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DIFFERENT LED LIGHT EFFECT ON GONAD MATURATION AND GENE EXPRESSION IN FEMALE SYNODONTIS BROODSTOCKS (*Synodontis* sp.)

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ABSTRACT

The study investigated the influence of the different LED light spectrums on the maturation of the gonads in female *synodontis* broodstock and examined the maturation-related gene expression levels. Female *synodontis* were exposed to four different LED light spectra, specifically: white, blue, green, and red, for a continuous period of 120 days rearing. This study employed a completely randomized design, consisting of four treatments and five individual fish as replication for each treatment. An analysis was conducted on the gonadosomatic index (GSI), hepatosomatic index (HSI), gonad histology, estradiol levels, *kiss2* and *gnrh2* mRNA expression levels. The utilization of blue LED light treatment is highly effective in enhancing the reproductive parameters in female *synodontis* fish. The results show that brain and gonad *kiss2* mRNA expression levels are not significantly different ($P < 0.05$), while *gnrh2* mRNA expression levels were significantly different ($P < 0.05$) and had the highest expression in the gonads. The results suggest that light exposure can induce changes in the expression levels of *kiss2* and *gnrh2* mRNA, as well as control reproduction.

KEYWORDS: Female *synodontis*; LED light; gonad maturation; *kiss2*; *gnrh2*

INTRODUCTION

Light is a crucial environmental component that controls the pace of gonad growth of fish (Zou *et al.*, 2022). Light stimulation impacts the secretion of hypothalamic hormones, which in turn triggers the release of gonadotropin hormones in the pituitary gland, promoting the maturation of the gland (Yuniar, 2017). Several research employed light modulation to mitigate stress and retinal damage in fish (Song *et al.*, 2016; Song & Choi, 2019), enhanced fish growth and lifespan (García *et al.*, 2020), influenced sex reversal in medaka fish (Hayasaka *et al.*, 2019), and impacted fish reproductive (Choi *et al.*, 2018). Multiple research employed light modulation to mitigate stress and retinal damage in fish (Mylonas *et al.*, 2010). Choi *et al.*

(2018) stated that environmental manipulation is crucial in stimulating the hypothalamus-hypophyse-gonad axis (HPG), which is responsible for the growth and reproduction of the gonad. Ikegami & Yoshimura (2016) asserted that the activation of the HPG axis commences with the secretion of gonadotropin-releasing hormone (*gnrh*) by the hypothalamus into the portal blood capillaries of the pituitary gland (estrogen and androgen). Hence, it is essential to assess the impact of various LED (light-emitting diodes) light colors on the reproduction of *synodontis* fish.

LEDs in power supply have advantages over other lamps. they consume less power to save costs. In addition, LEDs are also more efficient, easier to apply, and have a lifespan that is 10-times longer than that of any other type of lamp. According to studies conducted by Yoomak & Ngaopitakkul, (2018) and Rangari *et al.* (2021), LED light spectrum manipulation is a cost-effective solution. LED light spectrum manipulation has the advantage of being easy to ap-

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ply and manage, especially in ornamental fish production. Aquaculture can also benefit from LEDs as they offer a more efficient lighting system with spectral manipulation that can be adjusted to the sensitivity of the target species (Zou *et al.*, 2022).

The impact of light spectrum revealed that four-month-old female cookfish exhibited more mature ovaries when exposed to the green LED spectrum, as opposed to the red and white LED spectrum (Shin *et al.*, 2014). Furthermore, Bairwa *et al.* (2017) discovered that the green light LED spectrum accelerates the maturation of female Koi fish's gonads, as evidenced by the diameter of the ovum, vitellogenin, and its steroid hormones. The effects of blue and green LED light spectrums have also been conducted on female yellowtail damselfish, which revealed higher GSI and plasma levels of estradiol compared to the red-light spectrum (Shin *et al.*, 2013). Sinansari *et al.* (2024) discovered that in male synodontis, the blue LED light has the best performance of GSI (gonadosomatic index) and sperm quality. Red light can also make zebrafish less fertile (Adatto *et al.*, 2016), Nile tilapia more fertile (Volpato *et al.*, 2004; Volpato *et al.*, 2013), and tropical damselfish ovaries develop differently (Bapary *et al.*, 2011). According to Choi *et al.* (2018), different species of fish can respond to different colors of light, which means the light response depends heavily on the species.

Light spectrum can change the amount of gene expression linked to GnRH, FSH (follicle-stimulating hormone), and LH (luteinizing hormone) reproduction in the brain tissue and pituitary of grass buffer fish during fertilization (Choi *et al.*, 2018). The results show that the abundance of mRNA sbGnRH (seabream) and FSH α in the green spectrum is significantly higher than in the white, red, and blue spectrums ($p < 0.05$). In addition, light is an environmental factor regulating physiological functions such as reproduction and gonad maturation (Bairwa *et al.*, 2017). Retinal photoreceptors and the extra retina enable fish to detect differences in light intensity and spectrum. According to Bayarri *et al.* (2002) and Vera *et al.* (2007), fish are capable of detecting differences in light intensity and spectrum. Sensitivity to light varies between species, affecting behavior, somatic growth, reproductive activity, and survival (Volpato *et al.*, 2004; Bapary *et al.*, 2011).

Synodontis, a species of catfish from Africa, is a type of featherfin catfish. Most information about this species comes from the literature on catfish, with a few references in scientific literature (Kusrini & Cindelar, 2011). Research on the behavior of synodontis is crucial for determining reproduction quality. The availability of mature gonad broods, ready

for breeding outside the peak season, remains a key factor in the cultivation of synodontis. A study by Nurhidayat *et al.* (2017) on the process of gonad maturity in fish found that the rate of gonad maturity is not the same for male and female broods in the reproductive environment. Using LED lamps to manipulate the light spectrum can be a breakthrough strategy for preparing synodontis broods. The study aims to evaluate the effects of various LED light spectra on gonadal maturation and to analyze the expression of the genes *kiss2* and *gnrh2* in female synodontis broodstock.

MATERIAL AND METHODS

Research design

This research was conducted experimentally using a completely randomized design. Four treatments and five individual fish as replications for each treatment were exposed to the white (450 and 50 nm), green (530 nm), blue (460 nm), and red (630 nm) LED light spectrum. The rearing stage involves the treatment of fish for a period of 120 days. The LED light spectrum was used for 24-hour lighting in the laboratory.

LED Light Wavelength Measurement

The study used LED lights with white, red, green, and blue light spectrums. Wavelength measurements were done using the Ocean Optics USB2000 and Ocean Optics Spectrasuite application. Microsoft Excel converted the data into a curve diagram. Figure 1 displays the peak wavelength and range of the light spectrum.

Experimental fish and setup

The female broods synodontis, each over one year old, were obtained from a farmer in the Ciseeng area, Bogor District. Fish were reared in 4 fiber tubes measuring 75 cm \times 60 cm \times 60 cm, which were placed in a row. Aras *et al.* (2016) research also utilized the CE 101 3-eye LED lamp, which operates at 12 volts and comes with a 12 volt, 10 A adapter. LED light installation followed by (Sinansari *et al.*, 2024).

Fish diet and maintenance

Fish were fed a commercial diet containing 40% of protein twice daily (09.00 AM and 17.00 PM) at a rate of 3% of their body weight. Temperature in cultivation containers are measured twice every day, in the morning at 07.00 AM and in the afternoon at 05.00 PM. Carbonate hardness (KH) and general hardness (GH) were measured at the water source entering the maintenance container and with measurement

taken three times during the maintenance period. Temperature, pH, dissolved oxygen, and ammonia levels were measured in the rearing tank to monitor optimal water quality conditions. The water was changed once a week by as much as 70%, and water was added at least twice a week. The water quality during the investigation exhibited a temperature range

of 25.8-28.1°C, a pH range of 6.33-7.9, dissolved oxygen levels ranging from 2.38-5.98 mg/L, ammonia 0.5-1 mg/L, KH range of 1-3 dKH, and GH range of 3-4 dGH. This water quality range aligns with the established protocols for rearing *synodontis* fish, as described by (Nurhadi *et al.*, 2021).

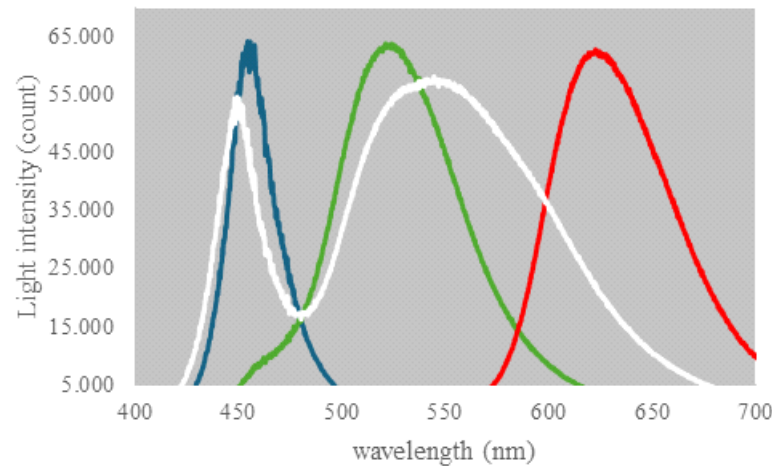


Figure 1. Spectral profiles of (a) white LED, and (b) blue (B), green (G) and red (R) LEDs used in this experiment. Dotted line shows the spectral profile of a white fluorescent light.

Parameter observations

Gonadosomatic Index (GSI), Hepatosomatic Index (HSI), and gonad histology

Gonads and liver were collected at the initial and end of fish rearing to examine the gonadal morphology, calculate the GSI and HSI, and analyze the gonadal histology. The fish were euthanized using ethylene glycol monophenyl ether (phenoxy ethanol) at a concentration of 0.3 mL per liter of water for 5–10 minutes. The total fish, gonad, and liver weight were measured using a digital scale with 0.01 g accuracy. Gonad and liver were dissected and weighed ($n = 3$ for each treatment replication). The gonads were fixed in Bouin's solution and then stored in 70% ethanol, followed by histological processing using Gunarso's procedure (1989). The histological structure of the preparations was examined under a microscope at 40x10 magnification, and the gonads were quantified using the Indomicro View program and ImageJ software at the Laboratory of the Research Institute for Ornamental Fish Culture, Depok.

Estradiol Hormone Analysis

During the experiment, blood collection was conducted on days 12, 24, 36, 48, 60, 72, 84, 96, 108, and 120. Prior to weighing, the 0.1–0.2 mL of blood was collected from the dorsal aorta located at the base of the tail. The procedure employed a heparin-treated syringe. The blood sample was then trans-

ferred to a 1.5-mL microtube and centrifuged at 10,000 rpm for 10 minutes at 5°C. The plasma was isolated and stored in new microtubes in a freezer set to -20°C. This procedure was carried out to assess the estradiol concentration using the commercial estradiol ELISA kit (Catalog No. EIA2693) manufactured by DRG Instruments GmbH, Germany.

Egg diameter and fecundity

Eggs were observed at the end of rearing using a cannulation system. The eggs were placed on a glass slide and treated with 1 mL of Serra solution. Then the eggs were separated using a syringe to prevent them from sticking together. The egg diameter was examined ($n=100$) using a microscope with 40x10 magnification. Measurements were taken using a micrometer scale (100 micrometers) and analyzed using ImageJ software from the National Institute of Health in the USA. The relative fecundity was calculated by comparing the number of eggs produced to the fish body weight (measured in kilograms). To calculate this, 0.5 grams of ovulated eggs were measured and counted. Egg collection was performed three times and the average number of eggs was determined. This average value was then multiplied by the weight of the ovulated eggs and then divided by the mother's body weight.

gnrh2 and kiss2 mRNA Expression

Total RNA was extracted from brain and gonad tis-

sues. Three female broodstock in all treatments were anesthetized and monitored until unconscious. The fish was then dissected to take the brain and gonad. The tissues were weighed and put in an RNA Lateral solution (Qiagen) to stabilize the RNA condition and prevent degradation.

RNA was extracted from the tissues (0.25 mg) using a 1 ml Tri-reagent Kit (MRC) according to the manufacturer's protocol. After total RNA was obtained, the cDNA synthesis process was then carried out using the NZY First-Strand cDNA Synthesis Kit (Nzytech). The composition of the premix for cDNA synthesis is NZYRT 2× Mastermix 10 µL, NZYRT Enzyme Mix 2 µL, 1 ng total RNA concentration, and DEPC-treated H₂O to a total volume of 20 µL. The premix solution was mixed, incubated at 25 °C for 10 minutes, at 50 °C for 30 minutes, and inactivated at 85 °C for 5 minutes. One microliter of NZY RNase E.

coli was added and incubated at 37°C for 20 minutes. The cDNA samples were stored at -20 °C.

Each sample was run in a final volume of 10 µL containing 0.5 µL cDNA, 0.5 µL of primary, and 5 µL of master mix (THUNDERBIRD™ Next SYBR® qPCR Mix). As a control, testing was done without a template and reverse transcriptase. No amplification was observed in control studies. Cycle threshold (Ct) values were obtained from the exponential phase of PCR amplification and target gene expression normalized against the expression of the β-actin gene to produce 2^{-ΔΔCt} values to measure the genetic abundance of the target gene (Livak & Schmittgen, 2001). Real-time quantitative PCR (qPCR) was conducted using ABI Prism 7500 thermal cycler (Applied Biosystems, Foster, CA, USA) at 95°C (15 s), 60°C (1 minute) 40 cycles (Chaube *et al.*, 2020). The oligonucleotide primers for qPCR are presented in Table 1.

Table 1. Primary sequences used in the analysis of gonad maturation-related gene expression in female synodontis with real-time quantitative PCR method

Primer	Sequence (5'-3')	Size	Reference
<i>gnrh2</i> -F	GTTCAGCACAGACGAGGCA	145 bp	Chaube <i>et al.</i> (2020)
<i>gnrh2</i> -R	CTGATGTGTTCTCCAGGGCA		
<i>kiss2</i> -F	CAGACAGACCAGGATGTCCA	165 bp	Chaube <i>et al.</i> (2020)
<i>kiss2</i> -R	AGATCGGGATCGAGGAAAAT		
<i>β-actin</i> -F	TGGCCGTGACCTGACTGAC	157 bp	Chaube <i>et al.</i> (2020)
<i>β-actin</i> -R	CCTGCTCAAAGTCAAGAGCGAC		

Data analysis

Parameter data for evaluating the reproductive performance of female synodontis broods (GSI, HSI, egg diameter, fecundity, estradiol levels, and gene expression) were analyzed quantitatively. The data were tabulated (mean±SD) in tabular form and analyzed statistically (IBM SPSS Statistics 23), and a one-way ANOVA test was performed at a 95% confidence interval. Differences between treatments were evaluated using Duncan's advanced test. Graphs and images present descriptive data from observations of estradiol hormone levels and gonad histology.

RESULTS AND DISCUSSION

Reproductive performance

The study's findings revealed that exposure to LED light can affect the maturation of female synodontis fish gonads (Table 2). After 120 days of maintenance, the exposure to LEDs was shown to significantly increase GSI, HSI, egg diameter, and fecundity (p<0.05). Based on statistical analysis, GSI values, egg diameter, and fertility have the same pattern, with the highest result in blue LED treatment and the lowest

in green LED treatment, whereas the HSI value generated in white LED treatment is a real difference (P<0.05) with green, red, and blue LED treatments with values of 1.64±0.06%, 1.46±0.20%, 1.50 0.12%, and 1.59±0.10%. In various studies, blue light has different effects on female broods' GSI and HSI.

According to Choi *et al.*, (2018), found that light activates the brain-pituitary-gonad (BPG) axis, causing the hypothalamus to produce and secrete more gonadotropin-releasing hormone (GnRH), which elevates steroid hormone levels and affects gonadal maturation and fish spawning. The study found that using blue and red LED lights effectively increases the maturity of female synodontis gonads, with blue light treatment showing higher values, despite no statistically significant difference between the two. In line with research on Yellowtail Dam (Shin *et al.*, 2013) and Koi (Bairwa *et al.*, 2017), exposure to blue light results in higher levels of GSI and increased ovarian maturation, indicating positive effects on reproductive performance and maturation processes. By contrast, research on zebrafish shows that blue light exposure causes a decrease in GSI in the ovaries. Yuan *et al.* (2017).

Table 2. Reproductive performance of female synodontis broodstock under lighting conditions using white, green, red, and blue LEDs for 120 days of rearing

Parameters	Treatments			
	Blue	Red	White	Green
Initial GSI (%)	0.88±0.04	0.88±0.04	0.88±0.04	0.88±0.04
Initial HSI (%)	0.64±0.06	0.64±0.06	0.64±0.06	0.64±0.06
Final GSI (%)	12.05±0.35 ^b	10.93±1.51 ^b	2.62±1.58 ^a	1.59±1.43 ^a
Final HSI (%)	1.59±0.10 ^{ab}	1.50±0.12 ^{bc}	1.64±0.06 ^a	1.46±0.20 ^c
Egg diameter (mm)	1.34±0.13 ^b	0.98±0.16 ^b	0.50±0.36 ^a	0.31±0.08 ^a
Fecundity (eggs)	62,462±6,018 ^b	58,575±16,263 ^b	13,084±9,271 ^a	15,799±5,471 ^a

Remarks: Numbers followed by the same superscript letters in the same row indicates no significant difference ($P>0.05$).

Blue and red LED lights improve female synodontis gonad egg diameter and fertility, with blue light treatment exhibiting greater values despite no statistically significant difference ($P<0.05$) after 120 days of rearing (Table 2). Bapary *et al.* (2011) found that LED light's effectiveness in promoting ovarian development in fish may be influenced by the sensitivity of specific photoreceptors, with long wavelengths like red and blue more effectively absorbed by the pineal organ, which regulates the reproductive cycle. Additionally, synodontis fish that inhabit rivers prefer dim waters, as they are nocturnal and prefer to search for sustenance at the bottom of the water (Kusrini & Cindelaras, 2011).

The average egg diameter is highest on the blue LED treatment, followed by the red, white, and green LEDs with respective successive values of 1.34 ± 0.13 mm, 0.98 ± 0.16 mm, 0.5 ± 0.036 mm, and 0.31 ± 0.08 mm. The maximum fecundity values on blue LEDs were followed by red, green, and white LEDs, with respectively successive values of $62,462\pm6,018$, $58,575\pm16,263$, $15,799\pm5,471$ and $13,084\pm9,271$

eggs. According to Bairwa *et al.* (2017), a bigger egg diameter in a certain group suggests early maturity and a higher GSI because of the larger size of the eggs, not because there is a rise in the number of oocytes. Volpato *et al.* (2004) demonstrated that exposing female tilapia fish (*O. niloticus*) to blue light greatly enhances their fecundity. Shin *et al.* (2013) confirm that the later stages of egg development in fish exposed to green and blue light underscore the significance of these wavelengths in boosting reproductive capacity. In addition, Bairwa *et al.* (2017) have found that the green light spectrum positively affects the egg cell diameter and reproductive performance in koi fish.

Blood estradiol hormone profile

Figure 2 shows that the concentration of estradiol-17 β in the blood of the synodontis fish fluctuated. The peak concentrations of the hormone estradiol-17 β (black arrow) form an identical pattern of three peaks that coincide with the occurrence of

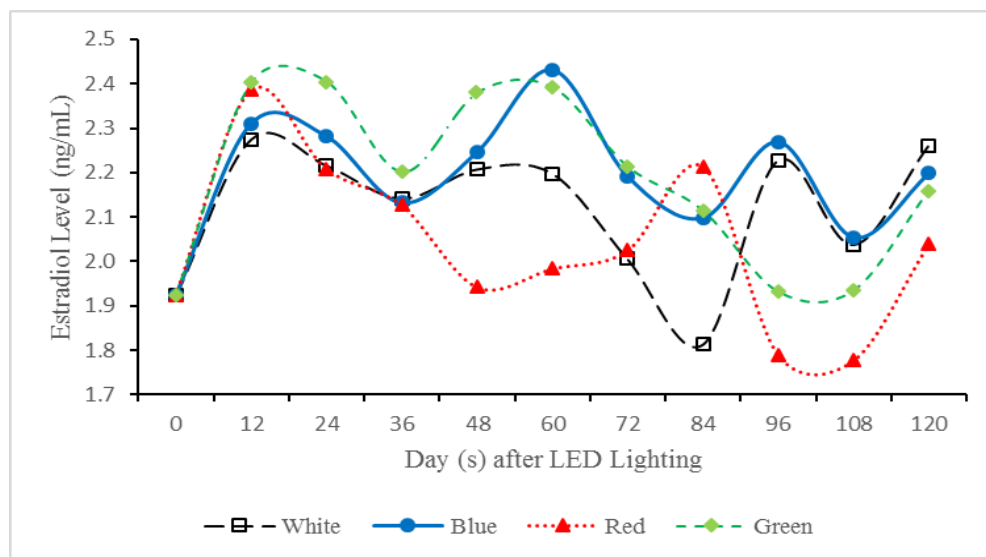


Figure 2. The blood estradiol concentration in synodontis under lighting conditions using white, green, red, and blue LEDs for 120 days of rearing.

gonad maturity in blue and white LED light treatment, while in other treatments only two peaks. Figure 2 shows that the hormone estradiol-17 β experienced an increase in concentration from the start of maintenance to a peak occurring on the 60th day, then fluctuating until the end of maintenance. The concentration of the hormone estradiol-17 β in the 120-day time range in blue and white LED light treatment can accelerate the maturation of the gonads three times, which is an average of 36 days, while for red and green LED light it is capable of accelerating gonad maturation twice, that is, on average 72 days. Sinansari *et al.* (2024) have found that the most significant level of gonad maturation was observed on the 60th, 84th, and 108th days of the blue LED treatment in male synodontis. Shin *et al.* (2013) have demonstrated that blue light significantly influences the estradiol concentrations of female fish (*Chrysiptera parasema*), blue light can raise the levels of vitellogenin (VTG) and estrogen receptor (ER), as well as plasma levels of the hormone estradiol-17 β . In the same way, research on mackerel chubs shows that long photoperiods and certain wavelengths of light raise plasma levels of estradiol-17 β and other sex hormones.

Gene expression levels

Figure 3 presents the results of measuring the mRNA expression levels of *kiss2* and *gnrh2* genes taken from brain and gonad tissues after 120 days of LED lighting. The expression of the *kiss2* gene showed that neither brain tissue nor gonad does not show any significant difference ($P > 0.05$). *Kiss2* gene expression in brain tissues is highest in blue LED treatments (1.90 ± 1.64), whereas in gonad tissues, the

highest expression is in red LED treatments (10.90 ± 10.86). The *gnrh2* expression showed statistically significant difference ($P < 0.05$) in both gonad and brain tissues. The results showed that after exposure to blue LED light, the gonad and brain tissue showed the highest level of *gnrh2* expression. The study's results indicated that following exposure to blue LED light, the peak level of *gnrh2* expression was seen in the brain at 3.55 ± 1.30 and in the gonads at 32.91 ± 4.96 . Different wavelengths of LED light can modulate the expression of neurohormones and related genes. For instance, teenage red-spotted crustaceans are significantly more likely to express *kiss2* and GPR54 when they are exposed to different LED spectrums, such as white, red, blue, and green. This is especially true when they are exposed to white and blue LEDs, which suggests that these wavelengths can stimulate the reproductive axis by increasing kisspeptin signaling (Xu *et al.*, 2023). Also, in goldfish, green and purple LEDs greatly raise the amounts of the hormones *kiss1*, *kiss2*, GPR54, and gonadotropin (GTH). This suggests that these wavelengths raise the production and release of reproductive hormones (Yun *et al.*, 2015). Furthermore, studies have shown that changes in photo period and temperature conditions in catfish, mimicking the effects of different light spectrums, stimulate the expression of *kiss2* and *gnrh2*, with long photoperiods and high temperatures producing maximum effects. This further supports the role of light in regulating these genes (Chaube *et al.*, 2020). Additionally, exposure to green LED light in goldfish was found to significantly increase GnRH, *kiss1*, GPR54, and GTH mRNA levels, as well as plasma LH and 17 α -hydroxypregnenolone levels, enhancing sexual maturity (Shin *et al.*, 2014). On the contrary, red LED lights and melatonin treatments have been shown to de-

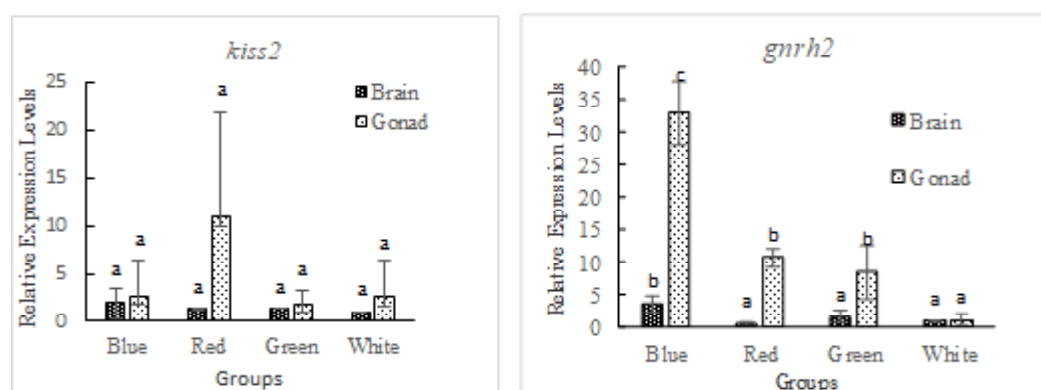


Figure 3. *Kiss2* and *gnrh2* gene expression levels in female synodontis after white, green, red, and blue LEDs exposure for 120 days of rearing. The results are expressed as normalized fold expression levels with respect to the β -actin levels in the same sample. The groups with different letters are significantly different and those with the same letters are not significant. All values are means \pm SD ($n=3$).

crease the regulation of *kiss1* and GPR54 expression, showing inhibitory effects on the production of reproductive hormones (Sipos *et al.*, 2019; Choi *et al.*, 2023).

Gonad histology

Gonad morphology in female synodontis after a 120-day rearing period by different LED lights is presented in Figure 4. This study investigated the impact of light spectrum on gonadal development in Synodontis fish by histological examination. The re-

sults demonstrated an increase in GSI expression levels, fertility, and egg diameter in fish exposed to blue LED light, followed by red, green, and white light. The use of blue LED light signifies the presence of mature gonads, evidenced by advanced oocyte development indicated by germinal vesicle breakdown (GVDB). The treatment of red LED light also indicates secondary development of vitellogenin, while the treatment of green and white LED light indicates advanced stages of gonadal maturation but is not at the same stage of development as blue and red LED lights.

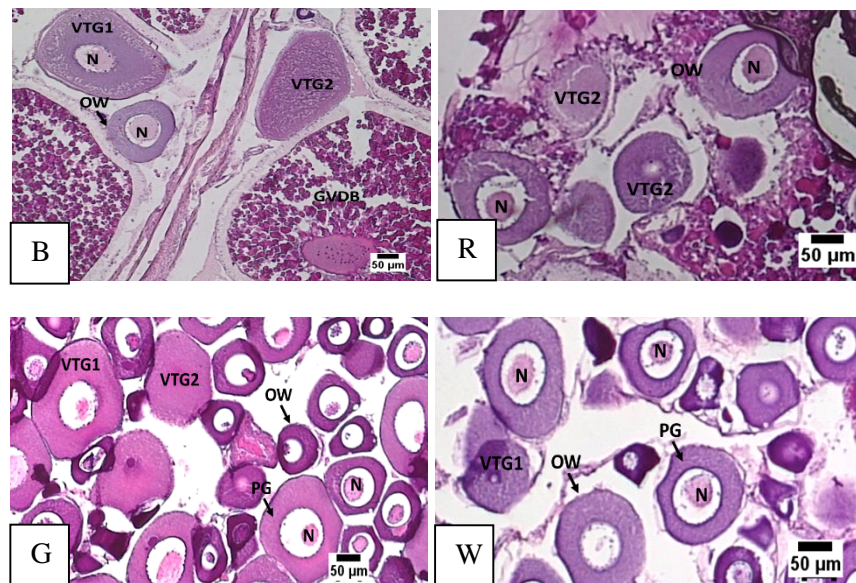


Figure 4. Histology of female synodontis gonads for all treatments under different lighting conditions using white (control/W), green (G), Red (R) and blue LEDs (B). The histology was observed at 4x magnification. PG= primary growth oocyte; POF= postovulatory follicle complex; N= nucleus; OW= ovarian wall; VTG 1= primary vitellogenic; VTG 2= secondary vitellogenic; VTG 3= tertiary vitellogenic; GVM= germinal vesicle migration; dan GVBD= germinal vesicle breakdown.

CONCLUSIONS

Blue LED light spectrum gave the best results based on the GSI, HSI, egg diameter, fecundity, estradiol concentration, and maturation-related gene expression of the synodontis female.

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