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Physiological Effect of Extended Photoperiod and Green Wavelength on the Pituitary Hormone, Sex Hormone and Stress Response in Chub Mackerel, *Scomber japonicus*

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Abstract: Chub mackerel, *Scomber japonicus*, is heavily farmed and harvested due to its demand as a high-quality protein source rich in fatty acids. However, the effects of environmental cues on sexual maturation of the fish remain understudied. We aim to elucidate the effect of light manipulation on the hormones related to reproduction and on the stress response in the species. Mackerel were exposed to different photoperiods (12 h light:12 h dark or 14 h light:10 h dark) and light wavelengths (provided by white fluorescent bulbs or green LEDs). Total RNA extracted from the brain was assayed with quantitative polymerase chain reaction (a powerful technique for advancing functional genomics) and blood plasma was analyzed via immunoassay using ELISA kits. The mRNA expression of gene-encoding gonadotropin-releasing hormone, gonadotropin hormone, follicle-stimulating hormone, and luteinizing hormone were significantly increased through the use of an extended photoperiod and green wavelength, which also increased testosterone and 17β-estradiol plasma levels. Plasma levels of cortisol and glucose, which are indicators of a stress response, were significantly decreased through green LED exposure. Our results indicate that environmental light conditions affect the production of pituitary and sex hormones, and reduce the stress response in *S. japonicus*.

Keywords: HPG/HPI axis; maturation; photoperiod; *Scomber japonicus*; stress response; wavelength

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1. Introduction

Environmental factors such as temperature and salinity (for freshwater/seawater adaptation) are critical in the timing of sexual maturation and spawning in fish [1–3]. Among such factors, light has a major influence on the physiology and behavior of fish, and the light cycle is essential for biological processes such as reproduction, migration and dispersal, as well as metabolic rate and body pigmentation [4–8]. Diurnal fish are most active during the day and less active during the night, while nocturnal fish are most active during the night and less active during the day [4]. The photoperiod and wavelength affect the timing of sexual maturation and spawning because they stimulate the release of melatonin from the pineal gland and retina, which in turn activates reproductive hormones [9,10]. Seasonal changes in photoperiod may have a major effect on the sexual maturity of fish by regulating gonadal maturation (which is associated with endogenous rhythms) and the synthesis/secretion of reproductive hormones [11].

The photoperiod and wavelength are critical environmental cues that initiate the breeding season in temperate fish species, and have a major impact on reproductive and aggressive behavior mediated by the hypothalamus–pituitary–gonadal axis [12]. Artificial lighting produces changes in endocrine hormones through the same mechanism, and the exact effect of varying photoperiod and wavelength depends on the fish species and developmental stage [7,13]. Photoperiod manipulation has been successfully used to promote the growth, development and survival of various juvenile fish, such as those of the gilthead seabream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*), and

barramundi (*Lates calcarifer*) species; it has also been successfully used to increase the rate of sexual maturation and shorten spawning time of adult *D. labrax*, *S. aurata*, rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), and Atlantic salmon (*Salmo salar*)] [14]. A longer photoperiod promotes fry growth, because it presents more feeding opportunities [15]. Furthermore, it cues stimulation of the endocrine system, which prolongs the spawning period, thereby increasing the number of eggs produced; therefore, both the periodicity (in terms of a daily cycle that varies seasonally and with latitude) and quality (the wavelength, absorbed by water) of light should be considered in fish reproduction [16,17].

The pituitary gland produces hormones that play major roles in the endocrine regulation of growth, the metabolism, homeostasis, reproduction, and stress responses [18]. The anterior pituitary gland secretes gonadotropin-releasing hormone (*GnRH*), gonadotropin hormone (*GTH*), follicle-stimulating hormone (*FSH*), and luteinizing hormone (*LH*), which influence cell development via signaling molecules and transcription factors [19]. In fish, light stimulates the central nervous system to promote pituitary secretion, which also affects the development of fish gonadal cells, and fish ovarian development is closely connected to the light in the environment [20]. Sex steroid hormones such as testosterone and estrogen regulate digestion and food utilization, growth, reproduction, gut transport, osmoregulation, intermediary metabolism, behavior, and immunity in fish, as well as genes and proteins that are involved in tissue and skeletal growth, metabolism, gonadal development, neuroplasticity, and cognition [21,22]. Sex steroid hormones especially control various behavioral and neural processes including aggression, reproduction, memory, and learning [23]. Therefore, in fish, sex steroid hormones may be an important indicator for evaluating the effects of varying photoperiods and light wavelengths, which are closely linked with gonadal development and reproduction [24]. The fish retina absorbs light of various wavelengths that is transmitted to the brain via photoreceptors and results in physiological and behavioral reactions, and some wavelengths may cause physiological stress [25]. In fish, an appropriate level of light can have effects such as improved food intake, accelerated nutrient assimilation, proper growth, improved immune system, increased enzyme activity and crude protein/crude lipid accumulation, which acts as a stressor leading to mass mortality [26]. The cycle and wavelength of light are major factors that induce physiological changes as a direct stress to fish, and these stress-related physiological indicators such as cortisol and glucose are widely used to evaluate the effects of environmental stress [27].

Chub mackerel, *Scomber japonicus*, occurs worldwide, including the warm and temperate coastal waters of the Pacific, Indian, and Atlantic Oceans. It is one of the most heavily caught and farmed fish species due to its demand in Korea, Japan, China, and Russia as a high-quality protein source rich in omega-3 fatty acids [28]. Although widely used as an aquaculture species, insufficient studies have been conducted on the sexual maturation of *S. japonicus*. Light is the most influential factor on the sexual maturity of fish, which is essential for stable seedling production. In particular, in recent studies, green wavelengths increased the immunity of fish, reducing stress responses, and had a greater impact on growth than red and blue wavelengths [29,30]. Therefore, the aim of this study was to assess the effect of light manipulation on levels of pituitary hormones, sex hormones, and stress indicators in *S. japonicus* via exposure to extended photoperiods and the green wavelength.

2. Materials and Methods

2.1. Experimental Fish

The study was conducted using sea cage fish farms measuring 7 m × 7 m × 7 m in size because it is the most commonly used sea border farming facility size in Korea. Chub mackerel (n = 480; full length, 33.7 ± 3.5 cm; weight, 453.5 ± 10.4 g) were obtained from Geomun Island, Yeosu, Korea, and randomly divided into four cages of forty exposed to natural light from the outside. Each cage was exposed to either a 12 h light and 12 h dark (12L:12D) photoperiod or a 14 h light and 10 h dark (14L:10D) photoperiod over a two-month experimental period, using either green LEDs (530 nm) or a white fluorescent

bulb (27 W) for lighting. These light sources were waterproofed and set in the center of their respective cages, and maintained at 150 cm under the water surface. The fish were provided commercial feed twice daily (at 09:00 and 17:00) and were reared under these conditions for two months.

2.2. Experimental Design and Sampling

Following the two months of rearing described in Section 2.1, the fish were sampled every month for the two subsequent months. We collected the brain, pituitary, and blood from fish in each group at 14:00 at the one- and two-month experimental periods (n = 40). Immediately after collection, the tissues were frozen in liquid nitrogen and stored at −80 °C until total RNA extraction was performed. Moreover, a blood sample was drawn from the caudal vasculature using a 1 mL syringe coated with heparin. After centrifugation (4 °C, 10,000× *g*, 10 min), the plasma was stored at −80 °C until analysis.

2.3. Pituitary Hormone

Quantitative polymerase chain reaction (qPCR) was conducted to determine the relative expression of *GnRH* and *GTHα* mRNA using total RNA extracted from the brain of chub mackerel. In general, this analysis technique was used because qPCR is used instead of semi-quantitative PCR as an analysis technique to quantify gene expression. The following qPCR primers were designed with reference to the known sequences of the chub mackerel (GenBank accession numbers: *GnRH*, HQ108195; *GTHα*, JF495131; and *β-actin*, GU731674): GnRH forward (5′-ACT GGT CCT ATG GAT GGC TAC-3′) and reverse (5′-TTC AGG AAG AGA CAC CAC ACC-3′) primers; GTHα forward (5′-ATC AAA CAT GGG CTG TGA GG-3′) and reverse (5′-TTT GAG TGG TGT CGG GTA GG-3′) primers; and β-actin forward (5′-ACC GGT ATT GTC ATG GAC TC-3′) and reverse (5′-TCA TGA GGT AGT CTG TGA GGT C-3′) primers.

2.4. Pituitary Gonadotropins

qPCR was conducted to determine the relative expression of *FSHβ* and *LHβ* mRNA using total RNA extracted from the brain of chub mackerel. The following qPCR primers were designed with reference to the known sequences of the chub mackerel (GenBank accession numbers: *FSHβ*, JF495132; *LHβ*, JF495133; and *β-actin*, GU731674): FSHβ forward (5′-TGT GAA GGA CAG TGT TAC CAC AGG G-3′) and reverse (5′-TCA TAG GTC CAG TCA CCG C-3′) primers; LHβ forward (5′-GAA ACA ACC ATC TGC AGC G-3′) and reverse (5′-AAA AGT CCC GAT ACG TGC AC-3′) primers; and β-actin forward (5′-ACC GGT ATT GTC ATG GAC TC-3′) and reverse (5′-TCA TGA GGT AGT CTG TGA GGT C-3′) primers.

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. qPCR was performed as follows: one cycle of denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 20 s, and annealing at 55 °C for 20 s. The assay was performed in triplicate for each experimental group to confirm consistency. β-actin was used as the internal control, and all data are expressed relative to the corresponding β-actin-calculated ΔCt levels. The calibrated ΔCt value (ΔΔCt) for each sample and the internal control were calculated as ΔΔCt = 2^−(ΔCtsample − ΔCtinternal control).

2.5. Sex Hormone

The levels of plasma testosterone and 17β-estradiol were analyzed via immunoassay with an ELISA kit (testosterone, catalog no. MBS933475; 17β-estradiol, catalog no. MBS221037; Mybiosource, San Diego, CA, USA). An anti-antibody that was specific to the respective hormonal antibodies was pre-coated onto a microplate. Then, 50 µL of blood plasma was added per well, followed by 50 µL of horseradish peroxidase conjugate and 50 µL of antibody. The contents were mixed well and then incubated for 2 h at 37 °C. During the subsequent wash process, any remaining wash buffer was removed via aspiration or

decantation. Next, 50 µL of substrate A and substrate B were added to each well, followed by incubation for 15 min at 37 °C in the dark. After incubation, 50 µL of stop solution was added to each well and the optical density of the wells was determined within 10 min, using a GloMax Explorer Multimode Microplate Reader (Promega, Madison, WI, USA) set to 450 nm.

2.6. Stress Hormone and Glucose

The levels of plasma cortisol were analyzed via immunoassay (catalog no. MBS007869; Mybiosource). An anti-antibody specific to the cortisol antibodies was pre-coated onto a microplate. The procedure followed for the remainder of the assay is identical to that described for the sex hormone analyses in Section 2.5. Levels of plasma glucose were measured with the dry multiplayer analytic slide method using a biochemistry auto-analyzer (FUJI DRI-CHEM 4000i; Fujifilm, Tokyo, Japan).

2.7. Statistical Analysis

Statistical analyses were performed using the SPSS/PC+ statistical package (SPSS Inc., Chicago, IL, USA). Significant differences between groups were identified using one-way ANOVA and Tukey's post hoc test for multiple comparisons. The significance level was set at $p < 0.05$.

3. Results

3.1. Pituitary Hormone

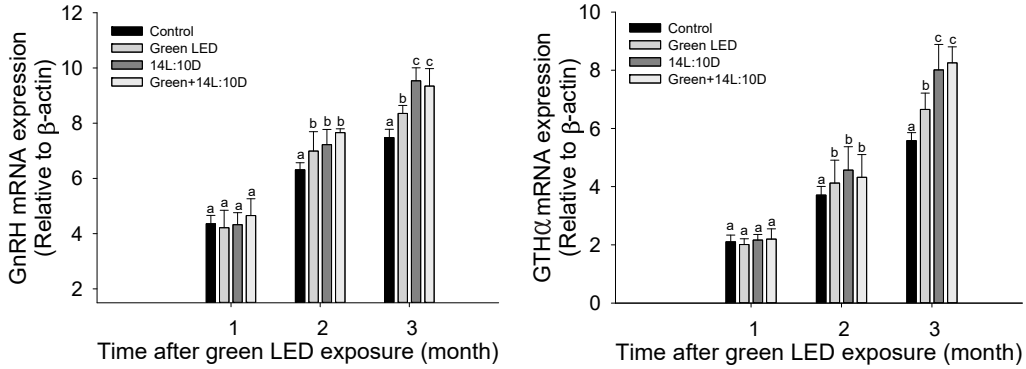
The expression of *GnRH* and *GTHα* mRNA in the brain of *S. japonicus* fish exposed to different photoperiods and light wavelengths is shown in Figure 1. The *GnRH* mRNA expression was significantly increased under green LED lighting and a 14L:10D photoperiod after 1 and 2 months of exposure, respectively. Fish exposed to a 14L:10D photoperiod for 2 months, under either a fluorescent bulb or green LEDs, displayed a significantly higher *GnRH* mRNA expression than fish under a 12L:12D cycle for that period. Similarly, *GTHα* mRNA expression was significantly increased with green LED and a 14L:10D photoperiod exposure at 1 and 2 months, respectively. Again, at the 2-month observation period, groups under a 14L:10D photoperiod indicated significantly higher *GTHα* mRNA expression than groups under a 12L:12D photoperiod.

3.2. Pituitary Gonadotropins

The mRNA expression of *FSHβ* and *LHβ* in the brain of *S. japonicus* fish exposed to different photoperiods and light wavelengths is depicted in Figure 2. The expression of both *FSHβ* and *LHβ* mRNA was significantly increased using green LED lighting and a lengthened 14L:10D photoperiod. The 14L:10D photoperiod produced a significantly higher expression of both *FSHβ* and *LHβ* mRNA than the 12L:12D photoperiod under either lighting, both at 1 and 2 months.

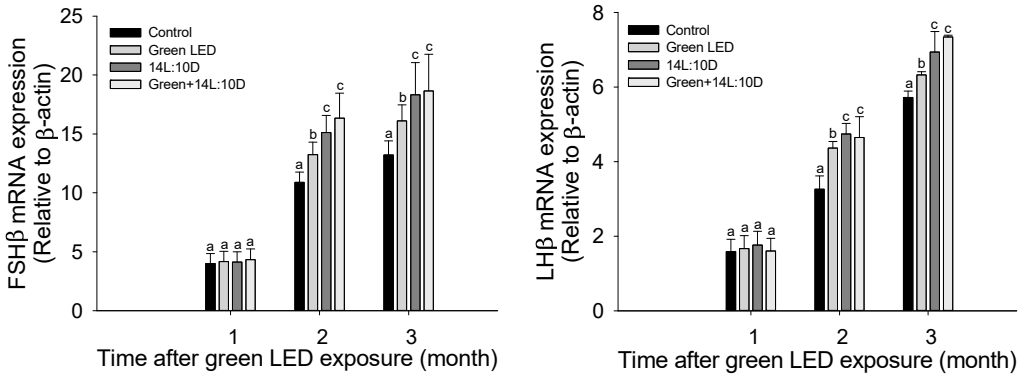
3.3. Sex Hormone

The levels of testosterone and 17β-estradiol sex hormones in the blood plasma of *S. japonicus* following exposure of the fish to different photoperiods and light wavelengths are presented in Figure 3. The plasma levels of both testosterone and 17β-estradiol were significantly increased under exposure to green LEDs and to a 14L:10D photoperiod of 1 and 2 months. Furthermore, the 14L:10D photoperiod produced significantly higher plasma levels of both sex hormones than a 12L:12D photoperiod after two months, regardless of light wavelength.



(**a**) (**b**)

Figure 1. Chub mackerel, *Scomber japonicus*, were exposed to a 12 h light and 12 h dark photoperiod, either under fluorescent lighting (Control) or under green LEDs (Green LED); mackerel were also exposed to an extended 14 h light and 10 h dark photoperiod under fluorescent lighting (14L:10D) or green LEDs (Green+14L:10D). Total RNA was extracted from the brains of fish (n = 40; full length, 33.7 ± 3.5 cm; weight, 453.5 ± 10.4 g per group) and subjected to quantitative polymerase chain reaction to assess the expression of (**a**) *GnRH* mRNA and (**b**) *GTHα* mRNA at onset of the experiment, after 1 month, and after 2 months. Vertical bars denote standard errors. Values with different superscripts (a, b, or c) within a given observation period indicate significant differences between groups ($p < 0.05$) as determined using Tukey's multiple range test.



(**a**) (**b**)

Figure 2. Chub mackerel, *Scomber japonicus*, were exposed to a 12 h light and 12 h dark photoperiod, either under fluorescent lighting (Control) or under green LEDs (Green LED); mackerel were also exposed to an extended 14 h light and 10 h dark photoperiod under fluorescent lighting (14L:10D) or green LEDs (Green+14L:10D). Total RNA were extracted from the brains of fish (n = 40; full length, 33.7 ± 3.5 cm; weight, 453.5 ± 10.4 g per group) and subjected to quantitative polymerase chain reaction to assess the expression of (**a**) *FSHβ* mRNA and (**b**) *LHβ* mRNA at onset of the experiment, after 1 month, and after 2 months. Vertical bars denote standard errors. Values with different superscripts (a, b, or c) within a given observation period indicate significant differences between groups ($p < 0.05$) as determined using Tukey's multiple range test.

3.4. Stress Response

Figure 4 indicates the stress responses of *S. japonicus* as measured by cortisol and glucose levels in the plasma following exposure to different photoperiods and lighting wavelengths. Plasma levels of both cortisol and glucose were significantly decreased in the groups that received green LED lighting, regardless of photoperiod, compared to the control group at 12L:12D and the 14L:10D group, which received fluorescent lighting.

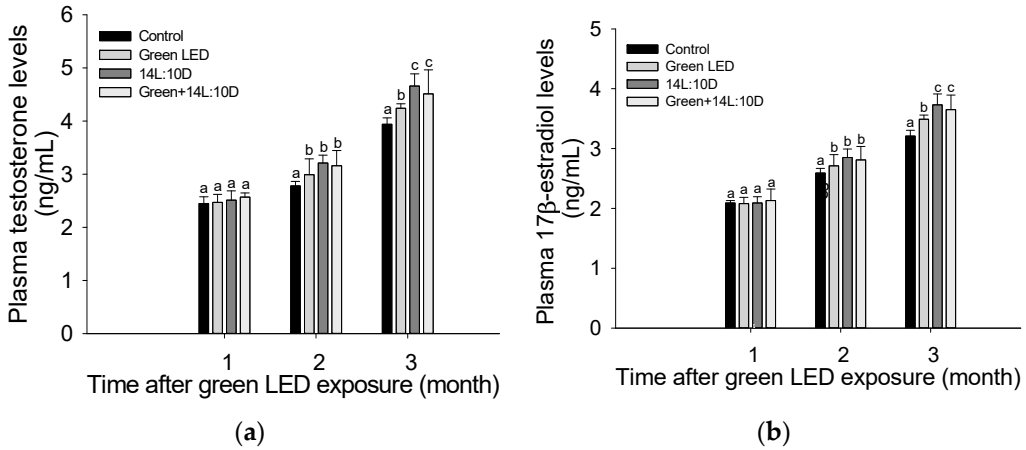


Figure 3. Chub mackerel, *Scomber japonicus*, were exposed to a 12 h light and 12 h dark photoperiod, either under fluorescent lighting (Control) or under green LEDs (Green LED); mackerel were also exposed to an extended 14 h light and 10 h dark photoperiod under fluorescent lighting (14L:10D) or green LEDs (Green+14L:10D). Blood was drawn from the caudal veins of fish (n = 40; full length, 33.7 ± 3.5 cm; weight, 453.5 ± 10.4 g per group) and subjected to immunoassay via ELISA kits to measure plasma levels of (**a**) testosterone and (**b**) 17β-estradiol at onset of the experiment, after 1 month, and after 2 months. Vertical bars denote standard errors. Values with different superscripts (a, b, or c) within a given observation period indicate significant differences between groups ($p < 0.05$) as determined using Tukey's multiple range test.

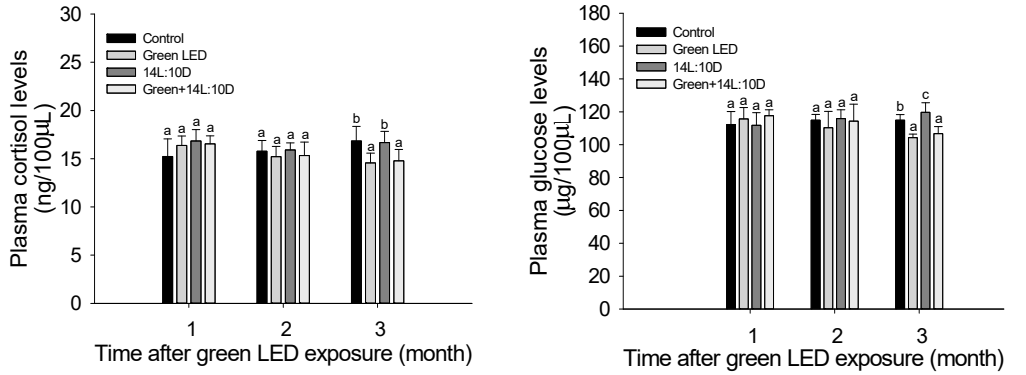


Figure 4. Chub mackerel, *Scomber japonicus*, were exposed to a 12 h light and 12 h dark photoperiod, either under fluorescent lighting (Control) or under green LEDs (Green LED); mackerel were also exposed to an extended 14 h light and 10 h dark photoperiod under fluorescent lighting (14L:10D) or green LEDs (Green+14L:10D). Blood was drawn from the caudal veins of fish (n = 40; full length, 33.7 ± 3.5 cm; weight, 453.5 ± 10.4 g per group) and subjected to immunoassay via ELISA kits to measure plasma levels of (**a**) cortisol and (**b**) glucose at onset of the experiment, after 1 month, and after 2 months. Vertical bars denote standard errors. Values with different superscripts (a, b, or c) within a given observation period indicate significant differences between groups ($p < 0.05$) as determined using Tukey's multiple range test.

4. Discussion

Sexual development and maturation in fish are regulated by various sex hormones produced in the hypothalamus–pituitary–gonadal axis, including *GnRH*, *GTH*, steroid hormones, and other neurohormones [31,32]. *GnRH* is critical for the initiation and maintenance of reproductive function as well as sexual behavior in fish, and the release of *GnRH* is influenced by the interaction of excitatory and inhibitory signals in the hypothalamus [33]. Subsequently, *GnRH* transfers the information to the gonadotropic cells of the pituitary,

thereby stimulating the synthesis and release of gonadotropic hormones such as *FSH* and *LH*. In this study, the *GnRH* mRNA expression of *S. japonicus* was significantly increased after exposure of the fish to green LEDs and a 14L:10D photoperiod; this increase was most pronounced for the 14L:10D photoperiod using a fluorescent bulb and green LEDs, which indicated that the photoperiod affected the experimental fish more than wavelength did. Ref. [34] suggested that the photoperiod promotes gonadal maturation in salmon species, reporting a significant increase in *GnRH* expression of masu salmon (*Oncorhynchus masou*) due to a lengthened photoperiod. Ref. [35] confirmed that the expression of *GnRH* mRNA in the hypothalamus of goldfish (*Carassius auratus*) was significantly higher in a long photoperiod (14L:10D) than in shorter photoperiods (12L:12D or 10L:14D). Similarly, ref. [36] reported that *GnRH* expression in the threespine stickleback (*Gasterosteus aculeatus*) significantly increased in a long photoperiod (16L:8D) compared to a short photoperiod (8L:16D), indicating sexual maturation through the photoperiod.

Furthermore, GnRH is a fundamental link in the brain–pituitary–gonad axis and regulates the production of two types of GTH (*GTH*-I, which is FSH-like, and *GTH*-II, which is LH-like) by the pituitary; together, these hormones have a major function in stimulating the maturation of gonads [37,38]. In this study, the *GTH* mRNA expression of *S. japonicus* was significantly increased by green LED exposure and a 14L:10D photoperiod. The increase was most pronounced in fish exposed to the 14L:10D photoperiod under fluorescent lighting and green LED. Ref. [39] suggested that a long photoperiod stimulates *GTH* synthesis and release in fish; they reported that the *GTH* of greater amberjack (*Seriola dumerili*) increased significantly under a long photoperiod (18L:6D) compared to other periods (natural or 8L:16D), which means that the long photoperiod can accelerate ovarian development. The stimulation of *GnRH* is closely related to a *GTH* increase and sexual maturation in various fish species, such as *C. auratus*, African catfish (*Clarias gariepinus*), *S. aurata*, eels (*Anguilla anguilla*), platyfish (*Xiphophorus maculatus*), and salmonids [40]. Ref. [36] reported that *G. aculeatus* remains immature in short photoperiods (8L:16D), but matures rapidly in long photoperiods (16L:8D). In this study, it was confirmed that a green LED wavelength could stimulate sexual maturation by inducing significant expression of *GnRH* and *GTH* mRNA in *S. japonicus* and that an increase in the photoperiod further increased *GnRH* and *GTH* expression.

FSH and *LH* are key hormones regulating fish reproduction; *FSH* is primarily responsible for gametogenesis initiation and gonadal growth, while *LH* regulates gonadal maturation, spermatogenesis, and ovulation [41]. The expression of *FSH-β* and *LH-β* in the pituitary gland is known to show a similar trend, but the cycle of synthesis and release of *FSH* and *LH* appears differentially: *FSH* increases in the early stages of yolk formation and spermatogenesis in fish, while *LH* increases in the final stage of maturation [42,43]. In this study, the *FSHβ* and *LHβ* mRNA expression in *S. japonicus* was significantly increased through green LED exposure and a longer photoperiod (14L:10D). This increase was most pronounced in fish exposed to a 14L:10D photoperiod under fluorescent lighting and in fish exposed to green LEDs, which suggests that both the photoperiod and green LED wavelength may play roles in inducing the production and release of sex hormones in *S. japonicus*. Ref. [39] showed that a long photoperiod (18L:6D) induced a significant increase in plasma *FSH* in previtellogenic *S. dumerili* compared to short photoperiods (8L:16D), suggesting that photoperiod cues are essential for inducing ovarian development in fish. Ref. [44] also reported that dark conditions (no light for 24 h) caused disturbances in ovarian growth in *D. labrax*, and that exposure to a long photoperiod significantly increased *FSHβ* expression. Ref. [45] argued that the photoperiod influences the expression of hormones such as *FSHβ* and *LHβ*, and they reported that a long photoperiod significantly increased *LHβ* levels in the cichlid fish, *Cichlasoma dimerus*.

As previously mentioned, *GnRH* promotes the synthesis of *FSH* and *LH*, which are transported to the gonads in the bloodstream where they bind to their respective receptors to induce the production of sex hormones [46]. Of the sex hormones, testosterone is not only essential for sperm production, but also acts as a precursor to estrogen biosynthesis;

thus, it is equally important in the female reproductive process [47]. Estradiol induces yolk formation by affecting vitellogenin gene expression in the liver and increases the granulosa cells of ovarian follicles [46]. In this study, we found that an increase in photoperiod and light wavelength resulted in significant increases in testosterone and 17β-estradiol levels in *S. japonicus*, indicating increased production of these sex hormones. Ref. [48] reported that a lengthening photoperiod caused by seasonal changes from winter to early spring promotes ovulation and spermatogenesis in *O. mykiss*, and they argued that photoperiod was associated with increases in plasma 17β-estradiol and plasma testosterone in female salmon. Ref. [39] reported a significant increase in 17β-estradiol levels in *S. dumerili* in a long photoperiod (18L:6D) compared to a short photoperiod (8L:16D), which could be recognized as a signal to initiate ovarian development. Ref. [49] reported that the green LED wavelength induced sexual maturation in the yellowtail damselfish (*Chrysiptera parasema*) and consequently observed a large number of mature oocytes, indicating that green LEDs induced ovarian maturation. Similarly, our findings suggest that this approach may be valuable for inducing sexual maturation and improving the reproductive ability of *S. japonicus*.

Photoperiod manipulation in fish can affect stress responses [50]. Neuroendocrine responses to stressors (such as toxicity) in fish entail activation of the hypothalamus–pituitary–interrenal axis, which results in increased cortisol production; therefore, cortisol is widely used as a stress indicator in fish [51]. In this study, the plasma cortisol levels of S. japonicus were significantly decreased in fish exposed to green LED lights, compared to fish in the control group (12L:12D) and fish exposed to fluorescent lighting (14L:10D). This suggests that the wavelength of green LEDs imposed less stress on fish compared to that of fluorescent bulbs. Ref. [52] reported that a gradual decrease in photoperiod affected the plasma cortisol of pintado (*Pseudoplatystoma corruscans*), which indicates that the photoperiod may modulate the stress response in fish. Ref. [53] reported that the change in daily photoperiod induced a significant change in plasma cortisol in *D. labrax*. Ref. [50] reported a significant increase in plasma cortisol of juvenile great sturgeon (*Huso huso*), which indicates that extreme photoperiod changes can induce stress. Plasma glucose is closely linked to carbohydrates that induce metabolic stress in fish, and high blood glucose is a critical indicator for evaluating stress, being the result of glycolysis in liver tissue to provide energy during stress conditions [54]. Ref. [55] suggested that a change in photoperiod acts as a stress factor, reporting a significant increase in plasma glucose of common dentex (*Dentex dentex*) as a result thereof. On the other hand, refs. [56,57] reported that changes in photoperiods did not affect stress indicators in striped knifejaw (*Oplegnathus fasciatus*) and red sea bream (*Pagrus major*). Ref. [58] concluded that an artificial photoperiod does not cause an acute stress response, such as heightened plasma cortisol and glucose levels, in Nile tilapia (*Oreochromis niloticus*). In this study, the stress response of *S. japonicus* was not affected by a change in the photoperiod. However, a green LED wavelength showed a tendency to decrease stress indicators, which indicates that the photoperiod and green LEDs do not affect the stress of fish, and that breeding using green LEDs may rather reduce the stress of fish.

5. Conclusions

In conclusion, an increase in the amount of light and the wavelength of green LEDs induced significant increases in *GnRH* and *GTH* mRNA expression in *S. japonicus*, which encode the major pituitary hormones in fish. In addition, an increase in photoperiod and green LED lighting stimulated a significant expression of gonadotropic *FSHβ* and *LHβ* mRNA and an increase in testosterone and 17β-estradiol sex hormone levels in *S. japonicus*. The levels of two key stress indicators, cortisol and glucose, were significantly lower in fish exposed to the green LED wavelength compared to those exposed to fluorescent lighting. The results of this study indicated that a longer photoperiod and green LED wavelength both stimulated the production of key factors related to the sexual maturation and development of *S. japonicus* and lowered stress induction.

Author Contributions: Data curation, Y.J.C., S.G.N.R.P., A.-H.J. and J.-H.K.; writing—original draft preparation, J.-H.K., S.G.N.R.P. and A.-H.J.; writing—review and editing, J.-H.K.; supervision, Y.J.C. and J.-H.K. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Ethics approval and consent to participate. The participants in this study were trained in animal protection, animal welfare, and animal experimentation by the National Institute of Fisheries Science. All experimental animals used in this study were maintained under a protocol approved by the Institutional Animal Care and Use Committee of the National Institute of Fisheries Science (NIFS-2022-30).

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Conflicts of Interest: The authors declare no conflict of interest.

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