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Original Research Articles

Effects of light spectrum, intensity and photoperiod on gonadal development and immunity of *Hexagrammos otakii*

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A 60-day trial was conducted to evaluate the effects of light spectrum, light intensity, and photoperiod on gonadal development, reproductive endocrine activity, and immune status of fat greenling (*Hexagrammos otakii*). An L9(3^3) orthogonal design tested nine of the twenty-seven possible factor combinations using three light spectra (red, 629 nm; green, 533 nm; and blue, 450 nm), three photometric setpoints (200, 1500, and 3000 lx), and three photoperiods (8L:16D, 12L:12D, and 24L:0D), together with a natural-light reference. Gonadal histology and plasma immunoglobulin M (IgM), complement component 3 (C3), 17β-estradiol (E2), and testosterone (T) were assessed. Compared with the natural-light reference, blue light at 200 lx under 8L:16D and green light at 200 lx under 24L:0D were associated with higher proportions of advanced germ cells and significantly higher plasma E2 and T concentrations ($P < 0.05$). Several combinations involving red light or the highest tested illuminance showed lower sex-steroid concentrations and less advanced gonadal development. Plasma IgM concentrations were highest under green light at 1500 lx with 12L:12D and blue light at 200 lx with 8L:16D, whereas C3 concentrations were highest under red light at 3000 lx with 24L:0D and blue light at 3000 lx with 12L:12D. Orthogonal range analysis identified 200 lx as the most favorable tested photometric setpoint, whereas estimated photoperiod main effects differed between ovarian and testicular development. The corresponding theoretical complete combinations were not directly tested and require further validation. These responses may involve melatonin-mediated modulation of the hypothalamic-pituitary-gonadal axis, although melatonin and gonadotropins were not directly measured. The findings provide a basis for refining artificial-light management in *H. otakii* broodstock culture while highlighting the need for validation under directly measured and matched photon irradiance.

INTRODUCTION

Light is one of the most important environmental factors regulating fish physiology. In aquaculture systems, artificial light is mainly determined by spectral composition, light intensity, and photoperiod. These factors can influence feeding activity, growth, endocrine rhythms, reproductive maturation, and immune status through retinal, pineal, and deep-brain photoreceptive pathways.[1-5] Therefore, light regulation has become a useful management tool in broodstock culture and controlled reproduction.

However, excessive or insufficient illumination can impair reproductive performance and health. Photoperiod is widely regarded as a key signal for synchronizing seasonal reproductive rhythms in teleosts. Changes in day length can alter melatonin secretion and regulate the hypothalamic-pituitary-gonadal axis, thereby affecting gonadotropin secretion, steroidogenesis, and gametogenesis.[6-12] Spectral composition has species-specific effects on fish reproduction. Blue, green, and red light have been reported to produce different responses in Nile tilapia, yellowtail damselfish, little yellow croaker, and red spotted grouper.[12-16] In addition, light intensity can modify physiological responses by changing stress status, energy allocation, and oxidative balance.[11,17-21] These differences are likely associated with species-specific habitat adaptation and visual

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sensitivity. However, the combined effects of spectrum, light intensity, and photoperiod are less well understood than the effect of every single factor.

Reproductive development is closely related to endocrine and immune status. Plasma 17β-estradiol (E2) and testosterone (T) are important indicators of ovarian and testicular endocrine activity in teleosts.[22-25] Immunoglobulin M (IgM) and complement component 3 (C3) are commonly used to evaluate humoral immunity and innate immune activation.[26-30] Because reproductive processes and immune responses are closely linked, simultaneous evaluation of gonadal histology, sex steroid hormones, and immune factors can provide a more complete understanding of broodstock physiological status.

Hexagrammos otakii is an economically important cold-temperate marine fish in China. Previous studies have mainly focused on artificial breeding, nutrition, intestinal health, and early development.[31-34] However, information on light-environment regulation of gonadal development and immunity in this species remains limited. Therefore, this study evaluated the effects of light spectrum, photoperiod, and intensity on gonadal maturation, steroid hormone secretion, and immune status in *H. otakii*, with the aim of providing practical guidance for broodstock light management.

MATERIALS AND METHODS

1. EXPERIMENTAL FISH AND FEEDING MANAGEMENT

Adult *H. otakii* were obtained from the Key Laboratory of Applied Biology and Aquaculture in Northern China, Dalian Ocean University. The average body length and body weight of the fish were 25.71 ± 5.58 cm and 381.97 ± 15.87 g, respectively. All broodstock were confirmed to be at a comparable gonadal developmental stage before the trial.

The experiment was carried out in 600 L tanks equipped with bottom-side drainage valves. A 14-day acclimation period was followed by a 60-day light-exposure trial. Nine artificial-light treatment groups and one natural-light reference group were established. Each group had three replicate tanks, and 30 broodstock were randomly assigned to each tank at a female-to-male ratio of 1:1. The tank was considered the experimental unit, and individual-fish measurements were averaged within each tank before statistical analysis. During the trial, water temperature was maintained at 16-20 °C, salinity at 27 ± 2 ppt, pH at 7.9 ± 0.4, and dissolved oxygen at 6.6 ± 0.7 mg/L. Total ammonia nitrogen and nitrite were maintained below 0.1 mg/L by exchanging one-third of the tank water twice daily. Fish were fed soft pellets twice daily at 07:30 and 16:00 to apparent satiation, and uneaten feed was removed after feeding.

2. LIGHT TREATMENTS

Three light spectra (red, 629 nm; green, 533 nm; and blue, 450 nm), three photoperiods (8L:16D, 12L:12D, and 24L:0D), and three light intensities (200, 1500, and 3000 lx) were arranged according to an L9(3^3) orthogonal design. The artificial-light treatments were designated R1-R3, G1-G3, and B1-B3. Specifically, the red-light treatments were R1 (1500 lx, 8L:16D), R2 (200 lx, 12L:12D), and R3 (3000 lx, 24L:0D); the green-light treatments were G1 (1500 lx, 12L:12D), G2 (200 lx, 24L:0D), and G3 (3000 lx, 8L:16D); and the blue-light treatments were B1 (1500 lx, 24L:0D), B2 (200 lx, 8L:16D), and B3 (3000 lx, 12L:12D). The natural-light reference was maintained under the ambient light conditions of the culture facility without artificial manipulation (Table 1). During the 60-day trial, the natural photoperiod varied from approximately 12L:12D to 15L:9D, and the natural light intensity reaching the water surface fluctuated between 1000 and 5000 lx depending on weather conditions and time of day. Because its spectrum, intensity, and photoperiod varied simultaneously, the natural-light group was treated as a practical husbandry reference rather than as a controlled orthogonal treatment.

Black shading cloth and light-blocking plates were used to prevent light leakage among tanks. Lighting fixtures were supplied by Shenzhen Anhong Ruida Technology Co., Ltd. The lamps were connected to an automatic timing-control system according to the assigned photoperiods. Light intensity was measured and calibrated daily using an illuminance meter (Zhejiang Yonghua Precision Electrical Manufacturing Co., Ltd., Yongkang, China). Because illuminance (lux) is weighted by the human photopic response, equal lux values at different wavelengths do not represent equal photon irradiance. To quantify the magnitude of these differences and aid interpretation, the recorded photometric setpoints were retrospectively converted to estimated theoretical photon flux density (PFD). The conversion was conducted using the following standard physical equation:

$$PFD = \frac{E_v \times \lambda \times 0.00836}{683 \times V(\lambda)}$$

where E◈ is the measured illuminance in lux, λ is the nominal peak wavelength of the LED (nm), and V(λ) is the standard relative visual sensitivity at that wavelength. The estimated PFD values for all experimental groups are presented in Table 2. These calculations assumed monochromatic emission at the nominal peak wavelength and did not account for the full spectral power distribution of the lamps. Spectral power distributions and photon flux densities were not measured directly; therefore, the calculated values are approximate and do not replace direct radiometric measurements.

3. SAMPLE COLLECTION

At the end of the 60-day trial, fish were fasted for 24 h and anesthetized with tricaine methanesulfonate (MS-222, 100 mgL^{-1}). Eighteen fish, comprising nine females and nine males, were randomly sampled from each replicate tank. Thus, 27 females and 27 males were sampled from the three replicate tanks in each treatment group. Blood was collected from the caudal vein using sterile syringes and centrifuged at 1000 × g for 15 min at 4 °C. The plasma was aliquoted and stored at -80 °C for the determination of sex steroid hormones and immune parameters. Plasma E2 was determined in the sampled females, whereas plasma T was

Table 1. Orthogonal Experimental Design Factors and Levels

Group	Factor		
	Spectrum	Intensity (lx)	Photoperiod
C	Natural sunlight	Uncontrolled	Natural photoperiod
R1	Red 629nm	1500	8L:16D
R2	Red 629nm	200	12L:12D
R3	Red 629nm	3000	24L:0D
G1	Green 533nm	1500	12L:12D
G2	Green 533nm	200	24L:0D
G3	Green 533nm	3000	8L:16D
B1	Blue 450nm	1500	24L:0D
B2	Blue 450nm	200	8L:16D
B3	Blue 450nm	3000	12L:12D

Note: Light intensities in this table represent the photometric setpoints (lux) used for the orthogonal design. Please refer to Table 2 for the estimated photon flux densities derived from the recorded illuminance values and nominal peak wavelengths.

Table 2. Estimated photon flux densities derived from the recorded illuminance values and nominal peak wavelengths of the LED treatments.

Set Illuminance (lx)	Red Light 629 nm (μmol m^-2^ s^-1^)	Green Light 533 nm (μmol m^-2^ s^-1^)	Blue Light 450 nm (μmol m^-2^ s^-1^)
200	5.7	1.5	29.0
1500	42.8	11.1	217.5
3000	85.5	22.2	435.0

Note: The theoretical photon flux densities were estimated using the CIE 1931 standard photopic luminosity function and Planck's equation, accounting for the human eye's differential sensitivity to red, green, and blue wavelengths. These values were estimated theoretically and not directly measured.

determined in the sampled males. Plasma IgM and C3 were determined in all sampled fish.

After blood collection, fish were dissected and gonads were removed. Sex was identified according to gonadal morphology. Portions of gonadal tissues were fixed in Bouin's solution for histological examination, while the remaining tissues were frozen in liquid nitrogen and stored at -80 °C.

4. HORMONE AND IMMUNE PARAMETER DETERMINATION

Plasma E2 (Cat. No. H102-1-2), T (Cat. No. H090-1-2), IgM (Cat. No. H109-1-2), and C3 (Cat. No. H186-1-2) concentrations were measured using the corresponding commercially available enzyme-linked immunosorbent assay (ELISA) kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), according to the manufacturer's instructions.

5. HISTOLOGICAL ANALYSIS

Ovarian and testicular samples were fixed, dehydrated through a graded ethanol series, cleared in xylene and embedded in paraffin. Sections were cut at 5 μm, stained with hematoxylin and eosin, mounted on glass slides and observed under an optical microscope. Ovarian and testicular developmental stages were assessed according to the standardized terminology for reproductive development in fishes.[35]

Nine females and nine males from each replicate tank were used for ovarian and testicular histological analyses, respectively. Therefore, 27 females and 27 males were examined histologically in each treatment group. For each fish, three non-consecutive sections were analyzed, and at least five randomly selected microscopic fields were counted per section. To minimize observer bias, all histological slides were assigned coded identification numbers before quantification. Germ-cell staging and counting were performed by an investigator blinded to treatment identity, and the treatment codes were revealed only after all histological measurements had been completed. The percentage of each developmental stage was calculated as the number of cells at that stage divided by the total number of germ cells counted and multiplied by 100. Fish-level percentages were averaged within each replicate tank to generate one tank-level value for statistical analysis.

6. STATISTICAL ANALYSIS

Data are expressed as mean ± standard deviation (SD). Normality and homogeneity of variance were assessed before analysis. Differences among treatment combinations were analyzed by one-way ANOVA followed by Tukey's multiple-comparison test. Orthogonal range analysis was used to evaluate the main effects of light spectrum, photoperiod, and light intensity. The natural-light reference was included in the one-way comparisons but excluded from the

orthogonal range analysis because its light conditions were uncontrolled. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using SPSS 29.0, and figures were prepared using GraphPad Prism 10.0. The tank was considered the experimental unit. Individual-fish measurements within each tank were averaged to generate one tank-level value; therefore, the sample size used for statistical inference was three replicate tanks per treatment (n = 3).

RESULTS

1. GONADAL HISTOLOGY

As shown in Fig. 1 and Fig. 3(a), gonadal development differed among light treatments. Ovarian tissues in all groups contained oocytes at different developmental stages. Stage I and stage II oocytes were dominant in most groups, while stage IV oocytes represented the most advanced maturation stage observed in this study.

The highest proportions of stage IV oocytes were observed under blue light at 200 lx with an 8L:16D photoperiod and green light at 200 lx with a 24L:0D photoperiod (B2 and G2, respectively), reaching approximately 25% and 23%, compared with approximately 17% in the natural-light reference. All three red-light combinations showed lower proportions of stage IV oocytes than the two 200-lx blue- and green-light combinations. Among the red-light treatments, red light at 200 lx under 12L:12D (R2) showed the lowest proportion, at approximately 6%. Ovaries exposed to the two 200-lx blue- and green-light combinations contained more vitellogenic oocytes with enlarged cytoplasm and abundant yolk accumulation, whereas the red-light combinations contained higher proportions of early-stage oocytes.

As shown in Fig. 2 and Fig. 3(b), a similar response was observed in testicular development. The highest proportions of stage III spermatogenic cells were observed under green light at 200 lx with a 24L:0D photoperiod and blue light at 200 lx with an 8L:16D photoperiod (G2 and B2, respectively), reaching approximately 25% and 27%. In contrast, red light at 3000 lx under 24L:0D (R3) produced the lowest proportion, at approximately 9%. Testes exposed to the two 200-lx green- and blue-light combinations contained higher proportions of advanced spermatogenic cells, whereas testes exposed to red light at 3000 lx under 24L:0D were dominated by earlier spermatogenic stages.

2. ORTHOGONAL RANGE ANALYSIS OF GONADAL DEVELOPMENT

The orthogonal range-analysis results are shown in Tables 3 and 4. For ovarian development, the level mean (K) for the combined proportion of stage III and stage IV oocytes was highest under blue light (43.33), followed by green light (42.33) and red light (28.33). Among the illuminance levels, 200 lx produced the highest mean value (49.33), whereas 1500 lx produced the lowest value (29.00). For photoperiod, 24L:0D produced the highest mean value (40.33), followed by 12L:12D (37.00) and 8L:16D (36.67). Based on the range (R) values, the main-effect ranking was illuminance (20.33) > spectrum (15.00) > photoperiod (3.66). The range analysis therefore suggested a theoretical combination of blue light, 200 lx, and 24L:0D for ovarian development; this complete combination was not directly tested.

For testicular development, the level mean (K) for the combined proportion of stage II and stage III spermatogenic cells was highest under green light (69.33), followed by blue light (65.67) and red light (59.67). Among the illuminance levels, 200 lx produced the highest mean value (73.67), whereas 3000 lx produced the lowest value (51.33). For photoperiod, 8L:16D produced the highest mean value (69.00), followed by 12L:12D (63.33) and 24L:0D (62.33). Based on the range values, the main-effect ranking was illuminance (22.34) > spectrum (9.66) > photoperiod (6.67). The range analysis, therefore, suggested a theoretical combination of green light, 200 lx, and 8L:16D for testicular development; this complete combination was not directly tested.

3. PLASMA IMMUNE PARAMETERS

As shown in Fig. 4, plasma IgM and C3 concentrations differed among the light-treatment combinations. The highest IgM concentrations were observed under green light at 1500 lx with a 12L:12D photoperiod and blue light at 200 lx with an 8L:16D photoperiod (G1 and B2, respectively), and these values were significantly higher than those in the natural-light reference and most other combinations ($P < 0.05$). Lower IgM concentrations were observed under red light at 1500 lx with 8L:16D and red light at 3000 lx with 24L:0D (R1 and R3, respectively). The remaining combinations showed intermediate IgM concentrations, with no significant difference from the natural-light reference ($P > 0.05$).

Plasma C3 showed a different response pattern. The highest C3 concentrations were observed under red light at 3000 lx with a 24L:0D photoperiod and blue light at 3000 lx with a 12L:12D photoperiod (R3 and B3, respectively). However, green light at the same illuminance level did not produce a comparable increase, indicating that the C3 response was associated with the complete treatment combination rather than illuminance alone. Lower C3 concentrations were observed under green light at 1500 lx with 12L:12D, green light at 200 lx with 24L:0D, and blue light at 1500 lx with 24L:0D (G1, G2, and B1, respectively). The natural-light reference and the remaining combinations showed intermediate values.

4. PLASMA SEX STEROID HORMONES

The highest plasma E2 concentrations were observed under green light at 200 lx with a 24L:0D photoperiod and blue light at 200 lx with an 8L:16D photoperiod (G2 and B2, respectively), reaching approximately 54 and 52 pg mL^{-1} (Fig. 5). These values were significantly higher than those in the natural-light reference and most other treatment combinations ($P < 0.05$). Green light at 1500 lx with a 12L:12D photoperiod (G1) also produced a relatively high E2 con-

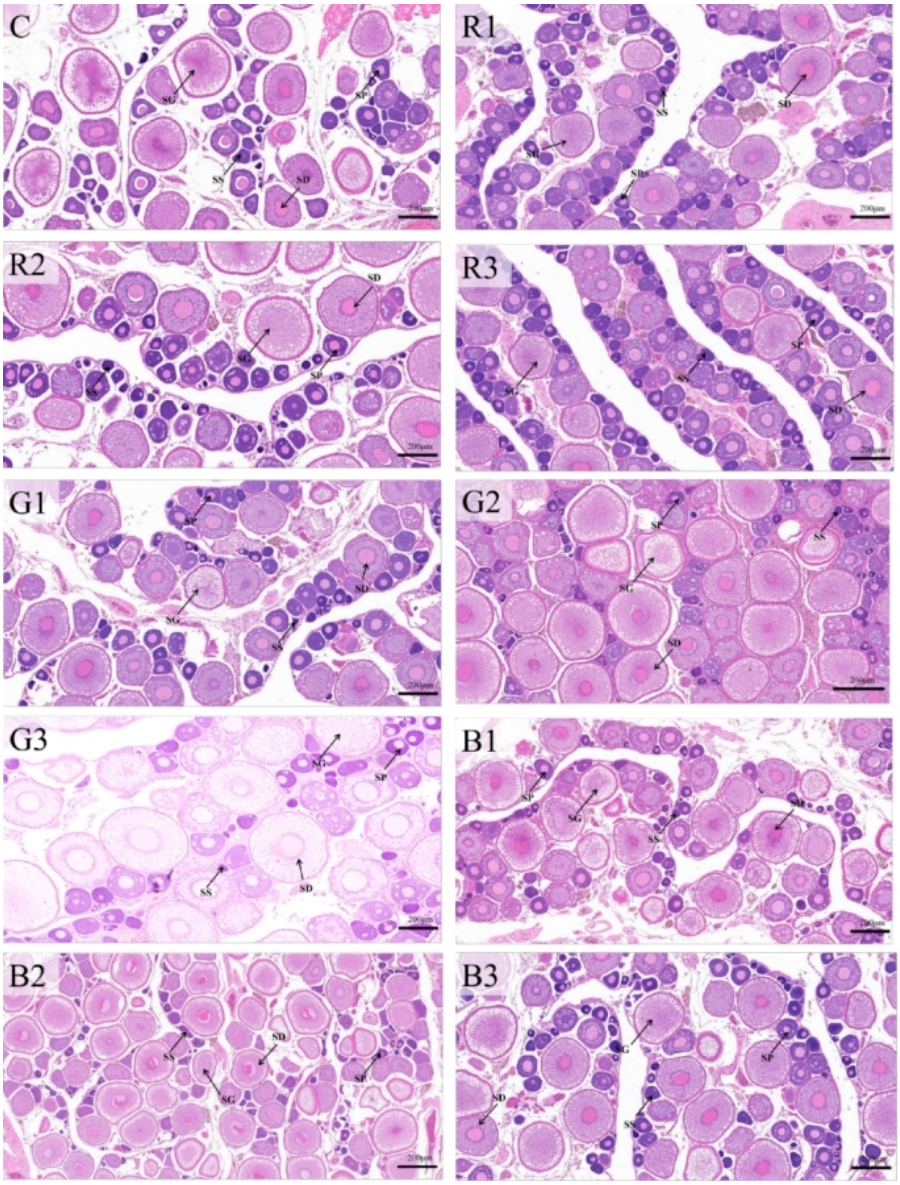

Fig.1. Representative cross-sectional images (scale = 200 μm) of ovarian tissue slices under different light environments, including control group, R1 group, R2 group, R3 group, G1 group, G2 group, G3 group, B1 group, B2 group, and B3 group.

Note: SS: Stage I; SP: Stage II; SD: Stage III; SG: Stage IV.

centration, whereas several red-light and high-illuminance combinations showed lower values.

Plasma T showed a similar overall pattern (Fig. 5). The highest concentrations were observed under green light at 200 lx with a 24L:0D photoperiod and blue light at 200 lx with an 8L:16D photoperiod (G2 and B2, respectively), with both treatments exceeding 1100 pg mL^{-1} and being significantly higher than the natural-light reference and most other combinations ($P < 0.05$). Green light at 1500 lx with 12L:12D (G1) also maintained a relatively high T concentration. Lower T concentrations were recorded under red light at 3000 lx with 24L:0D and blue light at 3000 lx with 12L:12D (R3 and B3, respectively), whereas the remaining combinations showed intermediate values.

DISCUSSION

The present study showed that the light environment was associated with gonadal development, sex-steroid secretion, and immune status in *H. otakii*. Blue light at 200 lx under 8L:16D and green light at 200 lx under 24L:0D produced higher proportions of advanced germ cells and higher plasma E2 and T concentrations than the natural-light reference. These two combinations differed in spectrum and photoperiod, and their estimated photon irradiances were not equivalent; therefore, their favorable performance cannot be attributed to a single factor without qualification. Their shared experimental feature was the 200-lx photometric setpoint. Several treatment combinations involving red light or the highest tested illuminance showed lower sex-steroid concentrations and less advanced gonadal de-

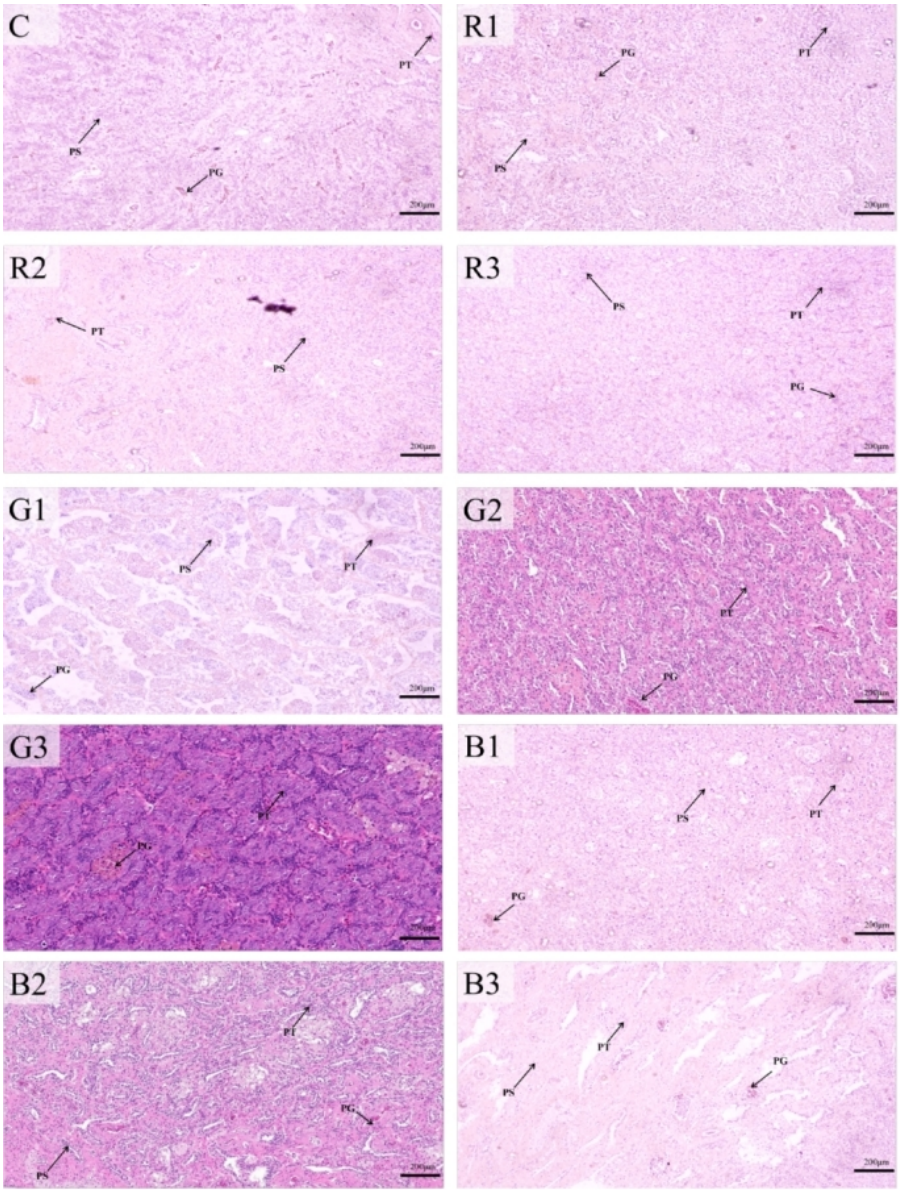

Fig.2. Representative cross-sectional images of testicular tissue slices under different light environments (scale=200 μm), including control group, R1 group, R2 group, R3 group, G1 group, G2 group, G3 group, B1 group, B2 group, and B3 group.

Note: PS: Stage I; PT: Stage II; PG: Stage III.

velopment. Directionally similar spectrum-dependent changes in gonadal development and reproductive hormones have been reported in red spotted grouper.[12] Differences in the magnitude of hormone responses among studies may reflect species-specific endocrine capacity, water temperature, reproductive stage, and sampling time.

Spectral effects on fish reproduction are species specific: red light promotes ovarian development in some species, whereas green or blue light is more effective in others.[12-16, 36-38] In the present study, the orthogonal range analysis suggested a blue-light-associated pattern for ovarian development and a green-light-associated pattern for testicular development under the tested photometric conditions. *H. otakii* is a demersal fish associated with rocky coastal and reef habitats in the northwestern Pacific.[39] In coastal waters, red wavelengths attenuate rapidly, whereas blue-green wavelengths generally penetrate more effectively, although transmission varies with colored dissolved organic matter and suspended particles.[40] This habitat may contribute to sensitivity to blue-green wavelengths. However, because photon irradiance was not matched across spectra, the observed spectral patterns cannot be completely separated from radiometric differences. Possible mechanisms include sex-related differences in retinal or deep-brain photoreceptor opsins and differences in neuroendocrine regulation of gonadotropin release,[41,42] with additional modulation by sex-steroid feedback at the pituitary level.[43] These mechanisms were not measured directly and should be regarded as hypotheses for future study.

Illuminance also contributed to the observed reproductive responses. Red light at 3000 lx under 24L:0D and blue light at 3000 lx under 12L:12D were associated with rela-

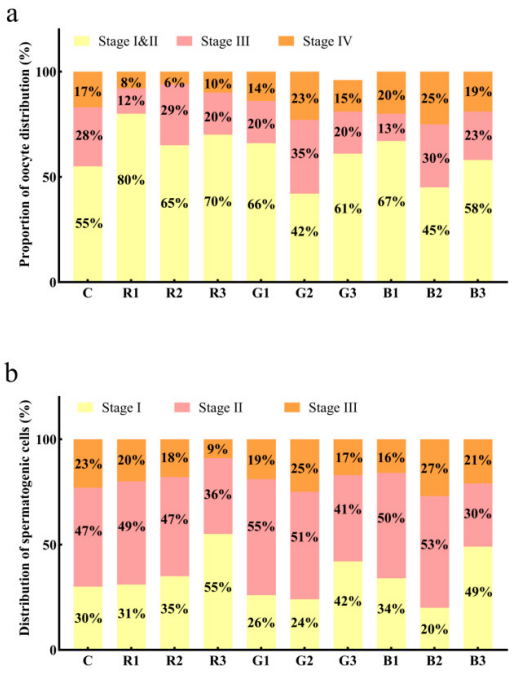

Fig.3. The composition ratio of oocytes (a) and spermatocytes (b) at different stages in different treatment groups.

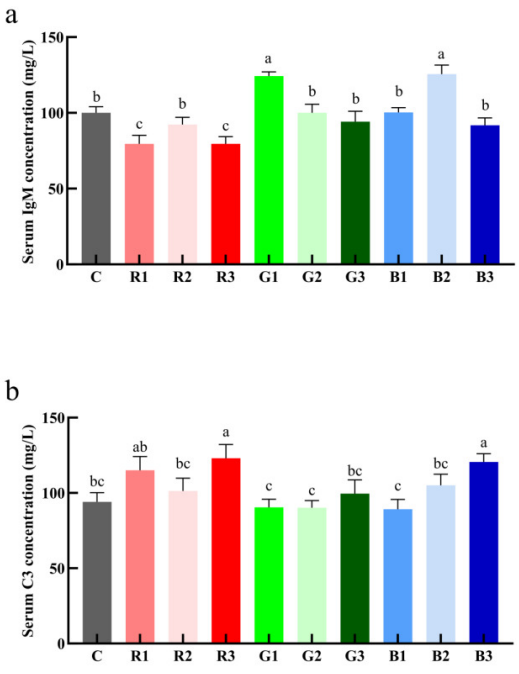

Fig.4. Comparison of IgM (a) and C3 (b) in the blood plasma of *H. otakii* under different spectra, light intensity, and photoperiod levels. Data are presented as mean ± SD (n = 3). Different lowercase letters indicate significant differences among groups ($P < 0.05$).

tively low T concentrations and less advanced gonadal development. Because equal lux values across different spectra represent markedly different photon irradiances, these observations should be interpreted as responses to the tested lamp settings rather than to radiometrically equivalent light exposures. Excessive illumination could increase metabolic demand or oxidative stress and thereby impair steroidogenesis and gametogenesis[19,38,44]; however, oxidative markers were not measured, so this explanation re-

Table 3. Range analysis of the combined proportion of stage III and stage IV oocytes

Item	Spectrum	Intensity (lx)	Photoperiod
K1 (Mean)	28.33 (Red 629nm)	29.00 (1500)	36.67 (8L:16D)
K2 (Mean)	42.33 (Green 533nm)	49.33 (200)	37.00 (12L:12D)
K3 (Mean)	43.33 (Blue 450nm)	35.67 (3000)	40.33 (24L:0D)
Range (R)	15.00	20.33	3.66
Influence arrangement	Blue > Green > Red	200 > 3000 > 1500	24L:0D > 12L:12D > 8L:16D
Optimal combination	Blue	200	24L:0D

Note: K1, K2, and K3 represent the average values for each respective level, and R represents the range (Max - Min).

Table 4. Range analysis of the combined proportion of stage II and stage III spermatogenic cells

Item	Spectrum	Intensity (lx)	Photoperiod
K1 (Mean)	59.67 (Red 629nm)	69.67 (1500)	69.00 (8L:16D)
K2 (Mean)	69.33 (Green 533nm))	73.67 (200)	63.33 (12L:12D)
K3 (Mean)	65.67 (Blue 450nm)	51.33 (3000)	62.33 (24L:0D)
Range (R)	9.66	22.34	6.67
Influence arrangement	Green > Blue > Red	200 > 1500 > 3000	8L:16D > 12L:12D > 24L:0D
Optimal combination	Green	200	8L:16D

Note: K1, K2, and K3 represent the average values for each respective level, and R represents the range (Max - Min).

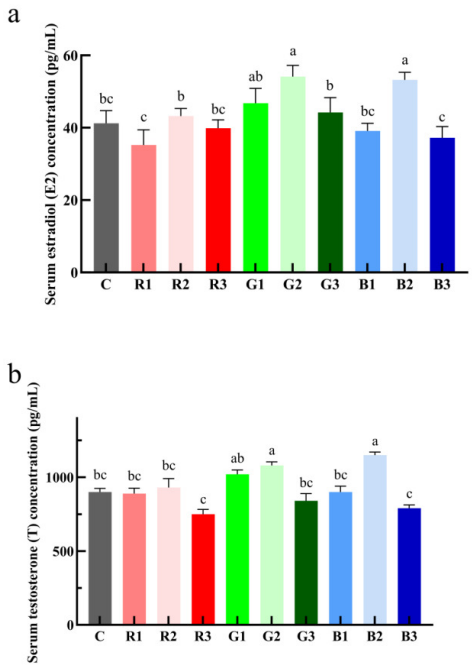

Fig.5. Comparison of E2 (a) and T (b) in the blood plasma of *H. otakii* at different spectral, photoperiod, and light intensity levels. Data are presented as mean ± SD (n = 3). Different lowercase letters indicate significant differences among groups ($P < 0.05$).

mains hypothetical. Within the tested range, the orthogonal analysis identified 200 lx as the most favorable photometric setpoint for both ovarian and testicular development.

Photoperiod effects were conditional on the accompanying spectrum and illuminance. The orthogonal main-effect analysis produced different theoretical photoperiod optima for ovarian and testicular development, namely 24L:0D and 8L:16D, respectively. The two empirically favorable combinations also used different photoperiods: blue light at 200 lx was paired with 8L:16D, whereas green light at 200 lx was paired with 24L:0D. Their common feature was therefore the 200-lx photometric setpoint rather than a specific day length. The favorable response under green light at 200 lx with 24L:0D indicates that continuous illumination was not consistently inhibitory. Conversely, the relatively poor response under red light at 3000 lx with 24L:0D cannot be attributed solely to continuous illumination because spectrum and illuminance differed simultaneously.

Previous studies have likewise shown that prolonged or continuous illumination can alter reproductive development and endocrine activity in teleosts, but the direction and magnitude of the response vary among species, developmental stages, and environmental conditions.[45-47] Photoperiodic regulation of fish reproduction is commonly associated with melatonin signaling and synchronization of the hypothalamic-pituitary-gonadal axis.[48-50] Melatonin responses may themselves be modified by light intensity and spectral composition.[51] However, plasma melatonin, circadian hormone profiles, and gonadotropin expression were not measured in the present study. The involvement of melatonin-mediated HPG-axis regulation, therefore, remains a plausible hypothesis rather than a mechanism demonstrated by the present data. A full-factorial experiment conducted under directly measured and matched photon irradiance is required to distinguish the independent and interactive effects of spectrum, illuminance, and photoperiod.

The immune responses were also associated with specific light combinations. Plasma IgM was highest under green light at 1500 lx with 12L:12D and blue light at 200 lx with 8L:16D, whereas C3 was highest under red light at 3000 lx with 24L:0D and blue light at 3000 lx with 12L:12D. Green light at 3000 lx did not produce a comparable C3 increase, indicating that the response was not a uniform effect of the highest illuminance. Increased IgM may indicate enhanced humoral immune capacity, whereas elevated C3 may reflect activation of innate complement pathways.[26, 28-30] Elevated C3 should not be interpreted automatically as improved immunity because it may also be associated with altered innate immune activity or light-related stress.[19,44] Additional stress, inflammatory, and complement-activation markers are required to clarify the biological significance of these changes.

Taken together, the orthogonal range analysis suggested that illuminance had the largest main-effect range for gonadal development in *H. otakii*, followed by spectrum and photoperiod. The favorable responses under both the blue-light/200-lx/8L:16D and green-light/200-lx/24L:0D combinations support an association with the 200-lx photometric setpoint under the conditions of this study. Nevertheless, because photon irradiance differed markedly among the nominal spectra and factor interactions were not fully resolved, 200 lx should be regarded as the most favorable tested photometric condition rather than as a universally established optimum. The complete theoretical optimal combinations also require direct experimental validation.

The natural-light group represented a practical baseline for conventional *H. otakii* culture rather than a strictly controlled orthogonal reference. Its spectrum, intensity, and photoperiod varied simultaneously, limiting mechanistic comparisons with the artificial-light treatments. In addition, the spectral power distributions of the lamps and photon irradiance at the fish swimming depth were not measured directly. Averaging individual measurements within tanks avoided pseudoreplication, but using only three replicate tanks per treatment limited the statistical power for multi-group comparisons. Future studies should include a standardized white-light reference, direct radiometric measurements, complete factorial combinations, and a larger number of replicate tanks.

CONCLUSION

This study showed that light spectrum, photoperiod, and illuminance were jointly associated with gonadal development, sex-steroid secretion, and immune status in *H. otakii*. Within the tested range, 200 lx was the most favorable photometric setpoint, whereas estimated photoperiod main ef-

fects differed between ovarian and testicular development. The range analysis suggested different spectrum-associated patterns for ovarian and testicular maturation under the tested conditions. Responses to the highest illuminance and continuous illumination depended on the complete factor combination. Because photon irradiance varied across spectra and the theoretically optimal combinations were not directly tested, specific spectrum- and photoperiod-management recommendations require validation through controlled factorial experiments and direct measurements of spectral power distribution and photon irradiance.

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COMPETING OF INTEREST – COPE

No competing interests were disclosed.

ETHICAL CONDUCT APPROVAL – IACUC

All animal experiments were approved by the Ethics Committee of Dalian Ocean University (Approval Code: DLOU2026061101; Approval Date: 7 June 2025). All animal procedures followed the "Guidelines for Ethical Treatment of Experimental Animals" prepared by the Ministry of Science and Technology of China.

INFORMED CONSENT STATEMENT

All authors have confirmed this manuscript for publication.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding authors upon reasonable request.

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REFERENCES

1. Boeuf G, Le Bail PY. Does light have an influence on fish growth? *Aquaculture*. 1999;177(1-4):129-152. doi:10.1016/S0044-8486(99)00074-5

2. Bromage N, Porter M, Randall C. The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. *Aquaculture*. 2001;197(1-4):63-98. doi:10.1016/S0044-8486(01)00583-X

3. Migaud H, Davie A, Taylor J. Current knowledge on the photoneuroendocrine regulation of reproduction in temperate fish species. *J Fish Biol*. 2010;76(1):27-68. doi:10.1111/j.1095-8649.2009.02500.x

4. Kjesbu OS, Jakobsen T, Fogarty MJ, et al. Applied fish reproductive biology: contribution of individual reproductive potential to recruitment and fisheries management. In: Jakobsen T, Fogarty MJ, Megrey BA, Moksness E, eds. *Fish Reproductive Biology: Implications for Assessment and Management*. Wiley-Blackwell; 2009:293-332. doi:10.1002/9781444312133

5. Saraiva JL, Arechavala-Lopez P, Sneddon LU. Farming fish. In: Knight A, Phillips C, Sparks P, eds. *Routledge Handbook of Animal Welfare*. Routledge; 2022:115-127. doi:10.4324/9781003182351-3

6. Maitra S, Seth M, Chattoraj A. Photoperiod, pineal photoreceptors and melatonin as the signal of photoperiod in the regulation of reproduction in fish. *J Endocrinol Reprod*. 2006;10(2):73-87.

7. Bayarri MJ, Rodríguez L, Zanuy S, et al. Effect of photoperiod manipulation on the daily rhythms of melatonin and reproductive hormones in caged European sea bass (*Dicentrarchus labrax*). *Gen Comp Endocrinol*. 2004;136(1):72-81. doi:10.1016/j.ygcen.2003.12.004

8. Hoar WS. Comparative physiology: hormones and reproduction in fishes. *Annu Rev Physiol*. 1965;27(1):51-70. doi:10.1146/annurev.ph.27.030165.000411

9. Norberg B, Brown CL, Halldorsson O, et al. Photoperiod regulates the timing of sexual maturation, spawning, sex steroid and thyroid hormone profiles in the Atlantic cod (*Gadus morhua*). *Aquaculture*. 2004;229(1-4):451-467. doi:10.1016/S0044-8486(03)00393-4

10. Falcón J, Migaud H, Muñoz-Cueto JA, et al. Current knowledge on the melatonin system in teleost fish. *Gen Comp Endocrinol*. 2010;165(3):469-482. doi:10.1016/j.ygcen.2009.04.026

11. Ruchin AB. Effect of illumination on fish and amphibian: development, growth, physiological and biochemical processes. *Rev Aquac*. 2021;13(1):567-600. doi:10.1111/raq.12487

12. Xu W, Zou H, Zeng J, et al. Effects of various LED light spectra on growth, gonadal development, and growth-/reproduction-related hormones in the juvenile red spotted grouper, *Epinephelus akaara*. *Animals*. 2023;13(13):2047. doi:10.3390/ani13132047

13. Castro A, Gonçalves-de-Freitas E, Volpato GL, et al. Visual communication stimulates reproduction in Nile tilapia, *Oreochromis niloticus* (L.). *Braz J Med Biol Res*. 2009;42(4):368-374. doi:10.1590/S0100-879X2009000400009

14. Shin HS, Kim NN, Choi YJ, et al. Light-emitting diode spectral sensitivity relationship with reproductive parameters and ovarian maturation in yellowtail damselfish, *Chrysiptera parasema*. *J Photochem Photobiol B*. 2013;127:108-113. doi:10.1016/j.jphotobiol.2013.07.026

15. Han MM, Zhan W, Liang X, et al. Effects of light spectra on the ovarian maturation and maternal immunity in the little yellow croaker (*Larimichthys polyactis*). *Aquaculture*. 2024;581:740405. doi:10.1016/j.aquaculture.2023.740405

16. Brüning A, Hölker F, Franke S, et al. Impact of different colours of artificial light at night on melatonin rhythm and gene expression of gonadotropins in European perch. *Sci Total Environ*. 2016;543:214-222. doi:10.1016/j.scitotenv.2015.11.023

17. Trippel EA, Neil SR. Effects of photoperiod and light intensity on growth and activity of juvenile haddock (*Melanogrammus aeglefinus*). *Aquaculture*. 2003;217(1-4):633-645. doi:10.1016/S0044-8486(02)00198-9

18. Wang K, Li K, Liu L, et al. Effects of light intensity and photoperiod on the growth and stress response of juvenile Nile tilapia (*Oreochromis niloticus*) in a recirculating aquaculture system. *Aquac Fish*. 2023;8(1):85-90. doi:10.1016/j.aaf.2020.03.001

19. Zhong Z, Zhang J, Tang B, et al. Transcriptome and metabolome analyses of the immune response to light stress in the hybrid grouper (*Epinephelus lanceolatus* male x *Epinephelus fuscoguttatus* female). *Animal*. 2022;16(2):100448. doi:10.1016/j.animal.2021.100448

20. Qu B, Zhao H, Chen Y, et al. Effects of low-light stress on aquacultural water quality and disease resistance in Nile tilapia. *PLoS One*. 2022;17(5):e0268114. doi:10.1371/journal.pone.0268114

21. Hettiarachchi SA, Hyeon JY, Byun JH, et al. Growth enhancement and gonadal development disruption using 500-nm LED light in commercial-scale olive flounder (*Paralichthys olivaceus*) aquaculture. *Aquac Int*. 2025;33(6):467. doi:10.1007/s10499-025-02121-6

22. Santhakumar K. Neuroendocrine regulation of reproduction in fish: mini review. *Aquac Fish*. 2024;9(3):437-446. doi:10.1016/j.aaf.2023.09.001

23. Tomy S. Estrogenic regulation of reproduction in teleosts. In: Pandey A, ed. *Recent Updates in Molecular Endocrinology and Reproductive Physiology of Fish: An Imperative Step in Aquaculture*. Springer; 2021:35-52. doi:10.1007/978-981-15-8369-8_3

24. Tenugu S, Pranoty A, Mamta SK, et al. Development and organisation of gonadal steroidogenesis in bony fishes: a review. *Aquac Fish*. 2021;6(3):223-246. doi:10.1016/j.aaf.2020.09.004

25. Sharma P, Purohit S, Kothiyal S, et al. Sex-specific transcriptional regulation of gonadal steroidogenesis in teleost fishes. *Front Endocrinol (Lausanne)*. 2022;13:820241. doi:10.3389/fendo.2022.820241

26. Yu Y, Wang Q, Huang Z, et al. Immunoglobulins, mucosal immunity and vaccination in teleost fish. *Front Immunol*. 2020;11:567941. doi:10.3389/fimmu.2020.567941

27. Tizard I. Comparative immunology. In: Tizard I, ed. *Infection, Resistance, and Immunity*. 2nd ed. CRC Press; 2022:247-264. doi:10.1201/9780203750964-13

28. Nakao M, Tsujikura M, Ichiki S, et al. The complement system in teleost fish: progress of post-homolog-hunting researches. *Dev Comp Immunol*. 2011;35(12):1296-1308. doi:10.1016/j.dci.2011.03.003

29. Bai H, Mu L, Qiu L, et al. Complement C3 regulates inflammatory response and monocyte/macrophage phagocytosis of *Streptococcus agalactiae* in a teleost fish. *Int J Mol Sci*. 2022;23(24):15586. doi:10.3390/ijms232415586

30. Wu M, Jia BB, Li MF. Complement C3 and activated fragment C3a are involved in complement activation and antibacterial immunity. *Front Immunol*. 2022;13:813173. doi:10.3389/fimmu.2022.813173

31. Song Y, Wei Z, Li X, et al. The effects of dietary tributyrin supplementation on growth performance, antioxidant capacity, immune function, and intestinal health of fat greenling (*Hexagrammos otakii*). *Fishes*. 2026;11(1):67. doi:10.3390/fishes11010067

32. Guo Z, Meng X, Xie J, et al. Effects of low fish meal diet supplemented with dimethyl-beta-propiothetin (DMPT) on growth, feeding behavior and intestinal health of *Hexagrammos otakii*. *Anim Feed Sci Technol*. 2026;342:116837. doi:10.1016/j.anifeedsci.2026.116837

33. Wu Z, Tan X, Wang L, et al. Study on artificial induction and early development of gynogenetic fat greenling *Hexagrammos otakii*. *Aquac Rep*. 2022;22:100975. doi:10.1016/j.aqrep.2021.100975

34. Fan Y, Wang Y, Li L, et al. Gut microbiota changes in *Hexagrammos otakii* during development and association with diets based on metagenomics sequencing. *Aquac Rep*. 2023;33:101881. doi:10.1016/j.aqrep.2023.101881

35. Brown-Peterson NJ, Wyanski DM, Saborido-Rey F, et al. A standardized terminology for describing reproductive development in fishes. *Mar Coast Fish*. 2011;3(1):52-70. doi:10.1080/19425120.2011.555724

36. Bhat IA, Rather MA, Ahmad I, et al. Impact of shifting abiotic factors in aquaculture on fish breeding and reproduction: a review. *Blue Biotechnol*. 2025;2(1):3. doi:10.1186/s44315-025-00027-9

37. Sinansari S, Sudrajat AO, Arfah H, et al. Different LED light effect on gonad maturation and gene expression in female *Synodontis* broodstocks (*Synodontis* sp.). *Indones Aquac J*. 2024;19(2):179-187. doi:10.15578/iaj.19.2.2024.179-187

38. Bapary MAJ, Amin MN, Takeuchi Y, et al. The stimulatory effects of long wavelengths of light on the ovarian development in the tropical damselfish, *Chrysiptera cyanea*. *Aquaculture*. 2011;314(1-4):188-192. doi:10.1016/j.aquaculture.2011.02.006

39. Ren G, Liu Q, Gao T, Yanagimoto T. Population demography and genetic structure of the fat greenling (*Hexagrammos otakii*) inferred from mtDNA control region sequence analyses. *Biochem Syst Ecol*. 2013;47:156-163. doi:10.1016/j.bse.2012.09.026

40. Jerlov NG. *Marine Optics*. Vol 14. 2nd ed. Elsevier Scientific Publishing Company; 1976.

41. Jayakumar Rajeswari J. *Neuroendocrine Regulation of Reproduction in Fish*. doctoral dissertation. University of Saskatchewan; 2020.

42. Trudeau VL. Neuroendocrine control of reproduction in teleost fish: concepts and controversies. *Annu Rev Anim Biosci*. 2022;10(1):107-130. doi:10.1146/annurev-animal-020420-042015

43. Fontaine R, Royan MR, Von Krogh K, et al. Direct and indirect effects of sex steroids on gonadotrope cell plasticity in the teleost fish pituitary. *Front Endocrinol (Lausanne)*. 2020;11:605068. doi:10.3389/fendo.2020.605068

44. Tian HY, Zhang DD, Xu C, et al. Effects of light intensity on growth, immune responses, antioxidant capability and disease resistance of juvenile blunt snout bream *Megalobrama amblycephala*. *Fish Shellfish Immunol*. 2015;47(2):674-680. doi:10.1016/j.fsi.2015.08.022

45. Mlingi FT, Puvanendran V, Burgerhout E, et al. Influence of short-to-continuous and continuous photoperiods combined with elevated temperatures on sexual maturation in lumpfish (*Cyclopterus lumpus*, L. 1758). *Aquaculture*. 2025;598:741981. doi:10.1016/j.aquaculture.2024.741981

46. Sharaf HE, Assem SS, Ismail RF, et al. Effect of prolonged photoperiod on growth, gonadal development, sex ratio, and skin coloration of red tilapia (*Oreochromis mossambicus* x *Oreochromis urolepis hornorum*). *Aquac Int*. 2024;32(5):6523-6538. doi:10.1007/s10499-024-01477-5

47. Cai H, Zhou Q, Liu Y, et al. Effects of nighttime LED supplementary lighting on growth, ovarian development and serum hormone levels of *Acipenser gueldenstaedtii* in a flow-through system. *Aquaculture*. 2025;596:741757. doi:10.1016/j.aquaculture.2024.741757

48. Falcón J, Besseau L, Sauzet S, et al. Melatonin effects on the hypothalamo-pituitary axis in fish. *Trends Endocrinol Metab*. 2007;18(2):81-88. doi:10.1016/j.tem.2007.01.002

49. Bhattacharya S, Chattoraj A, Maitra SK. Melatonin in the regulation of annual testicular events in carp *Catla catla*: evidence from studies on the effects of exogenous melatonin, continuous light, and continuous darkness. *Chronobiol Int*. 2007;24(4):629-650. doi:10.1080/07420520701534665

50. Oliveira C, Sánchez-Vázquez FJ. Reproduction rhythms in fish. In: Kulczykowska E, Popek W, Kapoor BG, eds. *Biological Clock in Fish*. Science Publishers; 2010:185-215. doi:10.1201/b10170-10

51. Bayarri M, Madrid J, Sánchez-Vázquez FJ. Influence of light intensity, spectrum and orientation on sea bass plasma and ocular melatonin. *J Pineal Res*. 2002;32(1):34-40. doi:10.1034/j.1600-079x.2002.10806.x