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Effects of light-emitting diode spectra on the vertebrate ancient long opsin and gonadotropin hormone in the goldfish *Carassius auratus*

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ABSTRACT

We determined the molecular mechanism underlying the environmental (photoperiodic) regulation of sexual maturation in fish, we examined the expression of sexual maturation-related hormones and vertebrate ancient long opsin (VAL-opsin) in goldfish (*Carassius auratus*) exposed to different light spectra (red and green light-emitting diodes). We further evaluated the effect of exogenous gonadotropin hormone (GTH) on the expression of VAL-opsin under different light conditions. Our results demonstrated that the expression of GTHs was higher in the fish exposed to green light, and VAL-opsin levels were increased in the fish receiving GTH injection. Therefore, we have uncovered a molecular mechanism underlying the environmental (light)-induced trigger for sexual maturation: VAL-opsin is activated by green light and GTH, which promotes the expression of sexual maturation genes.

1. Introduction

Light directly or indirectly affects the circadian rhythm, growth, and sexual maturation of fish [1–3]. As the light passes through the retina, it sends a signal to the neurons in the brain [4]. To date, studies on the biological mechanisms associated with light have generally focused on the visual light path (retina); however, little is known about the effects of light on non-visual pathways (i.e., the deep brain, pineal complex, and skin), despite their important roles in animal behavior and physiology [5,6].

Vertebrates, including fish and birds, have photoreceptor cells in the retina (rods, cones and ganglion cells) as well as in various organs such as the pineal complex, deep brain, and skin, which regulate activation of the hypothalamus–pituitary–gonad (HPG) axis [3,7,8]. Initial studies conducted in birds identified the role of opsin genes in promoting maturation through photoperiodic control, demonstrating the important role of deep-brain photoreceptors in the hypothalamus, such as opsin-like proteins, in regulation of the HPG axis [7,9,10].

The pioneering study on vertebrate deep-brain photoreception demonstrated that a light-induced change in skin color of the European minnow (*Phoxinus laevis*) is not abolished by removal of the eyes and pineal complex, and the light sensitivity was instead ascribed to the “deep-brain photoreceptor” located at the ependyma of the diencephalic ventricle [11]. The involvement of deep-brain photoreceptors in the photoperiodic response of gonadal development was also suggested in channel catfish (*Ictalurus punctatus*) [12–14].

To date, several opsins, including rhodopsin, cone-like opsin, vertebrate ancient (VA) opsin, VA-long (VAL) opsin, and melanopsin, have been found in the brains of fishes [15–19]. Among these, VA-opsin and VAL-opsin share a common core sequence in the membrane-spanning domains, although VAL-opsin has a much longer C-terminal tail than that of VA-opsin. Functional reconstitution experiments on the recombinant proteins showed that VAL-opsin bound with 11-cis-retinal is a green-sensitive pigment (λmax ~ 500 nm), whereas VA-opsin exhibited no photosensitivity even in the presence of 11-cis-retinal [15]. Furthermore, it has been demonstrated that the deep-brain stimulator VAL-opsin is closely related to maturation in birds [9].

Sexual development and maturation in teleosts are regulated by various sex hormones in the HPG axis, including gonadotropin-releasing hormone (GnRH), gonadotropin hormone (GTH), and

Abbreviations: FSH, follicle-stimulating hormone; GTH, gonadotropin hormone; HPG, hypothalamus–pituitary–gonad; LH, luteinizing hormone; LED, light-emitting diode; VAL-opsin, vertebrate ancient long opsin.

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steroid hormones, and are regulated elsewhere by neuroendocrine materials and gonadal hormones [20]. In particular, GTHs are pituitary hormones that are secreted by GnRH stimulation, and are reported to play important roles in the regulation of gonadal development and sexual differentiation and stimulate spawning times and steroid hormone regulation in vertebrates, including fish [21]. In general, in fish, follicle-stimulating hormone (FSH) is involved in early gametogenesis, vitellogenin synthesis, and spermatogenesis, whereas luteinizing hormone (LH) is known to regulate final gonad maturation, ovulation, ejaculation, and steroid hormone synthesis [22,23].

Light induce or inhibit the photoreceptors in organisms, thereby influence the physiological processes [24] and sexual maturation by strongly affecting neuroendocrine control and the HPG axis [25,26]. Recent research has provided novel insights into the effect of light on fish maturation [3,27–29]; however, understanding of the effect of the various wavelengths of light on photoreceptor activation and photoreceptor-related maturation remains incomplete.

So, a light-emitting diode (LED) has the dual advantage of being able to emit light within a specific wavelength range with easily adjustable sensitivity. Thus, an LED is highly effective in light-related research [30,31]. In addition, Migaud et al. [2] showed that most of the light energy is wasted in the long wavelengths such as red light, which is rapidly absorbed by water molecules. Thus, a fish can generally detect short wavelengths such as green light better than longer wavelengths such as red light. However, studies on the relationship between VAL-opsin, a green-sensitive photoreceptor, and maturation during circadian are very limited in the fish.

Therefore, in the present study, we investigated the physiological rhythms of VAL-opsin and GTHs in goldfish by exposure to two kinds of LED light (red and green) and a white fluorescent bulb (control), and the differences between VAL-opsin and GTHs expression with and without GTH injection were examined. This study is the first report to focus on the relationship between VAL-opsin and reproduction of teleost fish during circadian exposed to various wavelengths.

2. Materials and methods

2.1. Experimental fish and conditions

For each experiment, immature goldfish (*Carassius auratus*) ($n = 400$, length, 6.1 ± 0.5 cm; mass, 12.5 ± 0.4 g) were purchased from a commercial aquarium (Choryang, Busan, Korea) and maintained in five 300-L circulation filter tanks prior to experiments in the laboratory. The five experimental conditions were reared in duplicate and with 40 fish per tank. The goldfish were reared in automatic temperature regulation systems (JS-WBP-170RP; Johnsam Co., Buchoen, Korea); the water temperature was maintained at 22 °C, and the fish were allowed to acclimate to the experimental conditions for 24 h.

In the present study, the experimental design for light condition was modified from the methods of Shin et al. [31]. The light control group was exposed to light from a white fluorescent bulb (27 W, wavelength range 350–650 nm); placed 50 cm above the water surface and the light intensity at the water surface was approximately 0.96 W/m^2. The experimental groups were exposed to red (peak at 630 nm) and green (530 nm) LEDs (Daesin LED Co. Kyunggi, Korea); placed 50 cm above the water surface. The light intensity at the water surface was approximately 0.96 W/m^2 (Fig. 1). The fish in the control and experimental groups were exposed to a 12-h light:12-h dark photoperiod (lights on at 07:00 h and lights off at 19:00 h). The fish were reared under these conditions with daily feeding of a commercial feed until the day prior to the sampling.

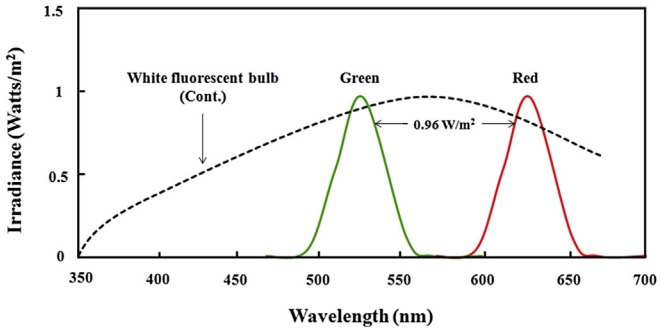

Fig. 1. Spectral profiles of the red (630 nm) and green (530 nm) light-emitting diodes (LEDs) used in this study. The dotted line shows the spectral profile of the white fluorescent bulb (Cont.). Reprinted from Shin et al. [28], with permission from *Comparative Biochemistry and Physiology Part A*. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)

The spectral analysis of the lights was performed using a spectroradiometer (FieldSpec®, ASD, CO, USA). The fish were anesthetized with 200 mg/L 2-phenoxyethanol (Sigma, St. Louis, MO, USA) to minimize stress prior to blood collection. The fish were euthanized by spinal transection (first sampling at 11:00 h) at 4-h sampling intervals (ZT4, ZT8, ZT12, ZT16, ZT20, ZT24, ZT36, and ZT48) to collect the hypothalamus, pituitary, and blood under dim light using an attenuated white fluorescent bulb. Plasma samples were separated from blood sample by centrifugation (4 °C, 10,000 × *g*, 10 min) and stored at −80 °C until analysis. The tissue samples were removed from the fish, immediately frozen in liquid nitrogen, and stored at −80 °C until analysis.

2.2. GTH injection

To investigate the effects of GTH on VAL-opsin expression, the fish were anesthetized with 2-phenoxyethanol (Sigma, St. Louis, MO, USA) prior to injection. GTH (Human chorionic gonadotropin; WAKO, Osaka, Japan) was dissolved in 0.9% physiological saline, and each fish was injected with GTH (5 μg/g and 10 μg/g, body mass [BM]) at a volume of 10 μL/g BM. The sham group was injected with an equal volume of 0.9% physiological saline (10 μL/g BM). The control groups are same as the control of light experiment. Each tank (each experimental group) included 40 fish. Four hours after the injection, the retina and hypothalamus samples were removed from the fish at 4-h sampling intervals for 2 days (first sampling at 11:00 h).

2.3. In vitro culture of cells

For cultures, the hypothalamus tissue was quickly removed at 07:00 h (lights-on time) and placed in 3 mL of ice-cold dispersion buffer (pH 7.4, Dulbecco's phosphate-buffered saline; Gibco-BRL, Rockville, MD, USA). The isolated hypothalamus tissues were then transferred to 6 mL of fresh dispersion buffer containing 0.25% trypsin (Type II-S from porcine pancreas; Sigma). The connective tissues and other impurities were removed, and the hypothalamus tissues were chopped into small pieces with a pair of scissors. The hypothalamus cells and minced hypothalamus tissue were transferred to a flask and incubated for 10 min at room temperature with slow stirring. The mixture of dispersed hypothalamus cells and tissues was filtered, and the culture medium (Gibco-BRL; osmolality adjusted to match the goldfish plasma osmolarity of 353 mOs) was added. The cell suspension was centrifuged at 800 × *g* for 10 min, and the cells were then resuspended in fresh culture medium. The hypothalamus cells at a concentration of approximately

1.2×10^6 cells/800 μL/well were transferred to a 24-well tissue culture plate (SPL Life Sciences, Seoul, South Korea). The cell culture was started at 07:00 h and then the cells were sampled at Zeitgeber time (ZT) 4, ZT8, ZT12, ZT16, ZT20, ZT24, ZT36, and ZT48. ZT4, ZT8, ZT12, and ZT36 are photophase with light, while ZT16, ZT20, ZT24, and ZT48 are scotophase without light. For the experimental groups, the cells were exposed to red and green LEDs, or treated to GTH (5 μg/μL and 10 μg/μL). The lights were set same condition with *in vivo* experiment. After the end of incubation, a cell dissociation reagent (trypsin/EDTA) was used to degrade the adhesion between the cells and wells. Then, cell suspension was centrifuged at 800 × *g* for 10 min, and the supernatant was stored at −80 °C until total RNA extraction and analysis.

2.4. Quantitative PCR (qPCR)

Total RNA was extracted from the retina and pituitary using the TRI reagent® (Molecular Research Center, Inc., Ohio, USA) according to the manufacturer's instructions. Total RNA (2 μg) was reverse-transcribed in a total volume of 20 μL, using an oligo-d$(T)_{15}$ anchor and M-MLV reverse transcriptase (Promega, Madison, WI, USA) according to the manufacturer's protocol. qPCR was conducted to determine the relative expression levels of VAL-opsin, GTHα, FSHβ, LHβ, and β-actin mRNA using cDNA reverse-transcribed from the total RNA extracted from the hypothalamus and pituitary. The primers used for qPCR are shown in Table 1. PCR amplification was conducted using a Bio-Rad CFX96™ Real-time PCR Detection System (Bio-Rad, Hercules, CA, USA) and iQ™ SYBR Green Supermix (Bio-Rad) according to the manufacturer's instructions. The qPCR program was as follows: 95 °C for 5 min, followed by 50 cycles of 95 °C for 20 s and 55 °C for 20 s. As internal controls, experiments were duplicated with β-actin, and all data are expressed relative to the corresponding β-actin threshold cycle (ΔCt) levels. The calibrated ΔCt value (ΔΔCt) for each sample and internal controls (β-actin) was calculated using the 2^{-}ΔΔCt method: $[\Delta\Delta Ct = 2^{\wedge}-(\Delta Ct_{sample} - \Delta Ct_{internal\ control})]$.

2.5. Western blot analysis

The fish were euthanized at 4-h sampling intervals (first sampling at 11:00 h) to collect the pituitary for western blot analysis. Total protein isolated from the pituitary of goldfish was extracted using a T-PER® Tissue Protein Extraction Reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's instructions. A total of 25 μg of protein was loaded per lane onto Mini-PROTEAN® TGX™ Gels (Bio-Rad), and a protein ladder (Bio-Rad) was used for reference. Samples were electrophoresed at 180 V, and the gels were immediately transferred to a 0.2-μm polyvinylidene difluoride membrane (Bio-Rad) at 85 V for 3 min using the Trans-Blot® Turbo™ Transfer System. Thereafter, the membranes were blocked with 5% skim milk in 0.04% Tris-buffered saline with Tween (TTBS) for 45 min and subsequently washed in TTBS. The membranes were incubated with a polyclonal rabbit antibody to GTHα antibodies (anti-goldfish GTHα; dilution, 1:4,000; courtesy of M. Kobayashi [23]) and β-tubulin (dilution, 1:4,000; ab6046, Abcam, Cambridge, UK), followed by horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody (dilution, 1:5,000; Bio-Rad) for 60 min. Bands were detected using a sensitive electrochemiluminescence system (ECL Advance; GE Healthcare Life Sciences, Uppsala, Sweden) and exposed for 2 min using a Molecular Imager® ChemiDoc™ XRS + Systems (Bio-Rad).

Table 1
Primers used for qPCR amplification.

Primers (Accession no.)	Primer sequences (5′–3′)
VAL-opsin (AB383149)	**F**: CAC CAC CTG CTT CAT CTT
	R: TCA TCA CAA CCA CCA TAC G
GTHα (AY800266)	**F**: CTC CTG TCT ATC AGT GTA TGG
	R: ACA AGC AGG CGT TTA ACT
FSHβ (D88023)	**F**: CGT GGA AAG TGA GGA ATG
	R: GTT CTG GTA AGA CAG CAT CA
LHβ (D88024)	**F**: TGT CCT ATT CTC TGT AAT TGT CC
	R: GTC TCA TTA ACT GGC TCA CA
β-actin (AB039726)	**F**: TGA TGT GGA CAT TCG TAA GG
	R: AGG AGC AAG GGA AGT GAT

2.6. Statistical analysis

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

3. Results

3.1. Changes in VAL-opsin mRNA expression levels

The expression of VAL-opsin mRNA using cDNA extracted from the goldfish hypothalamus was significantly higher in the photophase than in the scotophase in the control, red, and green LED groups. In particular, the VAL-opsin expression level in the green LED group was higher than that in the other groups (Fig. 2). Moreover, VAL-opsin mRNA expression levels in the GTH injection

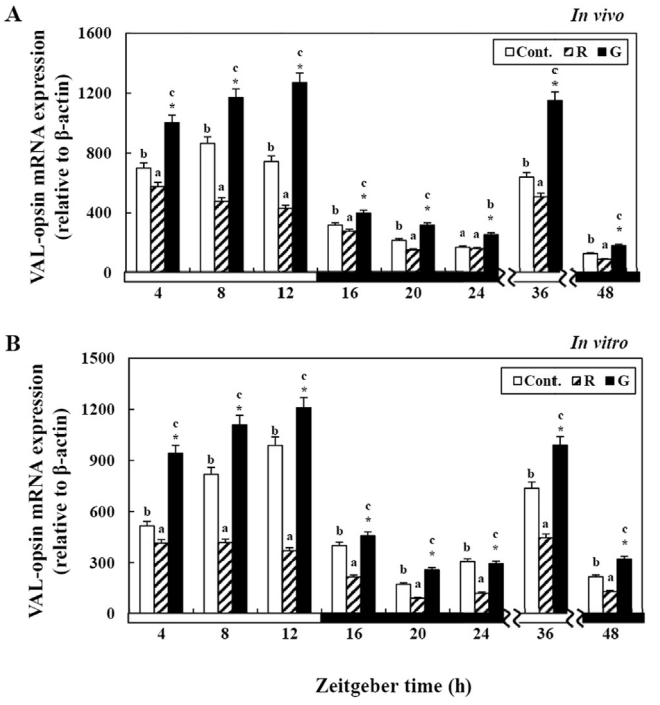

Fig. 2. Changes in the expression levels of vertebrate ancient long opsin (VAL-opsin) mRNA under red (R) and green (G) light-emitting diodes (LEDs) and a white fluorescent bulb (Cont.) in the hypothalamus (A; *in vivo*), hypothalamus cell culture (B; *in vitro*). Results are expressed as the normalized fold-change in expression levels with respect to the β-actin levels in the same sample. The white bar underneath the graph represents the photophase and the black bar represents the scotophase. Values with different characters are significantly difference exposed to the different LEDs spectra in fish within the same time ($P < 0.05$). The asterisks (*) indicates significant differences between times within the same LEDs spectra ($P < 0.05$). All values are means ± SE ($n = 5$).

group were significantly higher than those in the other groups. The results of the experiment using cultured hypothalamus cells treated with GTH were similar to those of the *in vivo* experiment (Fig. 3).

3.2. Changes in GTHs mRNA and GTHα protein expression levels

In the green LED group, the mRNA expression levels of GTHα and FSHβ from the goldfish pituitary at ZT12 and ZT36 were significantly higher than those in the other groups. Furthermore, in the green LED group, the mRNA expression levels of LHβ at ZT12, and ZT36 were significantly higher than those of the other groups. In addition, expression levels in the green LED group were higher than those in the other light groups (Fig. 4B–D).

GTHα protein expression was similar to the mRNA expression levels; in particular, levels in the green LED group were higher than those in the other groups (Fig. 4A).

4. Discussion

First, the goldfish were irradiated using white (control), red, and green LED, and the expression levels of VAL-opsin were evaluated. The VAL-opsin mRNA expression level in the green LED spectra group was significantly higher than that in the other groups, and was significantly lower in the red LED spectra group than in the control group. Kojima et al. [15], which demonstrated variety LED spectra, but a significant increase in VAL-opsin was detected in fish exposed to green LED spectra. In addition, the expression levels of Val-opsin gene over time were investigated in goldfish irradiated with white (control), red, and green LEDs. The VAL-opsin gene investigated were found to increase in the photophase. These results are in agreement with a previous study by Moore & Whitmore [32], which demonstrated that VAL-opsin mRNA expression levels in the photophase were significantly higher than those in the scotophase in zebrafish (*Danio rerio*). In the present study, VAL-opsin was highly expressed in the photophase, thereby confirming that this protein is sensitively also controlled by light in the goldfish.

Furthermore, previous study confirmed that the distinctive pattern of VA opsin expressing neurons is remarkably similar to the distribution of GnRH neurosecretory neurons in the avian brain [33]. Although it may suggest the relationship between VAL-opsin and sexual development and maturation, the mutual expression of VAL-opsin and maturation-related hormone is not clearly known. So, we further examined the expression of VAL-opsin following 5 μg/g and 10 μg/g GTH injections to investigate the association between HPG axis and VAL-opsin on sex maturation of goldfish. As the results, the VAL-opsin mRNA expression in the GTH injection groups was significantly higher than that in the non-injection group and stimulated by the higher dose (10 μg/g) of GTH than the lower dose (5 μg/g). Also, the results of cultured hypothalamus cells treated with GTH were similar to those of

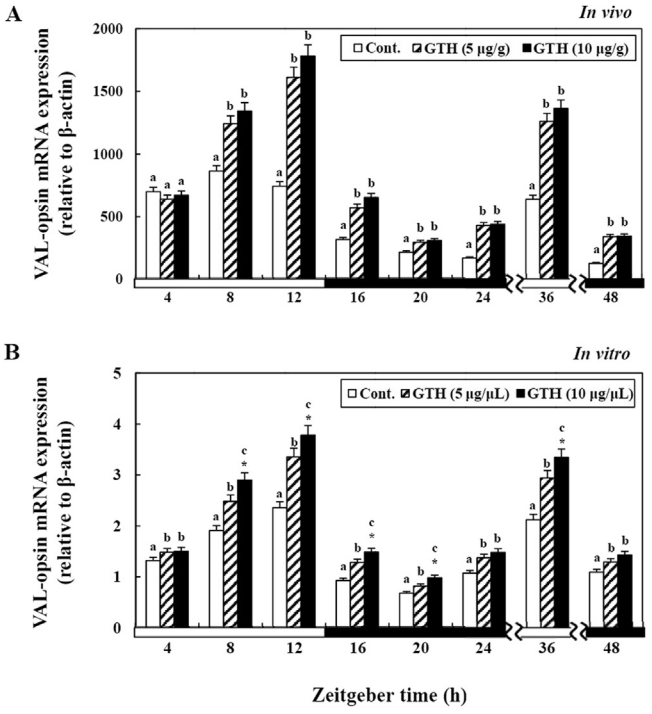

Fig. 3. Changes in the expression levels of vertebrate ancient long opsin (VAL-opsin) mRNA in the hypothalamus (A; *in vivo*) following GTH (5 μg/μL or 10 μg/μL) treatment, and hypothalamus cell culture (B; *in vitro*). The white bar underneath the graph represents the photophase and the black bar represents the scotophase. Values with different characters are significantly difference exposed to the different LEDs spectra in fish within the same time ($P < 0.05$). The asterisks (*) indicates significant differences within the same zeitgeber time ($P < 0.05$). All values are means ± SE ($n = 5$).

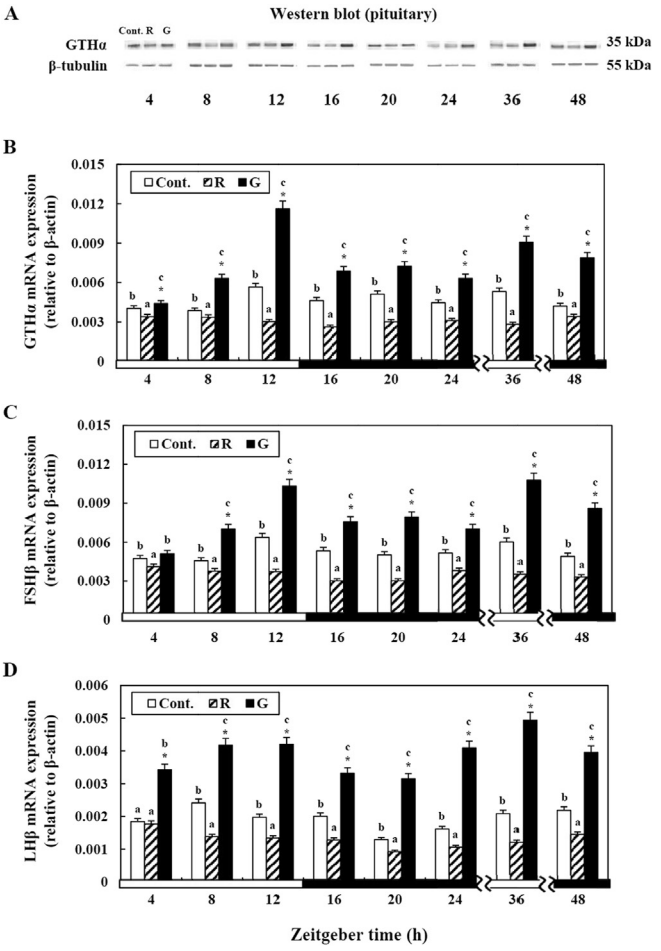

Fig. 4. Changes in the expression levels of GTHα protein (A) and GTHα (B), FSHβ (C), and LHβ (D) mRNA expression in the pituitary under red (R) and green (G) light-emitting diodes (LEDs) and a white fluorescent bulb (Cont.). The white bar underneath the graph represents the photophase and the black bar represents the scotophase. Values with different characters are significantly difference exposed to the different LEDs spectra in fish within the same time ($P < 0.05$). The asterisks (*) indicates significant differences between times within the same LEDs spectra ($P < 0.05$). All values are means ± SE ($n = 5$).

experiments conducted *in vivo*. This finding suggested that VAL-opsin genes was promoted the expression of GTHs in fish.

We further investigated the change in mRNA and protein levels of GTHs over time in fish exposed to red and green LED spectra. GTH mRNA and protein expression levels were significantly higher at ZT12 and ZT36 than at other time points. The present results are in agreement with a previous study by Karigo & Oka [27], which showed that light stimulation increased the synthesis and secretion of GnRH, leading to HPG axis activation to promote maturation. Additionally, in the present study, GTHs mRNA and protein expression levels were significantly higher in the green LED groups than the other groups, and those in the red LED group were significantly lower than the control levels. The present results are in agreement with those of a previous study by Shin et al. [28], which demonstrated that goldfish bred for 4 months under different LED wavelengths had significantly higher GnRH and GTH mRNA expression levels in the green LED groups than in the other groups, and the levels in the red LED groups were significantly lower than those in the other groups. Our results are also in agreement with a previous study by Choi et al. [34], in which long-afterglow phosphorescent pigment (luminous sheet) were used to emit green spectra, which accounts for both extended light conditions as well as green light, and showed that mRNA expression levels of GTHs of yellowtail damselfish (*Chrysiptera parasema*) increased and could promote sexual maturation.

In summary, we suggested that fish maturation is associated with light spectra in terms of the molecular mechanism and physiological response. Our findings support the hypothesis that VAL-opsin sensitively reacts to green spectra in goldfish. Furthermore, VAL-opsin and maturation-related hormones may interact to affect fish maturation. However, further studies on the effect of wavelength intensity on fish maturation and growth are required.

Conflict of interest

The authors declare no conflict of interest.

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References

[1] S. Doyle, M. Menaker, Circadian photoreception in vertebrates, Cold Spring Harb. Symp. Quant. Biol. (2007) 499–508.
[2] H. Migaud, M. Cowan, J. Taylor, H.W. Ferguson, The effect of spectral composition and light intensity on melatonin, stress and retinal damage in post-smolt Atlantic salmon, *Salmo salar*, Aquaculture 270 (2007) 390–404.
[3] H.S. Shin, J.A. Song, J.Y. Choi, N.N. Kim, Y.J. Choi, S.N. Sung, C.Y. Choi, Effects of various photoperiods on Kisspeptin and reproductive hormones in the goldfish, *Carassius auratus*, Anim. Cells Syst. 18 (2014) 109–118.
[4] R.M. Fischer, B.M. Fontinha, S. Kirchmaier, J. Steger, S. Bloch, D. Inoue, S. Panda, S. Rumpel, K. Tessmar-Raible, Co-expression of VAL- and TMT-opsins uncovers ancient photosensory interneurons and motorneurons in the vertebrate brain, PLoS Biol. 11 (2013) e1001585.
[5] S. Hattar, R.J. Lucas, N. Mrosovsky, S. Thompson, R.H. Douglas, M.W. Hankins, J. Lem, M. Biel, F. Hofmann, R.G. Foster, K.W. Yau, Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice, Nature 424 (2003) 76–81.
[6] R. Noseda, V. Kainz, M. Jakubowski, J.J. Gooley, C.B. Saper, K. Digre, R. Burstein, A neural mechanism for exacerbation of headache by light, Nat. Neurosci. 13 (2010) 239–245.
[7] R.G. Foster, B.G. Soni, Extraretinal photoreceptors and their regulation of temporal physiology, Rev. Reprod. 3 (1998) 145–150.
[8] H. Migaud, A. Davie, J.F. Taylor, Current knowledge on the photo-neuroendocrine regulation of reproduction in temperate fish species, J. Fish. Biol. 76 (2010) 27–68.
[9] S. Halford, S.S. Pires, M. Turton, L. Zheng, I. González-Menéndez, W.L. Davies, S.N. Perison, J.M. García-Fernández, M.W. Hankins, R.G. Foster, VA opsin-based photoreceptors in the hypothalamus of birds, Curr. Biol. 19 (2009) 1396–1402.
[10] Y. Nakane, K. Ikegami, H. Ono, N. Yamamoto, S. Yoshida, K. Hirunagi, S. Ebihara, Y. Kubo, A mammalian neural tissues opsin (Opsin 5) is a deep brain photoreceptor in birds, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 15264–15268.
[11] K. von Frisch, Beitraege zur physiologie der pigmentzellen in der fischhaut, Pflugers Arch. Gesamte Physiol. Menschen. Tiere 138 (1911) 319–387.
[12] K.B. Davis, C.A. Goudie, B.A. Simco, R. MacGregor, N.C. Parker, Environmental regulation and influence of the eyes and pineal gland on the gonadal cycle and spawning in channel catfish (*Ictalurus punctatus*), Physiol. Zool. 59 (1986) 717–724.
[13] S.K. Garg, Effect of pinealectomy, eye enucleation, and melatonin treatment on ovarian activity and vitellogenin levels in the catfish exposed to short photoperiod or long photoperiod, J. Pineal. Res. 7 (1989) 91–104.
[14] T. Masuda, M. Iigo, K. Aida, Existence of an extra-retinal and extra-pineal photoreceptive organ that regulates photoperiodism in gonadal development of an Osmerid teleost, ayu (*Plecoglossus altivelis*), Comp. Biochem. Physiol. A 140 (2005) 414–422.
[15] D. Kojima, H. Mano, Y. Fukada, Vertebrate ancient-long opsin: a green-sensitive photoreceptive molecule present in zebrafish deep brain and retinal horizontal cells, J. Neurosci. 20 (2000) 2845–2851.
[16] T. Minamoto, I. Shimizu, A novel isoform of vertebrate ancient opsin in a smelt fish, *Plecoglossus altivelis*, Biochem. Biophys. Res. Commun. 290 (2002) 280–286.
[17] O. Drivenes, A.M. Soviknes, L.O. Ebbesson, A. Fjose, H.C. Seo, J.V. Helvik, Isolation and characterization of two teleost melanopsin genes and their differential expression within the inner retina and brain, J. Comp. Neurol. 456 (2003) 84–93.
[18] T. Masuda, M. Iigo, K. Mizusawa, K. Aida, Retina-type rhodopsin gene expressed in the brain of a teleost, ayu (*Plecoglossus altivelis*), Zool. Sci. 20 (2003) 989–997.
[19] H.S. Shin, N.N. Kim, Y.J. Choi, J. Lee, G.-S. Kil, C.Y. Choi, Differential expression of rhodopsin and exo-rhodopsin genes in the retina and pineal gland of olive flounder, *Paralichthys olivaceus*, J. Appl. Anim. Res. 40 (2012) 229–246.
[20] D. Pati, H.R. Habibi, The involvement of protein kinase c and arachidonic acid pathways in the gonadotropin-releasing hormone regulation of oocyte meiosis and follicular steroidogenesis in the goldfish ovary, Biol. Reprod. 66 (2002) 813–822.
[21] G. Colombo, M. Chicca, Immunocytochemical studies on the pituitary gland of *Anguilla anguilla* L., in relation to early growth stages and diet-induced sex differentiation, Gen. Comp. Endocrinol. 131 (2003) 66–67.
[22] Y. Nagahama, M. Yoshikuni, M. Yamashita, T. Tokumoto, Y. Katsu, Regulation of oocyte growth and maturation in fish, Curr. Top. Dev. Biol. 30 (1995) 103–145.
[23] M. Kobayashi, T. Morita, K. Ikeguchi, G. Yoshizaki, T. Suzuki, S. Watabe, In vivo biological activity of recombinant goldfish gonadotropins produced by baculovirus in silkworm larvae, Aquaculture 256 (2006) 433–442.
[24] J.F. Baroiller, Y. Guiguen, A. Fostier, Endocrine and environmental aspects of sex differentiation in fish, Cell Mol. Life Sci. 55 (1999) 910–931.
[25] L. Rodríguez, I. Begtashi, S. Zanuy, M. Shaw, M. Carrillo, Changes in plasma levels of reproductive hormones during first sexual maturation in European male sea bass (*Dicentrarchus labrax* L.) under artificial day lengths, Aquaculture 202 (2001) 235–248.
[26] A.K. Biswas, M. Endo, T. Takeuchi, Effect of different photoperiod cycles on metbolic rate and energy loss of both fed and unfed young tilapia (*Oreochromis niloticus*) part I, Fish. Sci. 68 (2002) 465–477.
[27] T. Karigo, Y. Oka, Neurobiological study of fish brains gives insights into the nature of gonadotropin-releasing hormone 1-3 neurons, Front. Endocrinol. 19 (2013) 177.
[28] H.S. Shin, N.N. Kim, Y.J. Choi, H.R. Habibi, J.W. Kim, C.Y. Choi, Light-emitting diode spectral sensitivity relationship with reproductive parameters and ovarian maturation in yellowtail damselfish, *Chrysiptera parasema*, J. Photochem. Photobiol. B 127 (2013) 108–113.
[29] H.S. Shin, H.R. Habibi, C.Y. Choi, The environmental regulation of maturation in goldfish, *Carassius auratus*: effects of various LED light spectra, Comp. Biochem. Physiol. A 168 (2014) 17–24.
[30] N. Villamizar, A. García-Alcazar, F.J. Sánchez-Vázquez, Effect of light spectrum and photoperiod on the growth, development and survival of European sea bass (*Dicentrarchus labrax*) larvae, Aquaculture 292 (2009) 80–86.
[31] H.S. Shin, J. Lee, C.Y. Choi, 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. Physiol. A 160 (2011) 221–228.
[32] H.A. Moore, D. Whitmore, Circadian rhythmicity and light sensitivity of the zebrafish brain, PLoS One 9 (2014) e86176.
[33] J.M. García-Fernández, R. Cernuda-Cernuda, W.I. Davies, J. Rodgers, M. Turton, S.N. Peirson, The hypothalamic photoreceptors regulating seasonal reproduction in birds: a prime role for VA opsin, Front. Neuroendocrinol. 37 (2015) 13–28.
[34] Y.J. Choi, B.-S. Kim, C.Y. Choi, Effect of long-afterglow phosphorescent pigment on reproductive parameters and ovarian maturation in the yellowtail damselfish, *Chrysiptera parasema*, Comp. Biochem. Physiol. A 182 (2015) 113–120.