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Light-emitting diode spectral sensitivity relationship with reproductive parameters and ovarian maturation in yellowtail damselfish, *Chrysiptera parasema*

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ABSTRACT

The present study investigated the effects of exposure to different light spectra and intensities on ovarian maturation in yellowtail damselfish, *Chrysiptera parasema* over a 4-months period. We used a white fluorescent bulb and three different light-emitting diodes (LEDs: red, peak at 630 nm; green, 530 nm; blue, 450 nm), at three different intensities each (0.3, 0.6, and 0.9 W/m^2). The effects of different illuminations were assessed by measuring the mRNA and protein expressions of vitellogenin (VTG) and estrogen receptor (ER), gonadosomatic index (GSI), and plasma estradiol-17β (E_2) hormone level. For green and blue lights, significantly higher levels of VTG and ER expressions, GSI, and plasma E_2 were obtained, compared to the other light spectra. Histological analysis revealed the presence of vitellogenic oocytes in fish exposed to short wavelengths (green and blue) light. In addition, we observed significantly greater ovarian maturation in fish exposed to low and medium light intensities. The results indicate that exposure to green low intensity lighting accelerates gonadal maturation, and is likely to facilitate development of more energy-efficient aquaculture procedures.

1. Introduction

The influence of environmental factors on the growth and reproduction of fish has been extensively studied [1]. It is well known that light and temperature are among the most important natural environmental factors that regulate reproduction in fish. Lighting characteristics including wavelength (quality), intensity (quantity), and periodicity (daily cycle) are among factors that regulate seasonally dependent changes in reproductive and growth physiology of fish [1]. The reproductive physiology of fish is closely related with the perception of environmental factors by the sensory systems and the transduction of suitable signals to the hypothalamo–pituitary–gonadal axis [2,3]. The spectral composition (quality) of incident light are key properties affecting the physiological response of teleosts with, among others, effects on growth, reproduction, behavior and stress documented [1].

In various reproductive hormones, estrogen is an essential steroid hormone in reproduction, playing an important role in sexual maturation and differentiation, including oogenesis, vitellogenesis, and testicular development [4]. Estrogen activity is mediated by nuclear estrogen receptors (ERα and ERβ), and ERα is a member of a superfamily of transcription factors that induce target gene expression by binding *cis*-acting enhancer elements located in the promoter region of their responsive genes [5]. Furthermore, the induction of hepatic vitellogenin (VTG), which is a precursor yolk protein, in response to estrogens by an ER-mediated pathway has been well documented in several oviparous fish species [6,7]. Thus, VTG and ER might serve as indicators of reproduction and maturation in fish.

The application of artificial lighting in recirculating aquaculture systems requires appropriate combination of light hours (photoperiod), intensity and spectrum. There are numerous data related to photoperiod and light intensity effects on several farmed fishes and life stages [1]. In most studies fluorescent lamps are used, resulting in what humans perceive as white light, despite the fact that in natural fish habitat, wavelength of light penetrating water varies greatly, fish vision and spectrum perception are strongly adapted to each species natural habitat and living ethology [8], and recent studies indicate that light spectrum affects farmed fish growth performance [9], behavior [10] and physiological status [9].

To date, it has been shown that periodicity is a crucial determinant of reproductive success in fish, with extensive studies on its importance in the initiation and termination of gonadal

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development [11,2,3]. Also, the effects of light-intensity have been well studied over recent years and findings clearly suggest that exposure to threshold intensity levels is required to manipulate physiological functions in various teleosts [12–15]. Hence, it is important to evaluate the impact of different types of lighting on reproduction.

Metal halide bulbs are the present source of underwater artificial lighting used in the industry, but in many aspects they are not suitable for fish farming as they are neither environment nor species specific. They create a bright point source of light, involve high running costs and much of their light energy is wasted in the form of unsuitable wavelengths (i.e. longer wavelength yellow–red light) which are rapidly absorbed in the water column and therefore cannot be detected by fish [15,16]. Light-emitting diodes (LEDs) can output light at specific wavelengths [17]. Furthermore, LEDs have lower power requirements, electrical running costs and a longer life span than standard metal halide bulbs [17]. Narrow bandwidth high-energy short wavelength light may improve the efficiency of lighting systems compared to those currently used in the fish farming industry since it can be tuned more specifically in line with sensitivity of a target species [18]. Furthermore, it is known that the spectral composition of incidental light is differentially affected in underwater environments, and that rapid attenuation occurs with increasing depth [19].

Recently, several studies have investigated the utility of LED lights as photo-environmental factors, using different LED light wavelength light sources for aquaculture. For instance, Shin et al. [20] reported that green and blue light-emitting diodes (LEDs), which have short wavelengths, increased the level of antioxidants in response to oxidative stress in the yellowtail clownfish *Amphiprion clarkii*. In addition, Volpato and Barreto [21] reported that blue spectrum prevents stress in Nile tilapia *Oreochromis niloticus*. Meanwhile, red LED wavelength affects physiological function, and was found to induce oxidative stress in yellowtail clownfish [20]. However, studies on the effect of LED light wavelengths on fish reproduction remain very limited [22,23].

For energy-savings and the way to enhance the gonad development, in the present study, we examined the effects of LEDs on sexual maturation and development in yellowtail damselfish, *Chrysiptera parasema*. This species is a reef-associated damselfish that is widely distributed in shallow waters. It has commercial value as an ornamental fish and is widely used as a scientific experimental model. We investigated the effect of different types of lighting on ovarian maturation in this species. Fish were reared for 4 months under 3 LED wavelengths and three lighting intensities. Changes in the expression of VTG and ER mRNA, as well as expression of VTG and ER proteins were investigated. In addition, ovarian development was evaluated by measuring steroid hormone (estradiol-17β [E_2]) levels, and by determining oocyte development in relation to histological indices of gonadal maturation.

2. Materials and methods

2.1. Experimental fish

The immature yellowtail damselfish ($n = 600$; total length, 2.1 ± 0.4 cm; mass, 1.1 ± 0.2 g) were purchased from a commercial store. Fish length and weight were measured swiftly when the fish were divide to each experimental tanks, and then fish were allowed to acclimate for 1 week in 300-L circulation filter tanks with circular filtration prior to laboratory-based experiments under 12-h light:12-h dark photoperiod (lights on at 07:00 and light off at 19:00) using white fluorescent bulb at 27 °C. The water temperature and photoperiod were 27 ± 1 °C, with a 12L:12D photoperiod, and fed commercial feed twice daily (at 09:00 and 17:00).

The fish were exposed to a white fluorescent bulb (27 W; a simulated natural photoperiod; SNP) was used for the control group. In the experimental groups, fish were exposed to either blue (peak at 450 nm), green (530 nm), or red (630 nm) LEDs (Daesin LED Co. Kyunggi, Korea) for 4 months (Fig. 1). The LEDs were set 50 cm above the water surface, and irradiance at the water surface was maintained at approximately 0.3, 0.6, and 0.9 W/m^2, respectively (Fig. 1).

2.2. Sampling

At the end of the 4-month experimental period, blood was collected from the 30 fish per tanks ($n = 30$ groups; fluorescent bulb, plus red, green, and blue LEDs at three light intensities) using a 3-mL syringe coated with heparin from caudal vein after anesthetization. Plasma samples were separated by centrifugation (4 °C, 10,000 × *g*, 5 min) and stored at −80 °C.

The fish were euthanized by spinal transection for the collection of liver and gonads under dim white light using attenuated white fluorescent bulb. Immediately after collection, the liver were frozen in liquid nitrogen and stored at −80 °C until total RNA extraction was performed. No mortalities were observed.

2.3. Quantitative PCR (QPCR)

QPCR was conducted to determine the relative expression of VTG (accession No. JQ906787) and ERα (JQ906788) mRNA, using total RNA extracted from the liver of yellowtail damselfish, respectively. Primers for QPCR were designed in reference to known yellowtail damselfish sequences as follows: VTG forward primer (5′-ACC CGT CAG TGC TCA GTA-3′), VTG reverse primer (5′-TCG CTG CTG GTC TTA ATC A-3′), ERα forward primer (5′-TGA CTA GCA TGT CTC CTG AT-3′), ERα reverse primer (5′-ATG GTG ACC TCG GTG TAA-3′), β-actin forward primer (5′-GCA AGA GAG GTA TCC TGA CC-3′), and β-actin reverse primer (5′-CTC AGC TCG TTG TAG AAG G-3′). PCR amplification was conducted using a BIO-RAD iCycler iQ Multicolor Real-time PCR Detection System (Bio-Rad, CA, USA) and iQ™ SYBR Green Supermix (Bio-Rad, CA, USA), according to the manufacturer's instructions. QPCR was performed as follows: 95 °C for 5 min, followed by 35 cycles at 95 °C for 20 s and 55 °C for 20 s [24]. As internal controls, the experiments were duplicated with β-actin calculated threshold cycle (Ct) levels. The calibrated ΔCt value (ΔΔCt) for each sample and internal control (β-actin) was calculated using the formula: [$\Delta\Delta Ct = 2^{\wedge}-(\Delta Ct_{sample}-\Delta Ct_{internal\ control})$] [25].

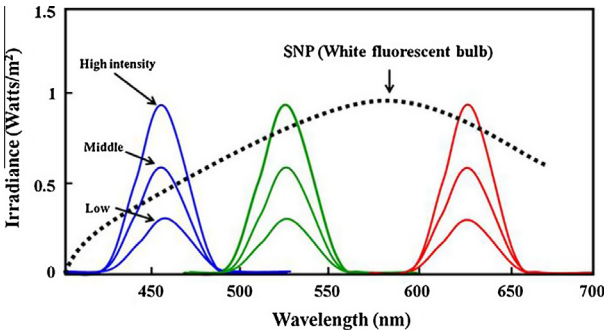

Fig. 1. Spectral profiles of the blue (B), green (G), and red (R) LEDs. Low (*L*, 0.3 W/m^2), medium (*M*, 0.6 W/m^2), and high (*H*, 0.9 W/m^2) light intensities were used for each type of LED in this study. Square dotted line shows the spectral profile of a white fluorescent light. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)