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Effects of different light wavelengths from LEDs on oxidative stress and apoptosis in olive flounder (*Paralichthys olivaceus*) at high water temperatures

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ABSTRACT

We investigated how different light spectra affect thermal stress in olive flounder (*Paralichthys olivaceus*), using light emitting diodes (LEDs; blue, 450 nm; green, 530 nm; red, 630 nm) at two intensities (0.3 and 0.5 W/m^2) at relatively high water temperatures (25 and 30 °C, compared to a control condition of 20 °C). We measured the expression and activity of antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), and the levels of plasma hydrogen peroxide (H_2O_2) and lipid peroxidation (LPO). Furthermore, the levels and mRNA expression of caspase-3 were measured, and terminal transferase dUTP nick end labeling (TUNEL) assays of liver and comet assays were performed. The expression and activity of antioxidant enzymes, as well as plasma H_2O_2 and LPO levels were significantly higher after exposure to high temperatures, and significantly lower after exposure to green and blue light. Caspase-3 levels and mRNA expression showed a similar pattern. The TUNEL assay showed that apoptosis markedly increased at higher water temperatures, compared with the 20 °C control. In contrast, green light irradiation decreased apoptosis rate. Furthermore, the comet assays showed that nuclear DNA damage was caused by thermal stress, and that green light irradiation played a role in partially preventing this damage. Overall, these results suggest that light with green and blue wavelengths can reduce both high temperature-induced oxidative stress and apoptosis, and that particularly green light is efficient for this. Therefore, green light can play a role in protecting in olive flounder from thermal stress damage.

1. Introduction

Water temperature is an important environmental factor for fish, as it affects physiological processes such as growth, reproduction, metabolism, and immune system functioning [1,2]. In fish, acute water temperature changes can induce oxidative stress, and an increased generation of reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-), and singlet oxygen ($^1O_2$) [3,4]. In addition, overproduction of ROS can increase the lipid hydroperoxide (LPO) levels, and negatively affect cell viability by causing cell membrane damage, DNA and proteins denaturation, and an acceleration of apoptosis [5,6].

Fish have various antioxidant systems to protect themselves from stress-induced ROS generation caused by acute environmental change. Generally, the antioxidant enzymes from these systems are activated to directly remove ROS [6]. Antioxidant enzymes involved in endogenous antioxidant mechanisms include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) [7,8]. The basic mechanisms of these antioxidant enzymes are as follows: SOD converts O_2^- into H_2O_2, and CAT and GPX convert the produced toxic H_2O_2 into water and molecular oxygen (O_2), thus eliminating the toxic effects [9,10]. These antioxidant enzymes are found virtually in all tissues of vertebrates, but show in general, high activity in the liver [11,12].

Although cells have numerous mechanisms to protect themselves against stress, enhanced stress caused by sudden changes in

Abbreviations: CAT, catalase; H_2O_2, hydrogen peroxide; LED, light-emitting diode; LPO, lipid hydroperoxide; SOD, superoxide dismutase; TUNEL, terminal transferase dUTP nick end labeling.

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the environment, such as acute water temperature changes, leads to disrupted cell signaling, extensive DNA damage, and cell apoptosis [13,14]. Apoptosis is regulated by the cysteine protease family caspases, and it is characterized by morphological events such as DNA fragmentation and cellular shrinkage [15]. Particularly, caspase-3 is known to play a central role in apoptosis, by influencing biochemical and morphological processes that respond to DNA damage and inflammation [16,17].

Light is known to affect fish physiological responses, including their growth and maturation [18,19]. Furthermore, light-induced changes, including a change in the photoperiod, can cause stress in fish [20,21]. Studies that investigated the influence of light on fish functioning recently showed that light-emitting diodes (LEDs) with specific wavelengths affect diverse physiological responses [22–24]. The wavelength of green light is particularly known for its influence in maintaining or regulating physiological homeostasis, and for alleviating stress in fish exposed to high water temperatures and toxic environments. In contrast, the wavelength of red light can cause stress in fish, and negatively affect fish physiological functioning [8,25]. Furthermore, the light emitted by LEDs can regulate homeostasis or the environmental sensitivity of different fish species, and control the generation of oxidative stress in fish. As a result, LED light may be an effective tool to use in the fish-farming industry [4,24,26].

In this study, we investigated how light with a particular wavelength controls oxidative stress and apoptosis in olive flounder (*Paralichthys olivaceus*) exposed to high water temperatures. For this, we exposed olive flounder to high water temperature (25 and 30 °C) and different wavelengths of light (white fluorescent bulb, 27 W; blue, 450 nm; green, 530 nm; red, 630 nm) and different light intensities (0.3 and 0.5 W/m^2). We measured the expression of mRNA, protein levels, the activity of SOD and CAT, as well as the changes in oxidative stress by measuring plasma H_2O_2 and LPO concentrations. Furthermore, we investigated the changes in caspase-3 mRNA expression and levels, in order to determine the effects of the different light wavelengths on apoptosis. Finally, we conducted terminal transferase dUTP nick end labeling (TUNEL) assays, as well as and comet assays, in order to determine how green light reduced the effects of DNA damage and apoptosis in the liver cells. Particular attention was given to green light, as this wavelength is known to affect ROS scavenging.

2. Materials and methods

2.1. Experimental fish and conditions

From a commercial aquarium (Jeju, Korea), we purchased olive flounders (*P. olivaceus*, length 11.5 ± 0.5 cm; mass 18.2 ± 0.8 g), and the fish subsequently acclimated in eight 300-L circulation filter tanks in the laboratory for two weeks. All fish were first exposed to 20 °C (the control temperature). The water temperature was subsequently increased from 20 °C to 30 °C, with daily increments of 1 °C, using an automatic temperature regulation system (JS-WBP-170RP; Johnsam Co., Seoul, Korea). At 20, 25, and 30 °C, fish were selected from the tanks to form different experimental groups with five replicate fish per group. The experimental groups were exposed to either blue (peak at 450 nm), green (peak at 530 nm), or red (peak at 630 nm) light from LEDs (Daesin LED Co., Kyunggi, Korea), whereas the control group was irradiated with a white fluorescent bulb (27 W; GX24Q-3, PHILIPS, Amsterdam, Netherlands) (Fig. 1). The photoperiod for all treatments was a 12-h light (L): 12-h dark (D) cycle (lights on at 07:00 and lights off at 19:00). The light control group was exposed to light from a white fluorescent bulb (wavelength range 350–650 nm); placed 50 cm above the water surface and the light intensity at the water surface

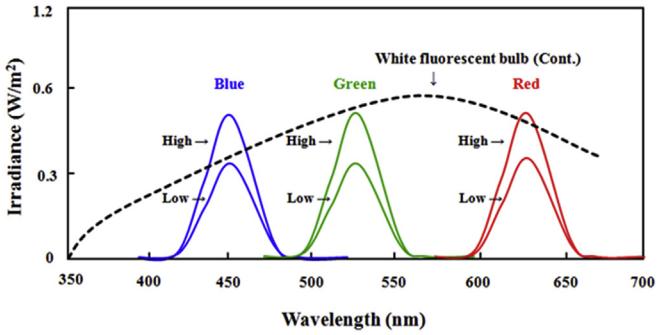

Fig. 1. Spectral profiles of light emitting diodes (LEDs; blue, 450 nm; green, 530 nm; red, 630 nm) and the white fluorescent bulbs (control) used in this study. For each LED light treatment, two different intensities were used (low, 0.3 W/m^2; high, 0.5 W/m^2). Reprinted from Shin et al. [23], with permission from *Comparative Biochemistry and Physiology Part A*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was approximately 0.96 W/m^2. The LEDs were placed 50 cm above the water surface, and irradiance levels at the water surface was maintained at approximately 0.3 or 0.5 W/m^2. The fish were kept for 24 h under experimental conditions, with a daily serving of commercial feed until the day before the sampling. Spectral analyses of the white fluorescent bulb and LEDs were performed with a spectroradiometer (FieldSpec, ASD Inc., CO, USA). The fish were anaesthetized with 200 mg/L tricaine methanesulfonate (Sigma, USA) in order to minimize the stress before collection of blood and liver tissue samples. Blood was collected from the caudal vein with a 1-mL syringe coated with heparin. From the blood samples, plasma samples were derived through centrifugation (4 °C, 10,000× *g*, 5 min), and the plasma samples were subsequently stored at −80 °C until the analyses. For total RNA and protein extraction, liver tissue immediately frozen in liquid nitrogen, and stored at −80 °C. Furthermore, for experiment of cell culture and assay of comet and TUNEL, several liver tissues immediately used the each experiment, according to the manufacturer's instruction.

2.2. Total RNA extraction, cDNA synthesis, and real-time quantitative polymerase chain reaction (qPCR) analysis

Total RNA was extracted from each sample using TRI Reagent® (Molecular Research Center, Inc., USA) and was treated with DNase, according to the manufacturer's instruction. Subsequently, 2 μg of total RNA was reverse transcribed in a 20 μL reaction volume, using an oligo-(dT)$_{15}$ anchor and M-MLV reverse transcriptase (Promega, Madison, WI, USA), according to the manufacturer's protocol. The resulting cDNA was diluted, stored at 4 °C, and subsequently used for PCR and real-time qPCR analyses.

The qPCR analysis was conducted to determine the relative expression levels of caspase-3 and the antioxidant enzymes SOD and CAT, using the total RNA extracted from the olive flounder

Table 1
Primers used for QPCR amplification.

Genes (accession no.)	Primer	DNA sequences
SOD (EF681883)	Forward	5′-CGT TGG AGA CCT GGG GAA TGT G-3′
	Reverse	5′-ATC GTC AGC CTT CTC GTGGAT C-3′
CAT (GQ229479)	Forward	5′-CCA AAC TAC TAT CCC AAC AGC-3′
	Reverse	5′-CCA CAT CTG GAG ACA CCT T-3′
Caspase-3 (JQ394697)	Forward	5′-GCA AAT CGC TGG TGG GAA A-3′
	Reverse	5′-CAT CGT CTA CAC TGT CTG TTT CG-3′
β-actin (HQ386788)	Forward	5′- GGA CCT GTA TGC CAA CAC TG-3′
	Reverse	5′- TGA TCT CCT TCT GCA TCC TG -3′