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Effect of Different Wavelengths of Light on the Antioxidant and Immunity Status of Juvenile Rock Bream, *Oplegnathus fasciatus*, Exposed to Thermal Stress

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Abstract – We investigated the effect of light wavelengths on antioxidant and immunity parameters in juvenile rock bream, *Oplegnathus fasciatus*, exposed to thermal stress (25 and 30°C). We exposed the fish to light emitting diodes (LEDs) emitting green (520 nm) and red light (630 nm) of 0.25 and 0.5 W/m^2 intensity, and measured the activity, and mRNA and protein expression levels of the antioxidant enzymes, superoxide dismutase, catalase, and glutathione peroxidase. We also determined the levels of plasma hydrogen peroxide (H_2O_2), melatonin, and lysozyme. Furthermore, the mRNA and protein levels of caspase-3 were measured and terminal transferase dUTP nick end labeling (TUNEL) assays were performed. We observed that mRNA expression and activities of antioxidant enzymes and plasma H_2O_2 levels were significantly higher after exposure to high temperatures. However, increases in these parameters were significantly lower after exposure to green LED light. The plasma melatonin and lysozyme levels were significantly lower in the different groups after exposure to high temperatures; however, in groups exposed to green LED light, their levels were significantly higher than those in the control group. The expression pattern of caspase-3 mRNA was similar to that of H_2O_2. The TUNEL assay showed that apoptosis was markedly higher at higher water temperatures than that at 20°C. These results indicate that high water temperatures induce oxidative stress and decrease the immunity in juvenile rock bream but green LED light inhibits the rise in oxidative stress and combats the decrease in immunity and should, thus, be useful in the culture of rock bream.

Keywords – antioxidant, immunity, light wavelength, juvenile rock bream, thermal stress

1. Introduction

Water temperature is an important factor involved in the growth, immunity, maturation, and in the physiological adjustment of fish (Maule et al. 1989; Bly and Clem 1992; Bowden 2008). Rapid change in temperature is an environmental stress for fish that causes an increase in reactive oxygen species (ROS), such as superoxide (O_2^-) anion, hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), and singlet oxygen ($^1O_2$) (Roch 1999). The excessive production of ROS as a result of environmental stress induces physiological disorders, such as a decrease in disease resistance and reproductive ability because of denaturation of cellular nucleic acids and proteins and loss of their functions, as well as because of the promotion of lipid peroxidation that adversely damages cell membrane and affects cell viability (Oldham and Bowen 1998; Pandey et al. 2003). In addition, ROS is known to have a negative effect on immune function because it decreases the activity of lysozyme (Wang et al. 2008).

Living organisms possess antioxidant defense mechanisms to protect themselves from oxidative stress caused by ROS and to maintain homeostasis. These antioxidant defense mechanisms mainly involve the activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) (Mcfarland et al. 1999). Such antioxidant enzymes are known to exert an anti-oxidative action, mainly in the liver and kidney of an organism (Basha and Rani 2003; Hansen et al. 2006). Firstly, SOD temporarily eliminates the active oxygen by converting O_2^- into O_2 and H_2O_2 ($2O_2^- + H^+ \rightarrow H_2O_2 + O_2$), and, thereafter, H_2O_2, which is also an active oxygen species, is converted to non-toxic H_2O and O_2 by CAT ($2H_2O_2 \rightarrow 2H_2O + O_2$) (Kashiwagi et al. 1997).

The immunity of fish is particularly influenced by external factors, such as change in water temperature due to the

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environmental characteristics of the habitat (Magnadottir 2010). Lysozyme, which is one of the important indicators of the level of immunity, is known to destroy the invading bacterial pathogens by damaging their cell wall during the process of phagocytosis (Saurabh et al. 2008; Shin et al. 2014). In addition, melatonin, a powerful antioxidant, has been reported to function in the direct removal of ROS as well as in enhancing immunity (Reiter et al. 1997; Gülçin et al. 2009; Carrillo-Vico et al. 2013).

Apoptosis, which is characterized by DNA fragmentation and cellular shrinkage, akin to morphological incidents, is regulated by caspases belonging to the cysteine protease family (Alnemri 1997). Caspase-3 is known to play an important role in the process of apoptosis, which is influenced by DNA damage and inflammation caused by biochemical and morphological processes (Häcker 2000).

The stress and immune responses of fish are mainly controlled by the endocrine system. Light is one of the environmental factors that greatly influence the endocrine system (Pierce et al. 2008; Jin et al. 2009). Recently, various physiological effects of different wavelengths of light, obtained from light emitting diodes (LEDs), on fish have been studied (Villamizar et al. 2009; Choi et al. 2012; Kim et al. 2016). Studies have shown that the wavelength and intensity of a specific LED play a role in homeostasis, immunity, maturation, and growth of fish (Karakatsouli et al. 2008; Kim et al. 2016). LEDs have been demonstrated to be effective for use in fish culture (Villamizar et al. 2009; Choi et al. 2015).

Rock bream is important to the aquaculture industry as one of the major species in East Asian coasts, including those of Korea and Japan. It is a typical sub-tropical fish that lives in shallow coastal areas with water temperatures around 20–22°C (Oh et al. 2007; Park et al. 2015). This species is sensitive to temperature changes; especially in the summer, immunity is reduced due to high temperature stress, and this frequently causes widespread death (Choi et al. 2010). Thus, this study was conducted to investigate the effects of high temperature changes on juvenile rock bream, because the juveniles are more sensitive to temperature changes than the adult fish (Zhang et al. 2013).

In the present study, we investigated the effects of specific wavelengths of light in the regulation of oxidative stress induced by an environment of high temperature, by analyzing the changes in mRNA and protein expression and the enzymatic activities of SOD, CAT, and GPX, in juvenile rock bream exposed to a high temperature environment (25 and 30°C) and different sources (fluorescent, green, and red LEDs) as well as different intensities (0.25 and 0.5 W/m^2) of light. In addition, we measured H_2O_2 levels to determine the level of fluctuation in stress under the different conditions, and also measured the concentrations of lysozyme and melatonin as immunological indicators. We also measured the DNA damage in liver cells of fish as a consequence of apoptotic activity by analyzing the changes in the expression and activity of caspase-3 mRNA and by conducting a terminal transferase dUTP nick end labeling (TUNEL) assay.

2. Materials and Methods

Experimental fish and environmental conditions

For each experiment, juvenile rock bream (n = 225; length, 10.6 ± 1.1 cm; mass, 8.7 ± 0.7 g) were purchased from a commercial aquarium (Jeju, Korea) and were allowed to acclimate in eleven 100-L circulation filter tanks in the laboratory. There were 45 tanks (three tanks each for exposure to 5 different wavelengths at 20, 25, and 30°C), with five fish in each tank. The fish in the control group were exposed to a white fluorescent bulb. For the experimental groups, the fish were exposed to either green (520 nm) or red (630 nm) LEDs (Daesin LED Co. Kyunggi, Korea), maintained at an intensity of approximately 0.25 or 0.5 W/m^2 in both cases (Fig. 1). The LEDs were placed 50 cm above the water surface and the depth of the water column was 50 cm. The irradiance level in the water column, in the tanks illuminated with the external light source was maintained at approximately 0.25 or 0.5 W/m^2, as determined using a spectrometer (MR-16; Rainbow Light Technology Co. Ltd., Taoyuan, Taiwan)

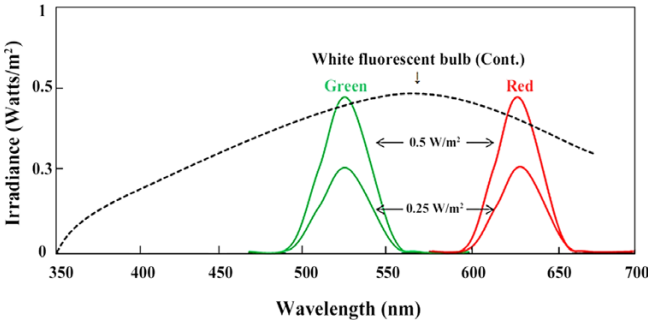

Fig. 1. Spectral profiles of light emitting diodes (LEDs; green, 520 nm; red, 630 nm) and white fluorescent bulb (Cont.) used in this study. Each LED light source was set at two intensities (low, 0.25 and high, 0.5 W/m^2). Reprinted from Shin et al. (2011), with permission from Comparative Biochemistry and Physiology, Part-A

and PHOTO-RADIOMETER (HD 2102.1; Delta OMH CO., Caselle di Selvazzano, Italy). The photoperiod consisted of a 12-h light (L):12-h dark (D) cycle, with the photo-phase lasting from 07:00 to 19:00 h (the lights were turned on at 07:00 h and turned off at 19:00 h). The juvenile rock bream were reared in the presence of an automatic temperature regulation system (JS-WBP-170RP; Johnsam Co., Seoul, Korea) and were allowed to acclimatize to the conditions for 24 h after transfer to the tanks. The fish were acclimated in the tanks for 24 hours, and the control group was sampled immediately after adaptation. Thereafter, the tanks were illuminated with light of different wavelengths in 12-h light:12-h dark cycles. The water temperature was increased by 1°C per day 20°C to 25 and 30°C in each tank. There was no death of fish in any of the groups. Moreover, because the period of this study was short (10 days), no change in the growth of rock bream was observed. The fish received commercial feed twice daily until the day prior to sampling. The sampling was performed at the experimental temperatures (20, 25, and 30°C). All the fish were anesthetized using tricaine methanesulfonate (MS-222; Sigma, St. Louis, MO, USA) and were decapitated prior to tissue collection. Liver samples were collected, immediately frozen in liquid nitrogen, and stored at -80°C until total RNA was extracted for analysis. Blood samples were separated by centrifugation (4°C, 10,000 × g for 5 min) and stored at -80°C until the analysis.

Total RNA extraction, cDNA synthesis, and quantitative real-time PCR (qPCR)

Total RNA was extracted from each sample (15 fish per experimental group) using the Trizol kit (Molecular Research Center, Inc., Cincinnati, OH, USA), according to the manufacturer's instructions. The concentration and purity of the RNA samples were determined by UV spectroscopy at 260 and 280 nm. Two micrograms of total RNA was reverse transcribed in a total volume of 20 μL, using an oligo-d (T) anchor and M-MLV reverse transcriptase (Promega, Madison, WI, USA), according to the manufacturer's protocol. The resulting cDNA was diluted and stored at 4°C for use in quantitative PCR. The qPCR analysis was conducted to determine the relative expression levels of the mRNAs of the antioxidant enzymes, SOD, CAT, GPX, and caspase-3, using the total RNA extracted from the liver of juvenile rock bream. The qPCR primer pairs were designed to span the spliced exon–exon junctions using the known juvenile rock bream sequences (Table 1). The qPCR amplification was performed using a Bio-Rad iCycler iQ multicolor real-time PCR detection system (Bio-Rad, Hercules, CA, USA) and the iQ SYBR green Supermix (Bio-Rad), following the manufacturer's instructions. As a control, β-actin gene was also amplified for each sample, and all the data were expressed in terms of their difference with the corresponding values calculated for the β-actin threshold cycle (Ct). The Ct values of the PCR products formed the basis for all the analyses. The Ct values were defined as the PCR cycle in which the fluorescence signal crossed a threshold during the exponential phase of the amplification curve. The calibrated ΔCt value (ΔΔCt) per sample and that for their internal control (β-actin) were calculated as follows: $[\Delta\Delta Ct = 2^{\wedge}\text{-}(\Delta Ct_{sample} - \Delta Ct_{internal\ control})]$. The qPCR data from three replicate samples were analyzed using CFX96™ Real Time System (Bio-Rad) to estimate the transcript copy numbers in each sample.

Western blot analysis

The total protein isolated from liver samples of juvenile rock bream (15 fish per experimental group) was extracted using a T-PER® Tissue Protein Extraction Reagent (Thermo

Table 1. Primers used for QPCR amplification

Genes (accession no.)	Primer	DNA sequences
SOD (JN593103)	Forward	5′-TGA CCT GAC CTA CGA CTA TG-3′
	Reverse	5′-GCC TCC TGA TAT TTC TCC TCT-3′
CAT (AY734528)	Forward	5′-GTG CTG AAC GAA GAG GAG-3′
	Reverse	5′-TTG TTG AGA AGA GTC TGA ACC-3′
GPX (AY734530)	Forward	5′-GAT GTG AAC GGA CAG GAT G-3′
	Reverse	5′-ACT GAC GGG ACT CCA AAT-3′
Caspase-3 (JQ315116)	Forward	5'-CTT CTT CTA CGC CTT CTC-3'
	Reverse	5'-TGA GTA GTA GCC TGT GGA-3'
β-actin (FJ975145)	Forward	5'-CAG AGC AAG AGA GGT ATC C-3'
	Reverse	5'-TCG TTG TAG AAG GTG TGA TG-3'

Fisher Scientific, Inc., Waltham, MA, USA), according to the manufacturer's instructions. A total of 30 µg protein was loaded in each lane of Mini-PROTEAN® TGX™ Gels (Bio-Rad), and a protein ladder (Bio-Rad) was used as a reference. The samples were electrophoresed at 180 V, and were immediately transferred from the gels onto a 0.2-µm polyvinylidene difluoride membrane (Bio-Rad) at 85 V for 3 min using a Trans-Blot® Turbo™ Transfer System. The membranes were subsequently blocked with 5% milk in Tris-buffered saline (TBS) (pH 7.4) for 45 min, after which they were washed in TBS. The membranes were then incubated with antibodies against SOD (1:2000 dilution, NBP1-47443, Novus Biologicals, USA), CAT (1:2000 dilution, SC-58332, Santa Cruz Biotechnology, USA), and GPX (1:2000 dilution, CPBT-35941RH, Creative Diagnostics, USA), and were incubated, thereafter, with horseradish peroxidase-conjugated anti-mouse IgG secondary antibody (1:2000 dilution, Bio-Rad) for 60 min. β-tubulin (probed using an anti-β-tubulin antibody, ab6046, Abcam, UK, at 1:4000 dilution) was used as an internal control. The bands were detected using Western Bright™ ECL (Advansta, Menlo Park, CA, USA) and were visualized by 30-s exposure in a Molecular Imager® (ChemiDoc™ XRS+ Systems, Bio-Rad). The images of the blot were scanned using a high-resolution scanner and the band density was estimated using a computer program (Image Lab™ Software, version 3.0, Bio-Rad).

Analysis of plasma parameters

Plasma (from five fish per experiment group in triplicate) was separated from blood samples by centrifugation (4°C, 10,000 × g, for 5 min). The H_2O_2 levels were measured using a modified version of the methods described by Nouroozzadeh et al. (1994), and in the instruction manual of PeroxiDetect kit (Sigma). The absorbance was read at 560 nm, and the concentration of H_2O_2 was interpolated from a standard curve. The concentrations were expressed as nM/mL.

The SOD, CAT, and GPX activities were determined by immunoassays using specific ELISA kits (SOD, CSB-E15929fh; CAT, CSB-E15928fh; Cusabio Biotech Co., Ltd., China; GPX, MBS0924388; Mybiosource Inc., San Diego, California, USA).

The plasma melatonin, lysozyme, and caspase-3 levels were analyzed using immunoassay ELISA kits (melatonin, MBS013211; Mybiosource; lysozyme, CSB- E17296Fh, Cusabio Biotech; caspase-3, MBS012786, Mybiosource). The absorbance was read at 450 nm.

Terminal transferase dUTP nick end labeling (TUNEL) assay

To evaluate the apoptotic response of the fish liver cells to green LED light, we performed the TUNEL assay using a commercially available *in situ* cell death detection kit (catalogue number, 11 684 795 910, Roche, Switzerland). Polylysine-coated slides were used to prevent the loss of adherence of the apoptotic cells to the slides. The fish liver tissue was washed and fixed with 4% buffered paraformaldehyde, and was permeabilized with freshly prepared 0.1% Triton X-100 and 0.1% sodium citrate solution. This liver tissue was then incubated with the TUNEL reaction mixture for 1 h at 37°C in a humidified chamber. The slides were washed three times with phosphate-buffered saline (PBS), and the incorporated biotin-dUTP was detected under a fluorescence microscope (excitation filter 465–495 nm; Eclipse Ci, Nikon, Japan). For the paraffin-embedded tissue sections, the slides were dewaxed and fixed according to standard protocols, and then treated as described above. The green fluorescent cells indicated apoptosis.

Statistical analysis

All the data were analyzed 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 the differences in the data ($P < 0.05$). The values are expressed as means ± standard error (SE).

3. Results

Expression and activities of antioxidants (SOD, CAT, and GPX) in the liver

We investigated the changes in the enzymatic activities and in the expression of mRNAs and proteins of SOD, CAT, and GPX in liver tissues in response to the changes in water temperature (Figs. 2 and 3).

The mRNA and protein expression as well as the activities of SOD, CAT, and GPX were increased significantly with the rise in temperature in all the experimental groups. However, the mRNA and protein expression levels, and the activities of SOD, CAT, and GPX in the green LED irradiation groups were significantly lower than in the control groups exposed to illumination with white fluorescent bulb and there were no significant differences between the groups exposed to green light intensities of 0.25 and 0.5 W/m^2. However, the groups exposed to red LED showed significant

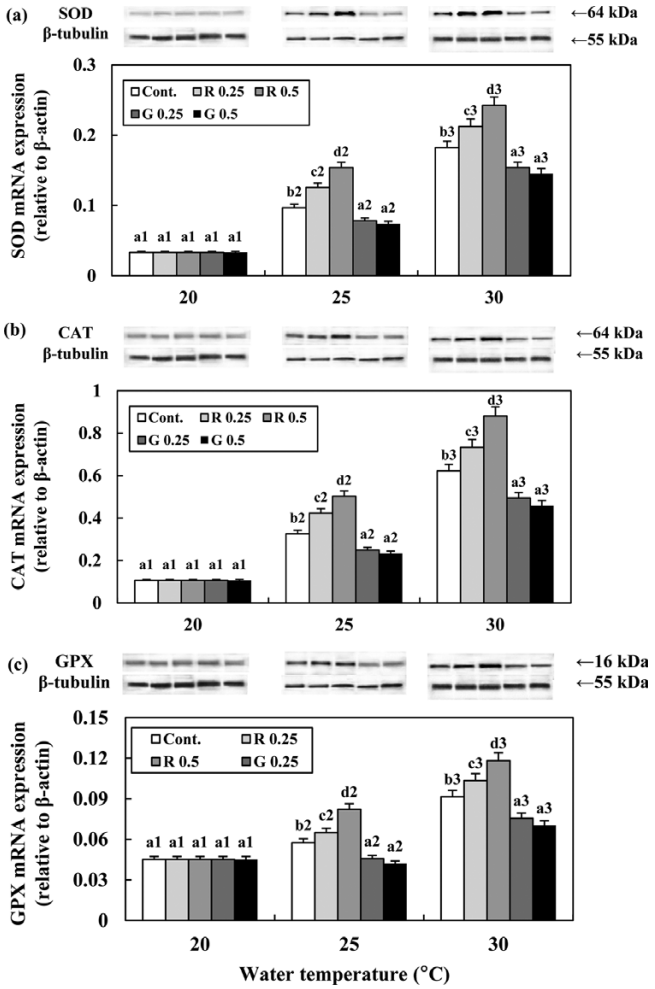

Fig. 2. Expression levels of SOD (a), CAT (b), and GPX (c) mRNAs and proteins in juvenile rock bream liver tissue during thermal changes under different lighting conditions using red (R) and green (G) LEDs at two light intensities (0.25 and 0.5 W/m^2), and white fluorescent bulb (Cont.), as measured by qPCR and western blotting. Total liver RNA (2 μg) was reverse-transcribed and amplified. Results are expressed as normalized fold expression levels with respect to the β-actin levels in the same sample. Western blots showing the expression of antioxidant enzymes [SOD (18 kDa), CAT (64 kDa), and GPX (16 kDa)] in the liver of juvenile rock bream; β-tubulin (55 kDa) was used as an internal control. Values with numbers are significantly different at the temperature within the same LED spectra ($P < 0.05$). The lowercase letters indicate significant differences between the different LED spectra within the same temperature ($P < 0.05$). All the values are means ± SE ($n = 15$)

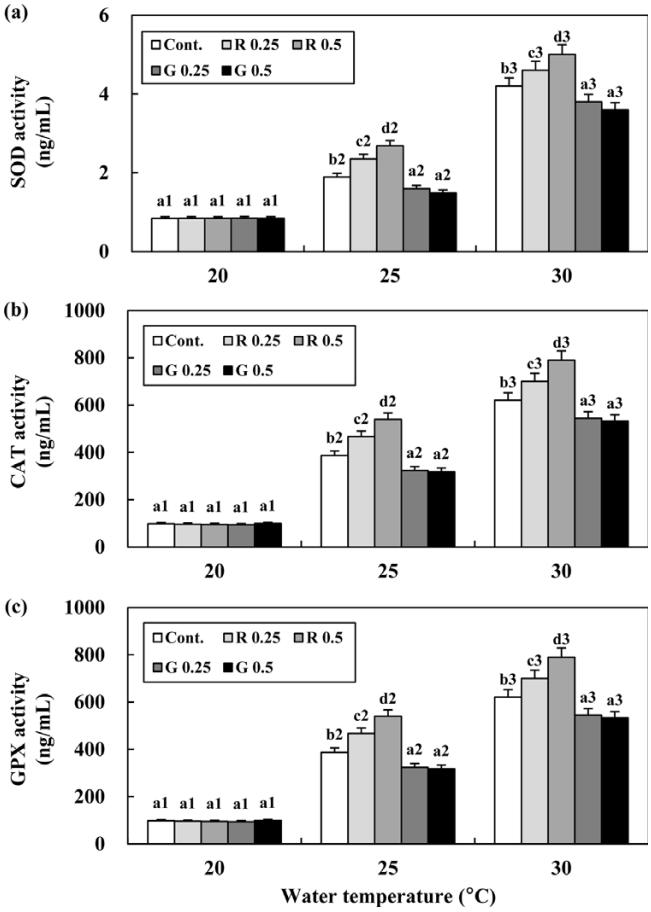

Fig. 3. Activities of plasma SOD (a), CAT (b), and GPX (c) in juvenile rock bream during thermal changes under different lighting conditions using red (R) and green (G) LEDs at two light intensities (0.25 and 0.5 W/m^2), and white fluorescent bulb (Cont.), as measured using microplate reader. Values with numbers are significantly different at the temperature within the same LED spectra ($P < 0.05$). The lowercase letters indicate significant differences between different LED spectra within the same temperature ($P < 0.05$). All the values are means ± SE ($n = 15$)

increase in the measured parameters with the increase in light intensity.

Plasma H_2O_2 level

The plasma H_2O_2 levels in all the experiment groups were increased at high water temperatures. However, the plasma H_2O_2 levels in the groups irradiated with green LEDs were significantly lower than in the control groups. However, the levels in the groups exposed to red LEDs were significantly higher than in the control groups (Fig. 4).

Plasma melatonin and lysozyme levels

The plasma melatonin and lysozyme levels were decreased significantly with the rise in temperature in all the experiment groups (Fig. 5). However, the levels of plasma melatonin and lysozyme in the green LED light groups were significantly higher than in the control groups, and there were no significant differences between the groups exposed to green light of different intensities. In contrast, the groups exposed

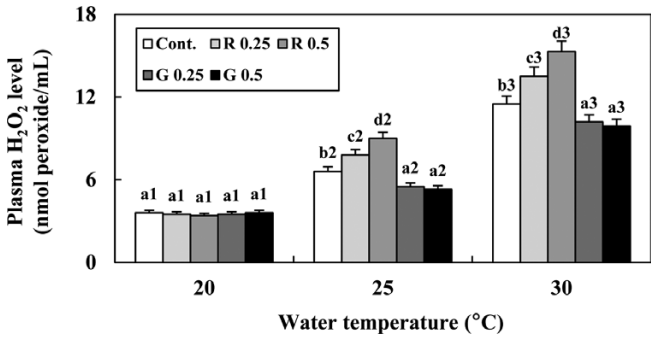

Fig. 4. Activities of plasma H_2O_2 in juvenile rock bream during thermal changes under different lighting conditions using red (R) and green (G) LEDs at two light intensities (0.25 and 0.5 W/m^2), and white fluorescent bulb (Cont.). Values with numbers are significantly different at the temperature within the same LED spectra ($P < 0.05$). The lowercase letters indicate significant differences between different LED spectra within the same temperature ($P < 0.05$). All the values are means ± SE ($n = 15$)

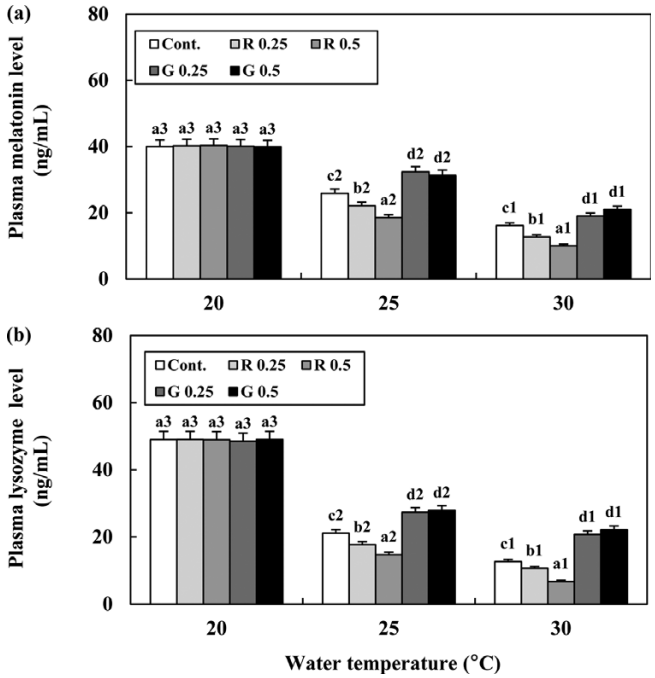

Fig. 5. Levels of plasma melatonin (a) and lysozyme (b) in juvenile rock bream during thermal changes under different lighting conditions using red (R) and green (G) LEDs at two light intensities (0.25 and 0.5 W/m^2), and white fluorescent bulb (Cont.). Values with numbers are significantly different at the temperature within the same LED spectra ($P < 0.05$). The lowercase letters indicate significant differences between different LED spectra within the same temperature ($P < 0.05$). All the values are means ± SE ($n = 15$)

to red LED light showed significant decrease in the plasma melatonin and lysozyme levels with the increasing intensity of light.

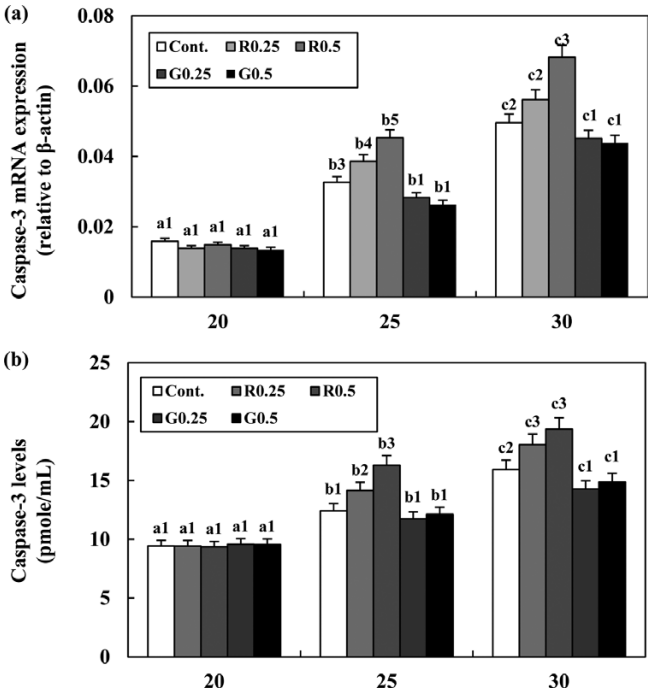

Fig. 6. Change in the levels of expression of caspase-3 mRNA (a) and plasma caspase-3 (b) during thermal changes under different lighting conditions using red (R) and green (G) LEDs at two light intensities (0.25 and 0.5 W/m^2), and white fluorescent bulb (Cont.). Results are expressed as normalized fold expression levels with respect to the β-actin levels in the same sample. Values with numbers are significantly different at the temperature within the same LED spectra ($P < 0.05$). The lowercase letters indicate significant differences between different LED spectra within the same temperature ($P < 0.05$). All the values are means ± SE ($n = 15$)

Expression and activity of caspase-3 in liver

We investigated the changes in the mRNA expression and activity of caspase-3 in liver tissue in response to the changes in water temperature (Fig. 6). We observed that the levels of mRNA and activity of caspase-3 increased significantly with the rise in temperature in all the experiment groups. In addition, the mRNA levels and the activity of caspase-3 in the groups irradiated with green LEDs were significantly lower than in the control groups and there were no significant differences between the groups irradiated with 0.25 and 0.5 W/m^2 of green light. However, the groups exposed to red light showed significant increase in caspase-3 with the increasing light intensity.

TUNEL assay

The TUNEL assay was used to investigate the presence of apoptotic cells (Fig. 7). There were significant visible differences

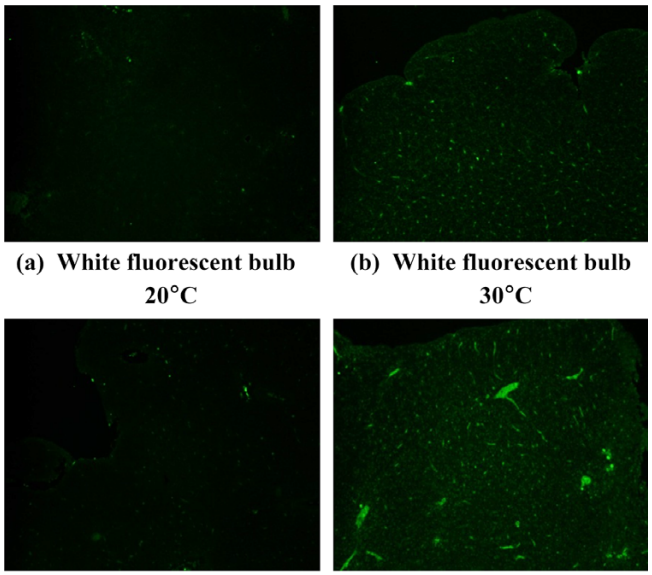

Fig. 7. TUNEL detection of juvenile rock bream liver cell apoptosis under different lighting conditions. The different panels are for the different groups, as follows: control group (Cont.) exposed to white fluorescent bulb at 20°C (a), group exposed to white fluorescent bulb at 30°C (b), group exposed to green LED (0.5 W/m^2) at 30°C (c), and group exposed to red LED (0.5 W/m^2) at 30°C (d). The cells were stained with acridine orange and visualized under a fluorescent microscope. Cells producing green fluorescence indicate apoptotic cells. Scale bars = 200 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

among the labeled cells in the TUNEL assay between the control (non-treated) and the experimental groups (fluorescent, red LED, and green LED) exposed to a temperature of 30°C. The frequency of apoptotic cells was lower after the exposure to green LEDs than that in the other experimental groups. In contrast, more apoptotic cells were detected after exposure to red LEDs.

4. Discussion

In this study, we investigated the effect of specific wavelengths (green, 520 nm; red, 630 nm) and intensities (0.25 and 0.5 W/m^2) of light on the antioxidant capacity and immunity of juvenile rock bream exposed to high water temperature by measuring the changes in expression and activity of antioxidant enzymes as well as in the plasma concentrations of H_2O_2, melatonin, and lysozyme. We also measured the changes in the expression and activity of caspase-3 and performed TUNEL analysis to investigate the effects of high temperature and specific wavelengths of light on cell death.

The results of the changes in the expression of protein, mRNA, and activities of SOD, CAT, GPX, which are typical antioxidative genes in organisms, revealed that these parameters were significantly increased with the increase in water temperature in each experimental group. When comparing the differences between the light sources, regardless of the light intensity (0.25 or 0.5 W/m^2), these values for parameters were observed to be significantly lower upon exposure to the green LED than their values in the control groups. However, the values for these parameters were observed to increase with the intensity of red light.

In a similar study, Kim et al. (2014) investigated the effects of various wavelengths (red, green, blue, and purple) on the oxidative stress in goldfish exposed to a high temperature environment. They observed that as the temperature increased, the expression of mRNAs and proteins, and the enzymatic activity of the antioxidant enzymes, SOD, CAT, and GPX showed an increasing trend. These parameters were significantly lower upon exposure to the green wavelength of light than their values in the control group, whereas they were significantly increased in the group exposed to the light of red wavelength.

Based on the results obtained in the present study, which are in agreement with those obtained in previous research, we suggest that the antioxidant gene expression was significantly increased due to the oxidative stress induced in the juvenile rock bream exposed to a high temperature environment, and that the green LED wavelength effectively reduced the oxidative stress regardless of the intensity of light. However, the red LED wavelength was observed to increase the oxidative stress.

We also determined the plasma H_2O_2 concentrations in juvenile rock bream exposed to a high temperature environment and observed that the H_2O_2 concentrations in plasma were significantly increased with the increase in water temperature. In addition, the plasma concentrations of H_2O_2 were significantly lower in the group exposed to green LED wavelengths than in the control. There was no significant difference between the different intensities of the green light used. However, the plasma concentrations of H_2O_2 increased significantly with the increase in the intensity of red light in the same range.

It was also demonstrated by Kim et al. (2014) that in goldfish exposed to a high temperature environment (25 and 30°C), as the water temperature increased, plasma H_2O_2

concentrations were significantly increased. However, the concentrations of H_2O_2 were significantly lower in the green and blue LED groups than in the control whereas they were significantly increased in the red LED groups. Shin et al. (2011) reported that the concentration of H_2O_2 in the green LED wavelength group was significantly lower than in the control group, when *Amphiprion clarkii* was exposed to different LED wavelengths (red, green, and blue). Therefore, in this study, green LED wavelength was found to be effective in reducing the plasma H_2O_2 concentrations by reducing the oxidative stress, as reported in previous studies.

Furthermore, we investigated the effect of high water temperature on the immunity of fish in the different groups. We measured the changes in the levels of lysozyme and melatonin in plasma and used them as indicators of immunity in the body. We observed that the concentrations of lysozyme and melatonin in the plasma were significantly decreased with the increase in water temperature. However, the lysozyme and melatonin concentrations were significantly higher in the green LED wavelength groups than that in the group exposed to the fluorescent bulbs. In a similar study, Choi et al. (2012) investigated the concentration of lysozyme in plasma after starvation of clownfish, *Amphiprion melanopus*, by inducing oxidative stress. They reported that the lysozyme concentration was significantly higher in the groups exposed to green LEDs.

We also analyzed the changes in the expression of mRNA and in the activity of caspase-3, which is a typical apoptosis marker in organisms. These parameters were significantly increased with the increase in water temperature in each experimental group. When comparing the differences between the different light sources, regardless of the light intensity, these parameters were significantly lower in the green LED group than their values in the control groups. However, these parameters were significantly increased with the increase in the intensity of red light.

In a similar study, Kim et al. (2016) showed that when individuals of the Olive flounder, *Paralichthys olivaceus*, were exposed to a high temperature environment, the expression of caspase-3 mRNA increased significantly with the increase in water temperature. However, compared to that in the control group, the expression of caspase-3 mRNA in the green LED group was significantly lower when compared to that in the light of other wavelengths (fluorescent bulbs, red and blue LEDs). In contrast, in the red LED wavelength group, the caspase-3 mRNA levels were significantly higher with respect to the expression levels in groups exposed to other wavelengths of light (fluorescent bulbs, green and blue LEDs). Yabu et al. (2001) showed that when zebrafish, *Danio rerio*, were exposed to a high temperature for an hour, apoptosis was significantly higher than that in the control groups.

In this study, when juvenile rock bream were exposed to a high water temperature (30°C), the caspase-3 mRNA expression and apoptosis were increased. Because the caspase-3 mRNA expression and apoptosis were decreased in the group exposed to green LEDs, we suggest that green LED wavelength has a role in reducing apoptosis.

In conclusion, the results of this study suggest that light of green wavelength has a role in reducing the oxidative stress in juvenile rock bream and it is also effective in increasing antioxidative capability as well as enhancing immunity even at a low intensity of 0.25 W/m^2. Moreover, the red wavelength causes oxidative stress that increases with the increasing intensity of light. In addition, the results show that light of green wavelength decreases apoptosis in contrast to that of red wavelength, which probably plays a role in increasing the apoptosis. Our results should aid in the judicious use of LED light of green and red wavelength in juvenile rock bream culture, especially under conditions of high temperature.

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References

Alnemri ES (1997) Mammalian cell death proteases: a family of highly conserved aspartate specific cysteine proteases. J Cell Biochem **64**(1):33–42

Basha PS, Rani AU (2003) Cadmium-induced antioxidant defense mechanism in freshwater teleost *Oreochromis mossambicus* (Tilapia). Ecotox Environ Safe **56**(2):218–221

Bly JE, Clem LW (1992) Temperature and teleost immune functions. Fish Shellfish Immunol **2**(3):159–171

Bowden TJ (2008) Modulation of the immune system of fish by their environment. Fish Shellfish Immun **25**(4):373–383

Carrillo-Vico A, Lardone PJ, Álvarez-Sánchez N, Rodríguez-Rodríguez A, Guerrero JM (2013) Melatonin: buffering the immune system. Int J Mol Sci **14**(4):8638–8683

Choi CY, Shin HS, Choi YJ, Kim NN, Lee J, Kil G-S (2012) Effect of LED light spectra on starvation-induced oxidative stress in the cinnamon clownfish *Amphiprion melanopus*. Comp Biochem Phys A **163**(3):357–363

Choi HS, Jee BY, Cho MY, Park M (2010) Monitoring of pathogens on the cultured Korean rockfish *Sebastes schlegeli* in the marine cages farms of south sea area from 2006 to 2008. J Fish Pathol **23**(1):27–35

Choi YJ, Yang S-G, Jung M-M, Kim B-S, Yun SG, Choi CY (2015) Effects of waterborne selenium on toxic and physiological stress response in goldfish, *Carassius auratus*. Mol Cell Toxicol **11**(1):35–46

Gülçin İ, BEYDEMİR Ş, Hisar O, Köksal E, Reiter RJ (2009) Melatonin administration increases antioxidant enzymes activities and reduces lipid peroxidation in the rainbow trout *(Oncorhynchus mykiss*, Walbaum) erythrocytes. Turk J Vet Anim Sci **33**(3):241–245

Häcker G (2000) The morphology of apoptosis. Cell Tissue Res **301**(1):5–17

Hansen BH, Rømma S, Garmo ØA, Olsvik PA, Andersen RA (2006) Antioxidative stress proteins and their gene expression in brown trout (*Salmo trutta*) from three rivers with different heavy metal levels. Comp Biochem Phys C **143**(3):263–274

Jin Y, Chen R, Sun L, Liu W, Fu Z (2009) Photoperiod and temperature influence endocrine disruptive chemical-mediated effects in male adult zebrafish. Aquat Toxicol **92**(1):38–43

Karakatsouli N, Papoutsoglou SE, Panopoulos G, Papoutsoglou ES, Chadio S, Kalogiannis D (2008) Effects of light spectrum on growth and stress response of rainbow trout *Oncorhynchus mykiss* reared under recirculating system conditions. Aquacult Eng **38**(1):36–42

Kashiwagi A, Kashiwagi K, Takase M, Hanada H, Nakamura M (1997) Comparison of catalase in diploid and haploid *Rana rugosa* using heat and chemical inactivation techniques. Comp Biochem Phys B **118**(3):499–503

Kim B-S, Jung SJ, Choi YJ, Kim NN, Choi CY, Kim J-W (2016) Effects of different light wavelengths from LEDs on oxidative stress and apoptosis in olive flounder (*Paralichthys olivaceus*) at high water temperatures. Fish Shellfish Immun **55**:460–468

Kim NN, Choi YJ, Shin HS, Lim JR, Han JM, Cho JH, Choi CY (2014) The effect of LED light spectra on antioxidant system by thermal stress in goldfish, *Carassius auratus*. Mol Cell Toxicol **10**(1):47–58

Oh DJ, Kim JY, Lee JA, Yoon WJ, Park SY, Jung YH (2007) Complete mitochondrial genome of the rock bream *Oplegnathus fasciatus* (Perciformes, Oplegnathidae) with phylogenetic considerations. Gene **392**(1):174–180

Oldham KM, Bowen PE (1998) Oxidative stress in critical care: Is antioxidant supplementation beneficial? J Am Diet Assoc **98**(9):1001–1008

Magnadottir B (2010) Immunological control of fish diseases. Mar Biotechnol **12**(4):361–379

Maule AG, Tripp RA, Kaattari SL, Schreck CB (1989) Stress alters immune function and disease resistance in chinook salmon (*Oncorhynchus tshawytscha*). J Endocrinol **120**(1):135–142

McFarland VA, Inouye LS, Lutz CH, Jarvis AS, Clarke JU, McCant DD (1999) Biomarkers of oxidative stress and genotoxicity in livers of field-collected brown bullhead, *Ameiurus nebulosus*. Arch Environ Con Tox **37**(2):236–241

Nouroozzadeh J, Tajaddinisarmadi J, Wolff SP (1994) Measurement of plasma hydroperoxide concentrations by ferrous oxidation-xylenol orange assay in conjunction with triphenylphosphine. Anal Biochem **200**:403–409

Pandey S, Parvez S, Sayeed I, Haque R, Bin-Hafeez B, Raisuddin S (2003) Biomarkers of oxidative stress: a comparative study of river Yamuna fish *Wallago attu* (Bl. & Schn.). Sci Total Environ **309**(1):105–115

Park IS, Gil HW, Yoo GY, Oh JS (2015) Effects of starvation in rock bream, *Oplegnathus fasciatus* and olive flounder *Paralichthys olivaceus*. Dev Rerprod **19**(2):97

Pierce LX, Noche RR, Ponomareva O, Chang C, Liang JO (2008) Novel functions for Period 3 and Exo-rhodopsin in rhythmic transcription and melatonin biosynthesis within the zebrafish pineal organ. Brain Res **1223**:11–24

Reiter RJ, Carneiro RC, Oh CS (1997) Melatonin in relation to cellular antioxidative defense mechanisms. Horm Metab Res **29**(8):363–372

Roch P (1999) Defense mechanisms and disease prevention in farmed marine invertebrates. Aquaculture **172**(1):125–145

Saurabh S, Sahoo PK (2008) Lysozyme: an important defence molecule of fish innate immune system. Aquac Res **39**(3):223–239

Shin HS, Kim NN, Choi YJ, Choi CY (2014) Retinal light input regulates clock genes and immune function in yellowtail clownfish (*Amphiprion clarkii*). Biol Rhythm Res **45**(4):541–556

Shin HS, Lee J, Choi CY (2011) Effects of LED light spectra on oxidative stress and the protective role of melatonin in relation to the daily rhythm of the yellowtail clownfish, *Amphiprion clarkii*. Comp Biochem Phys A **160**(2):221–228

Villamizar N, García-Alcazar A, Sánchez-Vázquez FJ (2009) Effect of light spectrum and photoperiod on the growth, development and survival of European sea bass (*Dicentrarchus labrax*) larvae. Aquaculture **292**(1):80–86

Wang F, Yang H, Gao F, Liu G (2008) Effects of acute temperature or salinity stress on the immune response in sea cucumber, *Apostichopus japonicus*. Comp Biochem Phys A **151**(4):491–498

Yabu T, Todoriki S, Yamashita M (2001) Stress-induced apoptosis by heat shock, UV and γ-ray irradiation in zebrafish embryos detected by increased caspase activity and whole-mount TUNEL staining. Fisheries Sci **67**(2):333–340

Zhang CI, Kwon HC, Kwon Y, Kim BY (2013) Age and growth of striped beak perch *Oplegnathus fasciatus* in the Jeju marine ranching area. Korean J Ichthyol **25**:25–32