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With immense pleasure and excitement, I welcome you to the *Tropical Aquaculture Journal*, the newest journal from UJAT Press. As the Editor-in-chief, it is an honor and a privilege to invite you to present cutting-edge research, thought-provoking analyses, and innovative ideas in tropical aquaculture.

Aquaculture ensures food security, economic growth, and sustainable resource management, especially in tropical regions. Our journal aims to stand as a beacon, guiding researchers, practitioners, policymakers, and enthusiasts in their pursuit of advancing the science and practice of aquaculture in tropical regions.

In this journal, you will find a diverse array of opportunities that explore various aspects of tropical aquaculture, ranging from breakthroughs in breeding techniques that enhance species' resistance to changing environmental conditions to novel approaches in disease management that uphold the health of aquatic organisms used in aquacultural practices. Our editorial experts have explored the intricacies of aquaculture in diverse systems and a variety of species, the optimization of feed formulations to minimize environmental impact, and the socio-economic implications of aquaculture development in tropical communities. The strict peer-review process followed by Tropical Aquaculture embodies the critical spirit of scientific communities with extensive backgrounds in aquaculture practiced in tropical countries. Our journal, oriented to the dissemination of new knowledge and scientific discoveries, also goes hand in hand with the philosophy of sustainable development and aquaculture practices that harmonize with the delicate ecosystems of tropical regions.

I want to express my deepest gratitude to the section editors for their invaluable contributions, the volunteer reviewers for their constructive insights, and the editorial team for their unwavering dedication to upholding the highest scientific publishing standards. Together, we ensure the authenticity and reliability of the knowledge disseminated through these pages.

We aim to foster an environment of open discourse and intellectual exchange. Through this process, we will pave the way for further breakthroughs, innovative solutions, and a deeper understanding of the complexities inherent in tropical aquaculture. I encourage you, our esteemed authors and readers, to engage with the articles, share your ideas, and join us on this journey of exploration and enlightenment. Together, we can contribute to improving aquaculture practices that empower communities in the tropics, support sustainable aquacultural practices, and secure a better future for future generations.




Thank you for your continued support, and I hope you find the *Tropical Aquaculture Journal* enlightening and inspiring.

Sincerely,

Wilfrido M. Contreras-Sánchez 

Editor-in-Chief


Manipulated Photoperiod Enhances Sperm Production and Quality in Male Gray Snapper *Lutjanus griseus* (Linnaeus, 1758): A Potential Approach for Tropical Aquaculture

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Abstract

Photoperiod manipulation has been used as an alternative non-invasive method to stimulate sexual maturation in fish. It consists of spatial and temporal manipulation of reproductive physiology. There are few studies on the effect of photoperiod on reproduction in male fish, particularly in tropical climates. Because of this, the present study focused on inducing gonad maturation in male gray snapper (*Lutjanus griseus*), by manipulating of photoperiod. From the fourth week of March (12h and 30 min light), the day length was increased by 2.4 minutes per day until the maximum estimated annual day length (13h and 42 min light) was achieved. All fish exposed to the artificial photoperiod produced sperm. In addition, greater fluidity and greater sperm motility were observed, with a higher number of sperm cells per ml ($5.14 \text{ million cells} \pm 1.64 e^6$) compared to fish exposed to the natural photoperiod ($3.63 \text{ million cells} \pm 0.36 e^6$). Current results indicate that manipulated photoperiod can be used to lengthen the duration of sperm production in gray snappers.

Keywords: Reproductive manipulation, controlled photoperiod, gray snapper, males

Introduction

Aquaculture has grown steadily at an average annual rate of 8% since 1970, producing over 80 million metric tons by 2016, thus contributing to food security, jobs, and income (Garlock *et al.* 2020). In comparison, the annual growth rate for land-based agriculture has been around 2.8% (Vela Vallejo and Gonzales Posada 2007). Additionally, aquaculture provides an alternative to agriculture by sustainably producing aquatic animals, avoiding the exploitation of some commercially important species (FAO 2022). In Mexico, Lutjanidae fish are marine species, like the common snook *Centropomus undecimalis* (Álvarez-Lajonchère and Tsuzuki, 2008), the Mexican pufferfish (*Sphoeroides annulatus*), and the spotted rose snapper (*Lutjanus guttatus*) are species with broad potential for cultivation (Álvarez-Lajonchère *et al.* 2010). However, the levels of laboratory and commercial-pilot fish production are low and far below the commercial scale (CONAPESCA 2014).

Fish production through aquaculture faces challenges such as adaptation, stress levels, maturation, and reproduction in captivity (Castelló 2013). To overcome these challenges, minimally invasive manipulation techniques of environmental factors such as photoperiod, temperature, and social behaviors are required. Successful aquaculture production requires the manipulation of seasonal environmental cues to achieve simultaneous reproductive maturation of broodstock (Muñoz-Cueto 2013). Three genera and ten species comprise the family Lutjanidae; however, only the genus *Lutjanus* (eight species) and one species of the *Hoplopagrus* are of commercial and recreational interest. Lutjanids have displayed remarkable plasticity, and studies of growth and

reproduction have reported favorable results when using controlled photoperiod and temperature (Arnold *et al.* 1978, Turano *et al.* 2000, Botero-Arango and Castaño-Rivera 2005, Papanikos *et al.* 2008 and Guerrero-Tortolero *et al.* 2008). In coastal regions of Tabasco, *L. griseus*, like other lutjanids, is appreciated in the market due to its high quality (García-Torcuato 2006) and has generated interest in aquaculture production to assess the potential for aquaculture production; this project examined gonad maturation in male *L. griseus* in response to photoperiod manipulation.

Materials and methods

Location and organisms

This research was conducted at the Marine Aquaculture Station (MAS) facilities of the Universidad Juárez Autónoma de Tabasco (UJAT) in Jalapita, Centla, Tabasco. Wild adults were captured using a cast net near areas with submersed vegetation in the Mecoacán Lagoon. The fish were transferred to the MAS and maintained for one year until the start of the experiment in 63.6 m³ circular fiberglass tanks (9 m Ø, 1 m height) containing seawater. Fish received a prophylactic treatment of formalin baths (1.5 mL L⁻¹) for one hour upon arrival. Feed (Skretting Marine MX®; 45% protein, 12% fat, 1.2% fiber, and 1.5% phosphorus) was provided to apparent satiation three times a day (9:00, 12:00, and 16:00 hours).

Experimental design

To determine the effect of the photoperiod manipulation on the maturation of male *L.*

griseus, a completely randomized design of one factor (photoperiod) was used. Two treatments (natural and controlled photoperiod) were implemented with two replicates. The first treatment consisted of keeping the fish in natural conditions without temperature and photoperiod control, with only 80% water replacement every third day. For the controlled photoperiod treatment, a room was covered with black polyethylene and fitted with artificial lighting (consisting of 200 W Volteck® lamps)

regulated by a timer. This setup successfully compressed and mimicked the natural photoperiod between March and July in just 60 days (Fig. 1). Changes in the illumination duration were set at 2.4 minutes/day. The experiment started with 12 hours and 20 minutes of light (March 27th), reaching a maximum daily illumination of 13 hours and 32 minutes (April 25th). Then it was maintained until the last day of the experiment (May 26th). (Fig. 2).

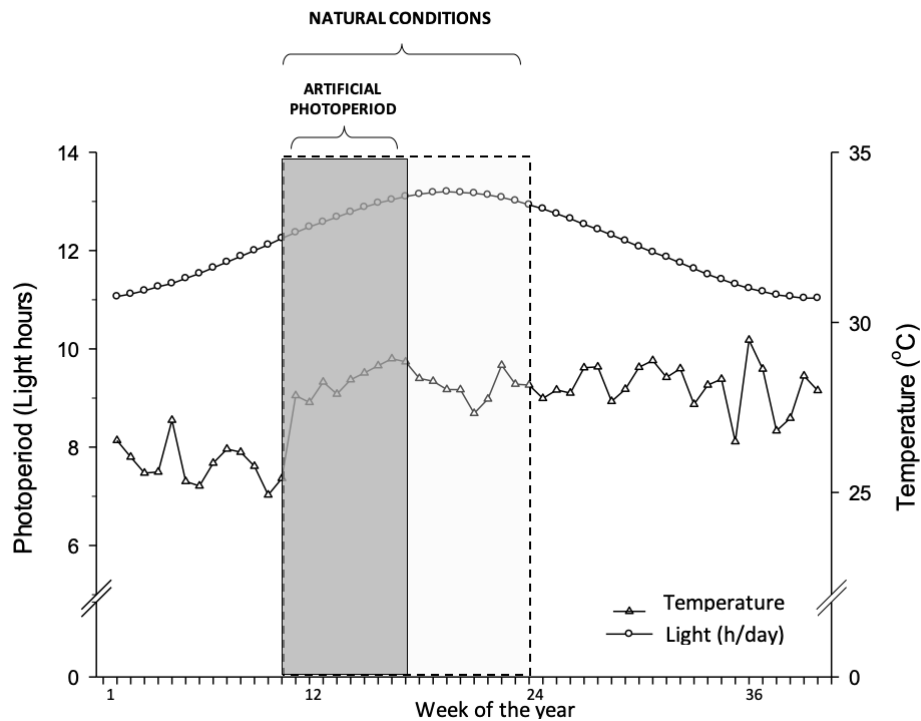


Figure 1. Weekly average values of photoperiod and temperature over one year in broodstock maturation ponds at the Marine Aquaculture Station. The dotted-line box indicates the time and photoperiod conditions to be simulated, and the shaded box represents the duration of the experiment.

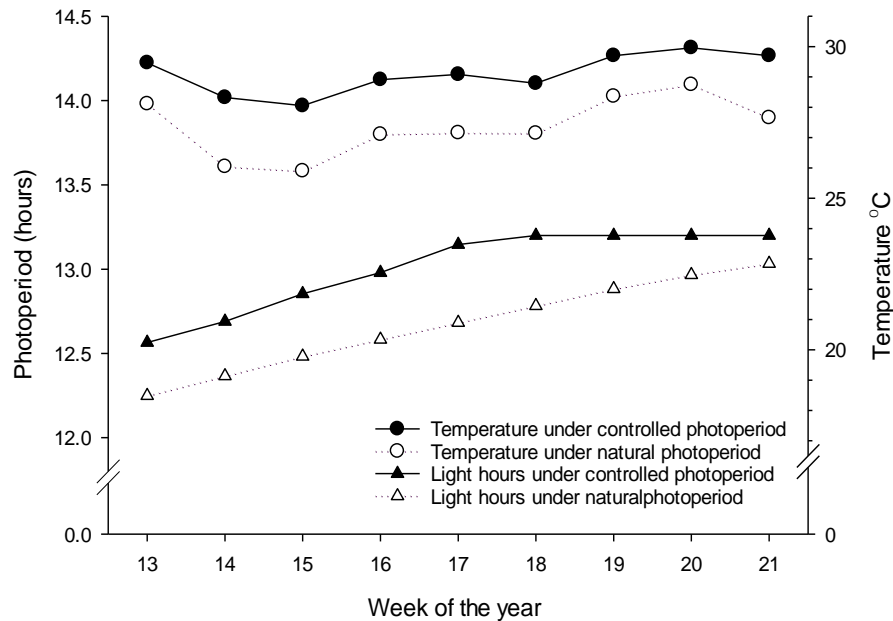


Figure 2. Average weekly values of photoperiod and temperature under natural conditions (open symbols) and controlled photoperiod (dark symbols). The weeks of the year indicate those in which the experiment was conducted.

The experimental units consisted of 7 m³ capacity (3 m Ø, 1 m height) fiberglass tanks containing filtered static seawater containing ten fish each. Water exchange was performed two to three times a week to maintain water quality, replacing 80% of the water. A daily siphoning of the bottom of each tank was performed two hours after the last feeding to eliminate feces and unconsumed food.

Forty randomly selected adult *L. griseus* were placed into the experimental units, averaging 180.47 g (\pm 37.66) in weight and 18.58 cm (\pm 1.53) in total length. At the beginning of the experiment, fish weight, length, and condition factor ($p > 0.10$) were similar across all experimental units. Because *L. griseus* does not exhibit sexual dimorphism, the sex of each fish was unknown at the beginning of the experiment.

Measurement of physicochemical parameters

Temperature, pH, salinity, and dissolved oxygen were measured daily. A pH meter (Eco Sense® 100A, USA) was used to measure pH; salinity was measured in mg/L using a refractometer (Aquafauna Bio marine Inc®, USA). Dissolved oxygen (DO) in mg/L and water temperature in °C were measured with an oximeter (YSI Model 55® Handheld Dissolved Oxygen System, USA). Ambient temperature was measured daily during the morning with an ambient thermometer. Ammonia (NH₄), nitrites (NO₂), and nitrates (NO₄) were measured in mg/L once a week using a photometer (HANNA instruments®, HI 83203, USA).

Manipulation of organisms

At the beginning of the experiment, each fish was implanted with a passive integrated transponder tag (12mm length; Avid Identification Systems, Inc.; Norco, California, USA). The transmitters were inserted using a hypodermic syringe near the dorsal fin. The needle used to insert the transmitter was slipped under the scales, using the tip to make an incision large enough to be inserted with minimal penetration to avoid damage.

The potential sex of each snapper was recorded based on the presence or absence of sperm. Measurements were made at the beginning of the experiment, after 30 days, and after 60 days at the end of the experiment. Weight was measured using an analytical balance (Ohaus®, Scout Pro SP-2001, USA) with an accuracy of 0.001 g; total length and maximum height (right behind the operculum) were measured with a conventional ichthyometer with an accuracy of 1 mm. Before handling, fish were anesthetized with clove oil at 0.015 mL/L. The abdominal region was cleaned with paper towels, and slight pressure was applied to obtain sperm with a 3 mL Plastipak® syringe and placed in 2 mL tubes, latter soon quality was evaluated. Survival was estimated at the end of the experiment by counting the remaining fish.

Sperm sampling

After three months of confinement, the effects of the treatments were determined. Once fish were anesthetized, slight abdominal pressure was applied to obtain sperm and determine its quality. Sperm was collected with a 3 mL syringe and placed in 2 mL Eppendorf tubes®. The samples were observed fresh.

Sperm evaluation

Sperm fluidity and consistency were evaluated immediately after leaving the urogenital pore following Álvarez-Lajonchère and Hernández-Molejón (2001) with some modifications. Four categories were used for fluidity: (low) when the slightly viscous sperm is observed by touch, (medium) when the fluid is whitish and is slightly viscous between the fingers, (high) when thick threads of semen formed between separated fingers and (very high) when the whitish color was obvious and thicker and highly malleable threads of sperm formed between separated fingers. The motility of sperm was measured in seconds (Rurangwua *et al.* 2004). At the same time, the percentage of active cells was determined. For the latter, the urogenital pore was dried to avoid activating the sperm cells with seawater or urine. A sample of 0.1 µL of semen was diluted in 200 µL of seawater to activate it and record the total activity time with a stopwatch when observed under an optical microscope (Carl Zeiss Primo Star®, Germany) at a magnification of 40X.

Sperm count

A 1:200 dilution of each semen sample was made with seawater. Each sample was left to stand for 3 minutes before counting. Sperm count was performed using a 0.10 mm deep Neubauer improved® cell counting chamber with two chambers. Five count fields were made for each of the chambers. The estimation method described by Clark and Hippel (2004) was used following the formula: $Y = N \times 200 \times 10 \times 400 / 80$, where: N is the number of spermatozoa in the sample, 200 is the dilution rate, 10 is the correction factor for chamber depth to determine the volume (mm³), 400 is the total number of frames in the

chamber, and 80 is the total number of frames counted.

Statistical analysis

The morphological state of the fish was evaluated using a multiple-condition analysis of Fulton (K) using the formula proposed by Ricker (1975). The normality of the data was verified using standardized coefficients of skewness and kurtosis, and the homoscedasticity was verified using Bartlett's test. Treatment mean pH, environmental temperature, and water temperature were compared using the Student's T-test (T). Treatment salinity, dissolved oxygen, weight, and total length were compared using Mann-Whitney (MW) tests. Treatment effects were evaluated using a Chi-square test (χ^2) for fluidity and a contingency table for consistency. The Student T-test was used to compare treatment motility, percentage of active cells, and number of sperm cells/mL. Percentage data were transformed to arcsine before analysis (Zar 1999). All statistical analyses were

performed using a confidence limit of $\alpha = 0.05$. Statgraphics Centurion XVIII[®] was used for all statistical analyses, and graphic representation of data used the SigmaPlot v.11[®] package. Data are presented as means (\pm standard deviation) for parametric variables and medians (\pm interquartile range) for non-parametric variables.

Results

At the end of the experiment, 16 of the 20 fish in the controlled photoperiod treatment were males. In comparison, 15 males were identified in the natural photoperiod treatment. At the end of the experiment, the weight of fish in the controlled photoperiod increased by an average of 15.43 g, twice the increase in weight for fish under the natural photoperiod (7.65 g); however, no statistical differences ($p > 0.10$) in weight, total length, or final condition factor were observed between the treatments (Table 1).

Table 1. Mean values (\pm SD) for weight, total length (TL), and Fulton's condition factor (K) from fish at the beginning and the end of the experiment.

TREATMENT	INITIAL			FINAL		
	WEIGHT (g)	TL (cm)	K	WEIGHT (g)	TL (cm)	K
Controlled Photoperiod	174.32 \pm 38.43	18.43 \pm 1.46	2.76 \pm 0.23	189.74 \pm 41.23	18.94 \pm 1.25	2.77 \pm 0.77
Natural Photoperiod	188.04 \pm 36.75	18.72 \pm 1.44	2.84 \pm 0.33	195.69 \pm 34.24	19.93 \pm 1.76	2.55 \pm 0.52

A significant effect of the controlled photoperiod on sperm production (χ^2 ; $p < 0.01$) was observed. Sperm was obtained from all (N = 16; 100%) males in the controlled photoperiod treatment. Sperm was obtained from only nine (60%) of the 15 males in the natural photoperiod treatment. Based on T-test results, sperm count (mm^3 ; $p = 0.03$) was

higher in the controlled photoperiod treatment (5.14 million cells per ml \pm 1.64 e^6) than in the natural photoperiod treatment (3.63 million cells per ml \pm 0.36 e^6).

Sperm fluidity was higher in the controlled photoperiod treatment than in the natural one (χ^2 ; $p < 0.01$). Eleven males in the controlled photoperiod treatment had high (3) or very

high (8) fluidity, compared to only two (one fish in each category) in the natural photoperiod treatment (Table 2). Sperm consistency was similar between treatments (χ^2 ; $p = 0.07$). However, the controlled

photoperiod treatment had more fish with high sperm consistency (32%) in contrast to the natural photoperiod treatment, with 4% of fish having high sperm consistency. Sperm motility was similar between treatments ($p > 0.10$).

Table 2. Frequency of sperm fluidity observations in organisms subjected to different photoperiod regimes.

TREATMENT	SPERM FLUIDITY				
	NO FLUIDITY	LOW (I)	MEDIUM (II)	HIGH (III)	VERY HIGH (IV)
Controlled Photoperiod	0	1	4	3	8
Natural Photoperiod	6	4	3	1	1

Physicochemical parameters were different between treatments. The mean water temperature in the controlled photoperiod treatment ($28.97 \text{ }^\circ\text{C} \pm 0.75$) was $1.77 \text{ }^\circ\text{C}$ higher than the natural photoperiod treatment ($27.20 \text{ }^\circ\text{C} \pm 1.48$; $p < 0.001$). Similarly, the mean dissolved oxygen concentration in the controlled photoperiod treatment ($5.69 \text{ mg/L} \pm 1.51$) was higher than the natural photoperiod treatment ($5.56 \text{ mg/L} \pm 1.30$; $p = 0.016$). Salinity was slightly higher in the natural photoperiod treatment ($34.01 \text{ ppm} \pm 4.00$) than in the controlled photoperiod treatment (33.00 ± 3.00 ; MW; $p < 0.01$). Environmental temperature and pH were similar between treatments (T , $p > 0.10$), with average values of $8.57 \text{ UI} (\pm 0.13)$ and $29.00 \text{ }^\circ\text{C} (\pm 0.82)$ in the controlled photoperiod treatment and $8.56 \text{ UI} (\pm 0.15)$ and $28.97 \text{ }^\circ\text{C} (\pm 1.21)$ in the natural photoperiod treatment. Based on ANOVA, concentrations of ammonia, nitrites, and nitrates ($p > 0.05$) were similar between treatments. The average values (\pm SD) of ammonia, nitrites, and nitrates in the controlled photoperiod treatment were $4.30 (\pm 3.27)$, $2.08 (\pm 2.46)$, and $5.02 (\pm 0.09 \text{ mg/L})$, respectively. Average values (\pm SD) of ammonia, nitrites,

and nitrates were $4.15 (\pm 3.65)$, $4.08 (\pm 5.8)$, and $4.98 (\pm 0.09) \text{ mg/L}$, respectively, for the natural photoperiod treatment.

Discussion

Early exposure of Gray Snapper males to eight weeks of long days resulted in the highest maturation levels. The controlled photoperiod treatment resulted in more *L. griseus* males producing sperm than the natural one. Similarly, sperm count and sperm fluidity were significantly higher in the fish maintained under the controlled photoperiod. These results support the out-of-season induction of maturation in *L. griseus* using a controlled photoperiod.

Environmental changes profoundly impact reproduction, primarily achieved through corresponding alterations in the activity of the GnRH-GtH-gonadal axis, a hormonal pathway involved in regulating reproductive processes (Bromage *et al.* 2001). Among those environmental changes, annual photoperiod variations can stimulate the pineal gland and hypothalamus of fishes to secrete and

synthesize hormones that are required for reproduction (Frantzen *et al.* 2004, Prayogo *et al.* 2012 and Aragón-Flores *et al.* 2014). Nonetheless, the impact of photoperiod changes on reproductive responses differs significantly across species. To develop suitable photoperiod regimes for manipulating the reproductive cycle of a specific fish species, it is essential to gather information about the timing of maturation and spawning events throughout the year since spawning occurs at a different phase of the light cycle, with different daylength (Bromage *et al.* 2001). While certain species necessitate longer daylight durations (flatfish, cichlids) as stimuli for triggering maturation, others (salmonids) respond to shorter daylight durations (Whitehead and Bromage 1980, Bromage *et al.* 1984, Bye 1984, Duston and Bromage 1988; Singh and Zutshi 2020). Nevertheless, various researchers have shown that to obtain the intended response in fish, the adjustment of daylength must consider whether the fish have previously experienced a shorter or longer photoperiod (Randall *et al.* 1991, Randall and Bromage 1998). For example, Atlantic cod females, *Gadus morhua*, had lower fecundity and produced smaller eggs due to inhibition of gonad development and did not spawn when exposed to extended light hours (Hansen *et al.* 2001 and Hildahl *et al.* 2013). This effect also occurred in Atlantic salmon *Salmo salar* (Aragon-Flores *et al.* 2014). On the contrary, higher fertilization, hatching, and larval survival rates were observed in the catfish *Ompok bimaculatus* using extended daylight (Ajithkumar *et al.* 2022). These authors indicated that Ovasis™ injection, photoperiod manipulation, and fixed light intensity stimulated advanced gonadal maturation and induced captive spawning in this species,

potentially supporting the year-round production of larvae.

Although photoperiod does not show major seasonal variations in tropical regions, it has been demonstrated that significantly influences the reproductive cycle of tropical fish species, in some instances in conjunction with temperature (Bromage *et al.* 2001, Basak *et al.* 2016 and Singh and Zutshi 2020). According to Guerrero-Tortolero *et al.* (2008), in the tropical fish *Lutjanus argentiventris*, females exhibited enhanced egg production and off-season fertility when exposed to long days (14 hours of light and 10 hours of darkness). Additionally, the study observed a notable rise in testosterone levels in these fish compared to those exposed to different photoperiod treatments that simulated shorter days. In *Lutjanus analis*, when confined to conditioning using photo and thermotropism, advanced and complete maturation was obtained, with 70% fertilization (Botero-Arango and Castaño-Rivera 2005). In *Oreochromis niloticus*, the response to long day length (18L:6D) significantly accelerated maturation, resulting in larger egg size and higher total and relative fecundity (Campos-Mendoza *et al.* 2004).

While manipulations of photoperiod can cause delays or advancements in gonadal recrudescence, maturation, and spawning among cultured fishes, research on the influence of photoperiod on tropical male fishes is limited, as most studies have focused on species from temperate zones and more intensively on controlling female reproduction (Prayogo *et al.* 2012 and Gonçalves-de-Freitas *et al.* 2014). In this regard, Mishra (2013) proposed that environmental cues perceived by the brain trigger the synthesis of gonadotropin-releasing hormone (GnRH), leading to the activation of the pituitary gland to release

gonadotropic hormone (GTH). GTH I, in turn, initiates testosterone production in the testes, initiating the proliferation and formation of gametes. Subsequently, GTH II prompts the release of maturation-inducing steroids (MIS) and maturation-promoting factors (MPF), facilitating the final maturation of gametes. In addition to this, some studies have demonstrated that 11-ketotestosterone is also affected positively or negatively by photoperiod changes (Frantzen *et al.* 2004, Ammar *et al.* 2015 and Basak *et al.* 2016). For example, Bhattacharya (1999) found that 11-ketotestosterone (11-KT) is strongly influenced by photoperiod changes, triggering or blocking spermatogenesis. Frantzen *et al.* (2004) highlighted that *Salvelinus alpinus* exhibited significantly higher levels of 11-ketotestosterone, and mature males were obtained two months earlier than those exposed to the natural photoperiod.

According to previous research, the quality of sperm can be influenced by various environmental factors, including temperature, salinity, and water chemistry (Estrada-Godínez *et al.* 2014 and Valdebenito II *et al.* 2015). Although our study did not specifically examine temperature as a variable, we noticed

slightly elevated temperatures in the controlled photoperiod treatment compared to the natural one. Given the demonstrated influence of temperature on sperm quality in other studies, it is important to acknowledge the potential implications of higher temperatures on sperm quality in future investigations.

Our findings indicate that by manipulating the photoperiod, we can effectively induce male maturation in *L. griseus*. This manipulation offers enhanced control over gamete production, enabling fish production outside their natural spawning season.

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