood sample by centrifugation ( $4^{\circ}\text{C}$ ;  $10,000 \times g$ ; 5 min) and stored at  $-80^{\circ}\text{C}$  until analysis.

### Quantitative real-time PCR (qPCR)

Total RNA was extracted from each sample using TRI Reagent<sup>®</sup> (Molecular Research Center, Inc., Cincinnati, OH, USA) according to the manufacturer's instruction. Reverse transcription was performed using M-MLV reverse transcriptase (Sigma, USA) according to the manufacturer's instructions. The resulting cDNA was diluted and stored at  $4^{\circ}\text{C}$  for use in PCR.

qPCR was conducted to determine the relative expression of SOD mRNA (GenBank Accession No. **KT229634**) and CAT mRNA (GenBank Accession No. **KT229635**) using total RNA extracted from the liver of gold-striped amberjack. Primers for qPCR were designed with reference to the known sequences of gold-striped amberjack: SOD forward (5'-GTC ATC GGC TCC ATC TTC-3') and reverse (5'-GTT TAT CGT ATT TGA GGC TGT G-3') primer set; CAT forward (5'-ATC TTA CAG GAA ACA ACA CCC-3') and reverse (5'-CGA TTC AGG ACG CAA ACT-3') primer set; and  $\beta$ -actin forward (5'-CCA ACA GGG AGA AGA TGA C-3') and reverse (5'-TAC GAC CAG AGG CAT ACA-3') primer set. PCR amplification was conducted using a Bio-Rad CFX96<sup>™</sup> Real-time PCR Detection System (Bio-Rad, Hercules, CA, USA) and an iQ<sup>™</sup> SYBR Green Supermix (Bio-Rad, USA) according to the manufacturer's instructions. qPCR included the following steps: one cycle of denaturation at  $95^{\circ}\text{C}$  for 5 min, 35 cycles each consisting of denaturation at  $95^{\circ}\text{C}$  for 20 s, and annealing and extension at  $55^{\circ}\text{C}$  for 20 s. All analyses were based on the cycle threshold ( $\Delta\text{Ct}$ ) values of the PCR products. The Ct was defined as the PCR cycle at which the fluorescence signal crossed a threshold line that was placed in the exponential phase of the amplification curve. After the PCR program, qPCR data from three replicate samples were analysed with PCR analysis software of the Bio-Rad CFX96<sup>™</sup> Real-time PCR Detection System (Bio-Rad, USA) to estimate transcript copy numbers for each sample. In addition, to ensure that the primers amplified a specific product, we performed a melting curve analysis, which confirmed that the products of each primer pair had a single melting point (only one temperature). Experiments with  $\beta$ -actin served as internal control; they were conducted in triplicates and all data were expressed relative to the corresponding  $\Delta\text{Ct}$  levels of  $\beta$ -actin. The calibrated  $\Delta\Delta\text{Ct}$  value ( $\Delta\Delta\text{Ct}$ ) for samples (SOD and CAT) and internal controls ( $\beta$ -actin) were calculated as:  $\Delta\Delta\text{Ct} = 2^{-(\Delta\text{Ct}_{\text{sample}} - \Delta\text{Ct}_{\text{internal control}})}$ .

### Western blot analysis

Total protein isolated from the liver of gold-striped amberjack was extracted using a T-PER<sup>®</sup> Tissue Protein Extraction Reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's instructions. A total of 30  $\mu\text{g}$  of protein was loaded per lane onto Mini-PROTEAN<sup>®</sup> TGX<sup>™</sup> Gels (Bio-Rad, USA), and a protein ladder (Bio-Rad, USA)

was used for reference. Samples were electrophoresed at 180 V, and the gels were immediately transferred to a 0.2- $\mu\text{m}$  polyvinylidene difluoride membrane (Bio-Rad, USA) at 85 V for 3 min using a Trans-Blot® Turbo™ Transfer System (Bio-Rad, USA). The membranes were blocked thereafter with 5% milk in Tris-buffered saline (TBS) (pH 7.4) for 45 min, after which they were washed in TBS. Membranes were incubated with SOD (1:2000 dilution, NBP1-47443, Novus Biologicals, Littleton, CO, USA) and CAT (1:2000 dilution, sc-58332, Santa Cruz Biotechnology, Dallas, TX, USA) antibodies, followed by horseradish peroxidase conjugated anti-mouse IgG secondary antibody (dilution 1:2000, Bio-Rad, USA) for 60 min each. The internal control was incubated with  $\beta$ -tubulin (dilution 1:5000, ab6046, Abcam, Cambridge, UK) antibody, followed by horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody (1:5000; Bio-Rad, USA) for 60 min each. Bands were detected using a WesternBright™ ECL substrate (Advanta, Menlo Park, CA, USA) and an exposure for 30 s in a Molecular Imager® ChemiDoc™ XRS + Systems (Bio-Rad, USA). The membrane images were scanned by a high-resolution scanner, and the band density was estimated using a computer program (Image Lab™ Software, version 3.0, Bio-Rad, USA).

### **Antioxidant activity analysis**

Plasma samples were used for the analyses of SOD and CAT levels. SOD and CAT activities were determined using a Fish Superoxide Dismutase (SOD) and a Fish Catalase (CAT) ELISA kits (Cusabio Biotech Co., Ltd., Wuhan, China).

### **Plasma parameter analysis**

Plasma samples were analysed for  $\text{H}_2\text{O}_2$ , LPO, lysozyme, melatonin and endorphin levels.

$\text{H}_2\text{O}_2$  concentrations were measured using a PeroxiDetect kit (Sigma, USA). Absorbance was read at 560 nm, and the concentration of  $\text{H}_2\text{O}_2$  was interpolated from a standard curve and expressed as  $\text{nM ml}^{-1}$ .

LPO, lysozyme, melatonin and endorphin levels were analysed by the immunoassay technique using ELISA kits: Fish lipid Peroxide (MyBioSource, San Diego, CA, USA), Fish lysozyme (Cusabio Biotech, China), Fish melatonin (MyBioSource, USA) and Fish beta endorphin (MyBioSource, USA), respectively. An anti-antibody specific to the antibodies of LPO, lysozyme, melatonin and endorphin was pre-coated onto a microplate. There was 50  $\mu\text{l}$  of plasma per well; subsequently, 50  $\mu\text{l}$  of horseradish peroxidase conjugate and 50  $\mu\text{l}$  of corresponding antibody was added to each well. The samples were mixed well and then incubated for 2 h at 37 °C. After the final wash, any remaining wash buffer was removed by aspirating or decanting. Subsequently, 50  $\mu\text{l}$  of both substrate A and substrate B were added to each well and the samples were incubated for 15 min at 37 °C in the dark. After incubation, 50  $\mu\text{l}$  of stop solution was added to each well. Finally, the optical density of each well was determined within 10 min using a microplate reader set to 450 nm.

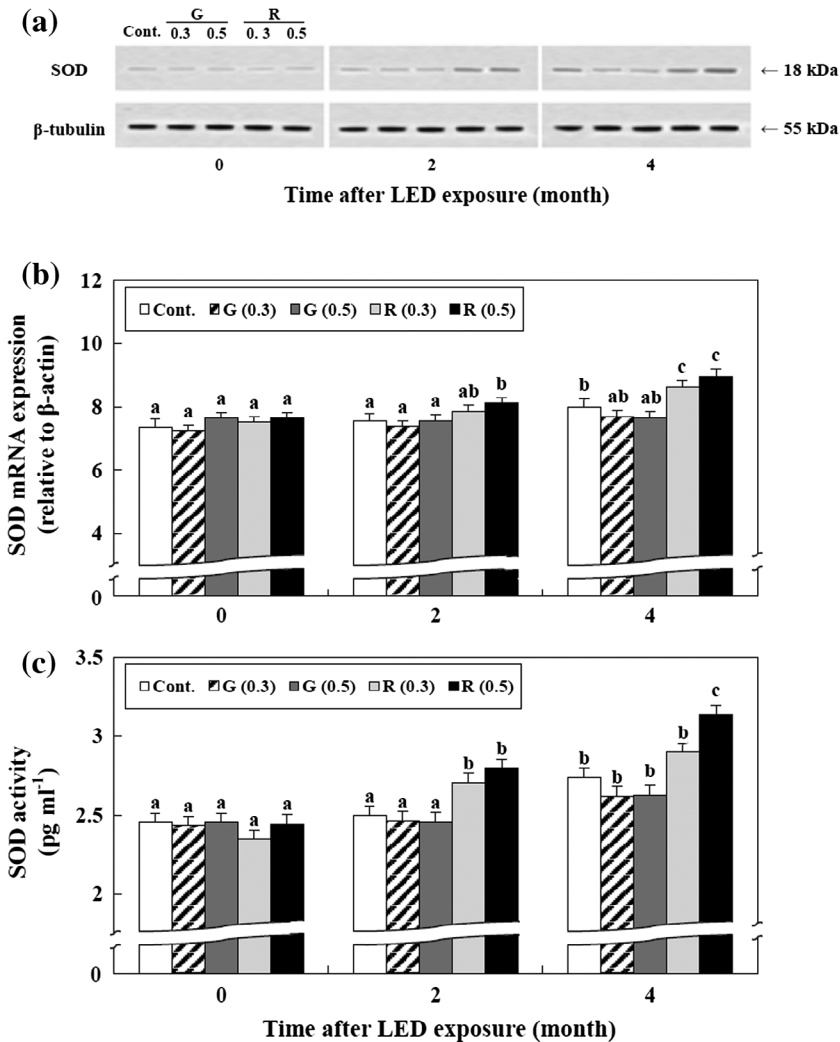
### **Statistical analysis**

All data were analysed using the SPSS statistical package (version 10.0; SPSS Inc., USA). A two-way ANOVA followed by Tukey's *post hoc* test was used to compare differences in the data ( $p < 0.05$ ). The values are expressed as the means  $\pm$  standard error (SE).

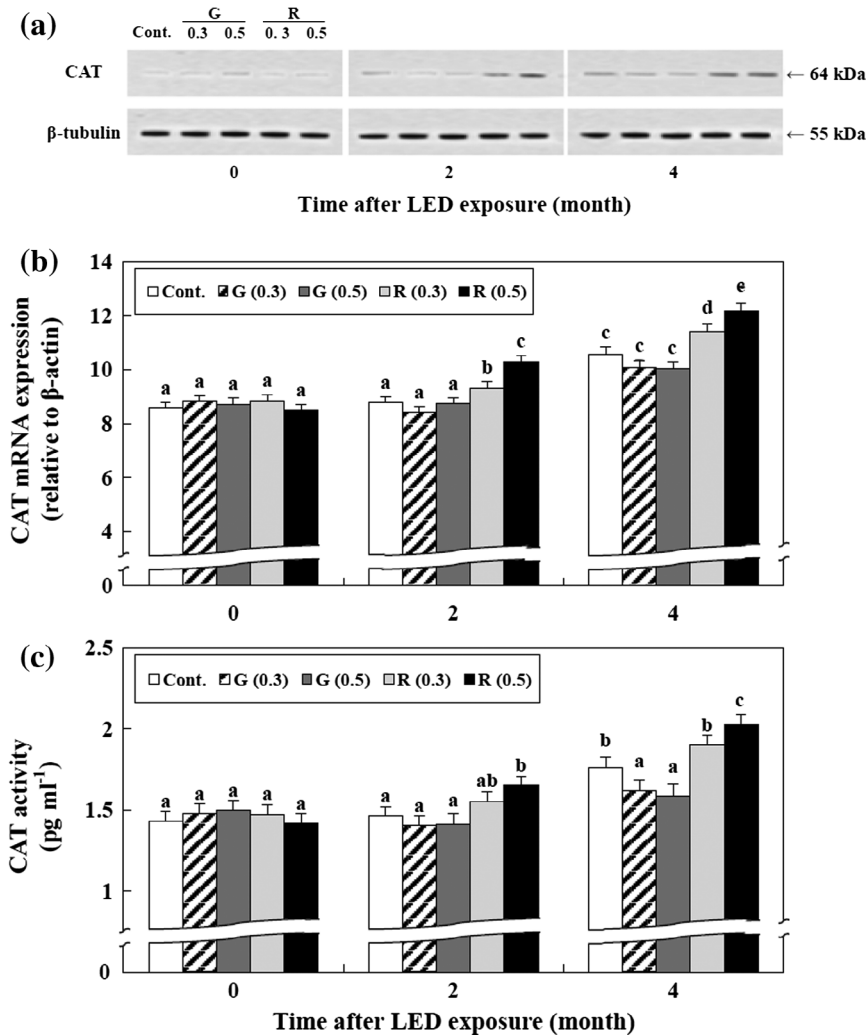
## Results

### Expression and activity of antioxidant enzymes (SOD and CAT)

The activity, mRNA expression and protein levels of SOD and CAT in liver tissue and plasma increased during the 4 months in red LED groups (Figures 2 and 3). The expressions of mRNA in fish exposed to  $0.5 \text{ W m}^{-2}$  red LED were especially significantly higher compared to those in fish exposed to  $0.3 \text{ W m}^{-2}$  red LED (Figures 2(b) and 3(b)). In contrast, in green



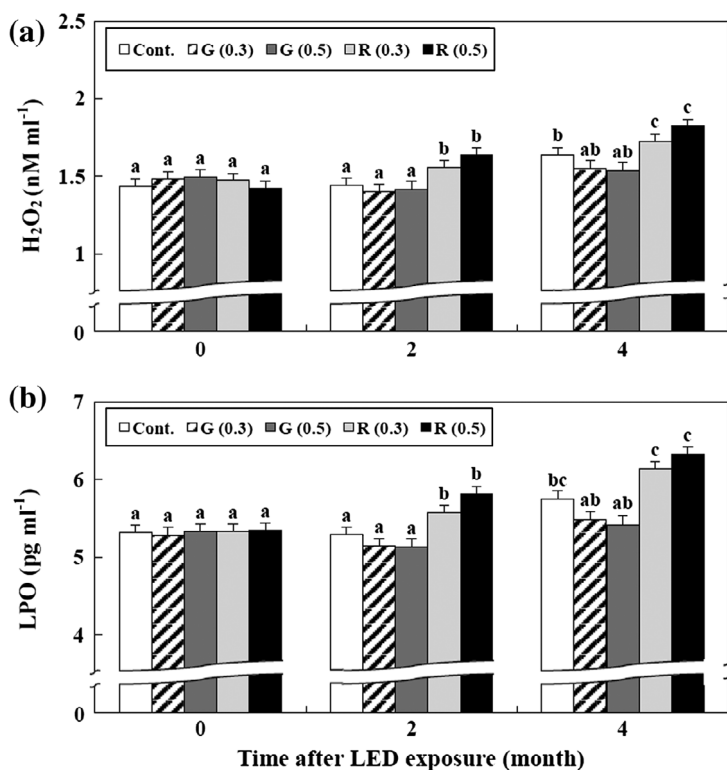
**Figure 2.** Expression and activity of superoxide dismutase (SOD) in the liver and plasma of gold-striped amberjack exposed to green (G) and red (R) light-emitting diodes (LEDs) at different light intensities ( $0.3$  and  $0.5 \text{ W m}^{-2}$ ) and to white fluorescent bulb (Cont.). (a) Western blotting using SOD ( $18 \text{ kDa}$ ) to examine protein expression in the liver,  $\beta$ -tubulin ( $55 \text{ kDa}$ ) was used as internal control. (b) SOD mRNA levels relative to  $\beta$ -actin mRNA levels in the same sample. (c) Plasma activity of SOD was also analysed with a plate reader. Small letters indicate significant differences among different wavelengths ( $p < 0.05$ ). All values are means  $\pm$  SE ( $n = 5$ ).



**Figure 3.** Expression and activity of catalase (CAT) in the liver and plasma of gold-striped amberjack exposed to green (G) and red (R) light-emitting diodes (LEDs) at different light intensities (0.3 and  $0.5 \text{ W m}^{-2}$ ) and to white fluorescent bulb (Cont.). (a) Western blotting using CAT (64 kDa) to examine protein expression in the liver,  $\beta$ -tubulin (55 kDa) was used as internal control. (b) CAT mRNA levels relative to  $\beta$ -actin mRNA levels in the same sample. (c) Plasma activity of CAT was also analysed with a plate reader. Small letters indicate significant differences among different wavelengths ( $p < 0.05$ ). All values are means  $\pm$  SE ( $n = 5$ ).

LED groups, the levels of antioxidant enzymes were significantly lower compared to those in the control and the red LED groups. Western blot analysis revealed two proteins with antioxidant enzyme-specific immunoreactivity and masses, which corresponded to the predicted mass of gold-striped amberjack antioxidant enzymes SOD, 18 kDa, and CAT, 64 kDa (Figures 2(a) and 3(a)). Furthermore, the protein expression patterns of SOD and CAT resembled the mRNA expression patterns in gold-striped amberjack liver. Similar to the mRNA expression of antioxidant enzymes, the activity of antioxidant enzymes was significantly higher following exposure to red LED at  $0.5 \text{ W m}^{-2}$  (Figures 2(c) and 3(c)).





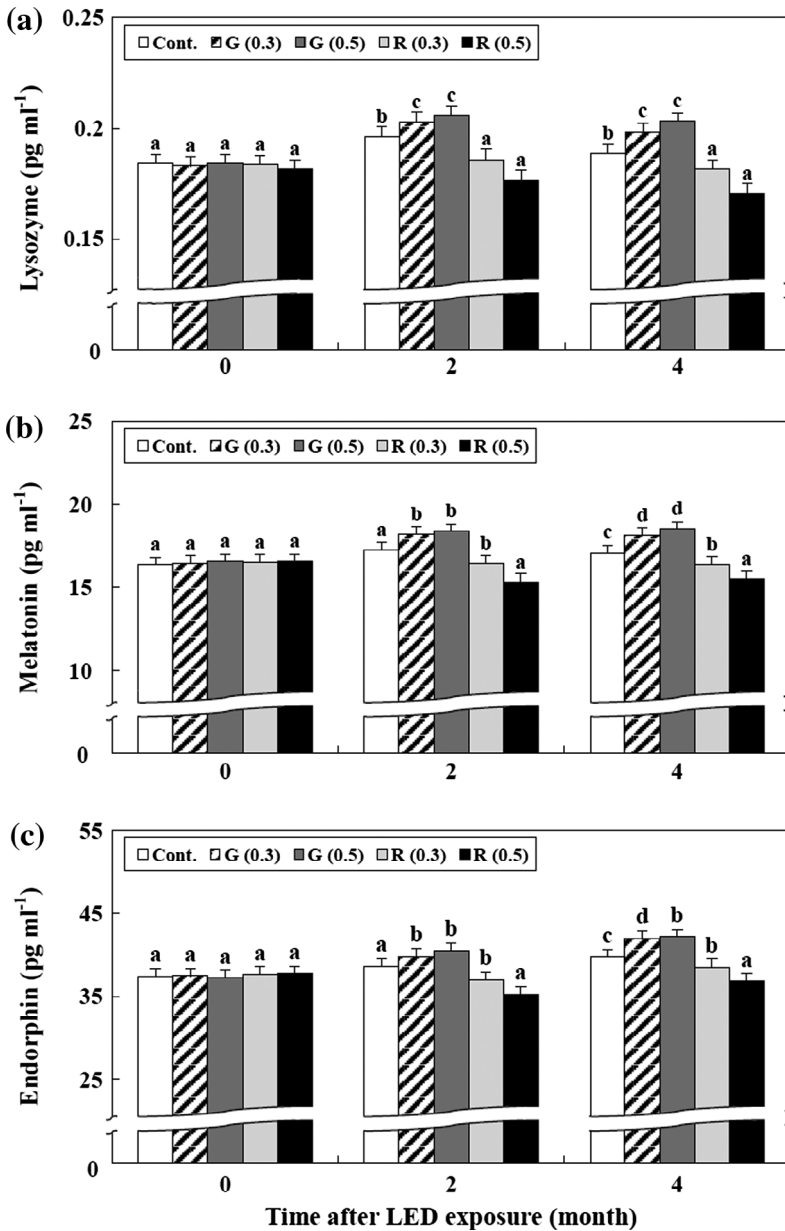
**Figure 4.** Plasma activity of H<sub>2</sub>O<sub>2</sub> (a) and lipid peroxidation (LPO) (b) in gold-striped amberjack exposed to green (G) and red (R) light-emitting diodes (LEDs) at different light intensities (0.3 and 0.5 W m<sup>-2</sup>) and to white fluorescent bulb (Cont.). Small letters indicate significant differences among different wavelengths ( $p < 0.05$ ). All values are means  $\pm$  SE ( $n = 5$ ).

### Plasma H<sub>2</sub>O<sub>2</sub> and LPO concentration

The plasma H<sub>2</sub>O<sub>2</sub> and LPO levels were  $1.43 \pm 0.04$  pg ml<sup>-1</sup> and  $5.31 \pm 0.09$  pg ml<sup>-1</sup>, respectively, at the start of the experiment (Figure 4). After 4 months of exposure to 0.5 W m<sup>-2</sup> red LED, the levels of H<sub>2</sub>O<sub>2</sub> and LPO increased to  $1.81 \pm 0.05$  nM ml<sup>-1</sup> (approximately 1.62-fold increase compared to the control) and  $6.32 \pm 0.12$  pg ml<sup>-1</sup> (approximately 1.2-fold increase compared to the control), respectively. The levels of H<sub>2</sub>O<sub>2</sub> and LPO in green LED group were significantly lower compared to those in the control group.

### Plasma lysozyme, melatonin and endorphin levels

The plasma lysozyme, melatonin and endorphin levels were  $0.18 \pm 0.01$  pg ml<sup>-1</sup>,  $16.35 \pm 0.45$  pg ml<sup>-1</sup> and  $37.44 \pm 0.89$  pg ml<sup>-1</sup>, respectively, at the start of the experiment (Figure 5). After 4 months of exposure to green LED (0.3 and 0.5 W m<sup>-2</sup>), the levels of lysozyme, melatonin and endorphin increased to  $0.20 \pm 0.01$  pg ml<sup>-1</sup> (approximately 1.11-fold increase compared to the control),  $17.47 \pm 0.48$  pg ml<sup>-1</sup> (approximately 1.62-fold increase compared to the control) and  $37.07 \pm 0.89$  pg ml<sup>-1</sup> (approximately 1.06-fold increase compared to the control group), respectively. However, in red LED group, the levels



**Figure 5.** Levels of plasma lysozyme (a), melatonin (b), and endorphin (c) in gold-striped amberjack exposed to green (G) and red (R) light-emitting diodes (LEDs) at different light intensities (0.3 and  $0.5 \text{ W m}^{-2}$ ) and to white fluorescent bulb (Cont.). Small letters indicate significant differences among different wavelengths ( $p < 0.05$ ). All values are means  $\pm$  SE ( $n = 5$ ).

of lysozyme, melatonin and endorphin were significantly decreased to  $0.16 \pm 0.01 \text{ pg ml}^{-1}$ ,  $14.35 \pm 0.45 \text{ pg ml}^{-1}$  and  $35.44 \pm 0.89 \text{ pg ml}^{-1}$ , respectively, after 4 months of exposure to red LED.

## Discussion

In this study, we examined the effects of long-term exposure (4 months) to white fluorescent bulb (control) and different intensities of green and red LED wavelengths (0.3 and 0.5 W m<sup>-2</sup>) on oxidative stress and immunity of gold-striped amberjack during its aquaculture.

We found that gene expression and activity of antioxidant enzymes SOD and CAT in the liver of gold-striped amberjack was significantly decreased after 4 months of exposure to green LED compared to the fluorescent bulb, regardless of light intensity. In contrast, the gene expression and activity of SOD and CAT was significantly increased as the intensity of red LED increased from 0.3 to 0.5 W m<sup>-2</sup>. Among the major antioxidant enzymes, SOD and CAT are known to remove directly ROS: first, SOD transfers O<sub>2</sub><sup>-</sup> through the process of dismutation to O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub>, and then H<sub>2</sub>O<sub>2</sub>, produced by SOD (2O<sub>2</sub><sup>-</sup> + H<sup>+</sup> → H<sub>2</sub>O<sub>2</sub> + O<sub>2</sub>), is sequentially reduced to H<sub>2</sub>O and O<sub>2</sub> by CAT (2H<sub>2</sub>O<sub>2</sub> → 2H<sub>2</sub>O + O<sub>2</sub>) (Kashiwagi et al. 1997).

This study corroborates the study by Choi et al. (2012) which reported that the expression of antioxidant genes and protein levels in green LED groups was significantly lower compared to the control and red LED groups during the exposure to fluorescent bulb (control) and red, green and blue LEDs in a 12-day starvation period in the cinnamon clownfish, *Amphiprion melanopus*.

Similar to the previous study, we suggest that green light decreases the oxidative stress in gold-striped amberjack, whereas red light induces oxidative stress. Furthermore, increasing the intensity of red light increases the levels of oxidative stress in gold-striped amberjack, but green wavelengths effectively reduce the oxidative stress regardless of light intensity. In the present study, the levels of plasma H<sub>2</sub>O<sub>2</sub> and LPO significantly decreased in the green LED experimental group but significantly increased in the red LED experimental group. The plasma H<sub>2</sub>O<sub>2</sub> and LPO levels in green LED groups did not, however, vary significantly with light intensity, whereas in red LED experimental groups, these levels increased significantly as the light intensity increased (0.3 → 0.5 W m<sup>-2</sup>).

The biologically produced H<sub>2</sub>O<sub>2</sub> is capable of damaging molecules of different biochemical classes including nucleic acids and amino acids, and exposure of proteins to ROS results in denaturation, loss of function, cross-linking, aggregation, and fragmentation of connective tissues such as collagen. However, the most damaging effect is the induction of LPO (Chance et al. 1979). The cell membrane, which is composed of polyunsaturated fatty acids, is a primary target for ROS leading to cell membrane damage (Boveris et al. 2008). Shin et al. (2011) reported that wavelengths emitted from red LED increased the plasma H<sub>2</sub>O<sub>2</sub> compared to other wavelength groups in clownfish, *Amphiprion clarkii*. Choi, Yang et al. (2015) also reported that high concentrations of selenium significantly induced the occurrence of oxidative stress and increased H<sub>2</sub>O<sub>2</sub> and LPO levels in goldfish, *Carassius auratus*. Similar to previous studies, the results presented here therefore suggest that red wavelengths induce the oxidative stress and green wavelengths reduce the oxidative stress effectively in gold-striped amberjack.

Furthermore, we observed the change in immunity measured through the levels of plasma lysozyme, melatonin and endorphin after exposure to different intensities of red and green LEDs. The plasma lysozyme, melatonin and endorphin levels of green LED groups increased significantly, although there was no significant difference in the levels between the two light intensities (0.3 and 0.5 W m<sup>-2</sup>). In red LED groups, however, as the

light intensity increased ( $0.3 \rightarrow 0.5 \text{ W m}^{-2}$ ), the plasma lysozyme, melatonin and endorphin levels significantly decreased. These results are in agreement with those of Choi et al. (2012), who, in their experiments with starved cinnamon clownfish exposed to fluorescent bulb (control) and LEDs (red, green and blue), reported that red LED significantly decreased the lysozyme levels and green LED induced the lysozyme levels. Wang et al. (2008) reported that environmental factors such as rapid change in salinity induce oxidative stress and decrease the plasma lysozyme levels in the sea cucumber, *Apostichopus japonicas*. We thus conclude that red light reduces the immunity as the intensity of the light increases, whereas green light enhances the immunity regardless of the light intensity.

In summary, we conclude the following: (1) Red wavelengths induce oxidative stress and oxidative stress increases with increasing the red light intensity. (2) Green wavelengths reduce oxidative stress, promote antioxidants synthesis and enhance immunity even at the low light intensity of  $0.3 \text{ W m}^{-2}$ . The results of this study provide a reference for future studies on the effects of different wavelengths and light intensities on physiological activity in other species.

### Disclosure statement

No potential conflict of interest was reported by the authors.

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