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Artificial Induction of Spawning in Threeline Grunt, *Parapristipoma trilineatum* Under Controlled Environmental Conditions

Jin Woo Park, Jeong-Hyeon Cho, Seong Il Baek, Kang-Hee Im and Yong-Woon Ryu *

Subtropical Fisheries Research Institute, National Institute of Fisheries Science, Jeju-do 63610, Republic of Korea; pjw1446@korea.kr (J.W.P.); cjh.jan23@gmail.com (J.-H.C.); qortjddlff@naver.com (S.I.B.); imk0408@naver.com (K.-H.I.)

* Correspondence: ryuyw@korea.kr; Tel.: +82-64-780-5470

Abstract: The threeline grunt (*Parapristipoma trilineatum*) is a recently introduced aquaculture species that has attracted considerable interest in Korea. However, research on its aquaculture potential and reproductive biology remains limited. This study aimed to investigate the natural reproductive cycle and the feasibility of manipulating the spawning period of the threeline grunt through environmental control. We designed an environmental changes protocol involving a gradual increase in water temperature (16 °C → 21 °C) and extension of photoperiod (10 L → 14 L) from January to April and compared the group exposed to these conditions to those maintained under natural conditions. The experimental group exhibited significantly higher plasma sex steroid hormone levels at 60 and 90 days and significantly higher gonadosomatic index at 100 days. In addition, the experimental group spawned between April and June, approximately 2 months earlier than that of the control group. Despite earlier spawning, no significant differences were found in egg diameter, hatching rate, and larval notochord length. These findings suggest that spawning can be accelerated through photothermal changes without negatively impacting egg and larval quality. This study provides valuable insights into the reproductive biology of the threeline grunt and highlights the potential of utilizing photothermal control to enhance its aquaculture production.

Keywords: Haemulidae; reproductive cycle; spawning induction; photothermal control

Key Contribution: This study demonstrates that advancing the sexual maturation and spawning of threeline grunts by manipulating water temperature and photoperiod can significantly enhance aquaculture productivity without compromising egg quality.

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1. Introduction

The sexual development and reproduction of aquatic organisms, including teleosts, are closely associated with periodic changes in environmental factors such as photoperiod, temperature, lunar cycle, and food availability [1–4]. In particular, seasonal variations in water temperature and photoperiod are major factors regulating sexual events in fish, including reproduction [5,6]. Fish reproduction typically occurs within a narrow window of two to three months a year, as the timing of reproduction is adapted to occur under optimal environmental conditions that enhance offspring survival [7].

Reproductive processes in fish are regulated by the hypothalamus–pituitary–gonad (HPG) axis in response to environmental changes, such as temperature and photoperiod, and are initiated by the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus [6,8]. GnRH released from the hypothalamus activates GnRH receptors located in the anterior pituitary and regulates the secretion of two distinct gonadotropic hormones, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), thus influencing gonadal development and maturation [9–11]. This reproductive mechanism in fish

suggests that changes in environmental factors, such as temperature and photoperiod, could potentially be used to control the timing of reproduction, either by promoting or suppressing sexual maturation. Zahangir et al. [12] reviewed studies evaluating the impact of temperature fluctuations on reproductive events in various fish species and confirmed that endocrine and neuroendocrine factors within the HPG axis are modulated under abnormal high and low-temperature conditions [12]. In addition, it has also been reported that photoperiod changes can either promote or delay reproduction and growth in fish, as documented across different species [13,14].

Reproductive regulation through environmental control plays a pivotal role in the sustainable advancement of the aquaculture industry. Given that aquaculture production is highly susceptible to fluctuations in environmental factors, optimizing these parameters can significantly reduce operational costs and enhance productivity [15]. However, as the environmental conditions required for sexual maturation and reproduction vary across species, a precise understanding of the reproductive cycle and optimal conditions for the target species is imperative for effective breeding management.

The threeline grunt, *Parapristipoma trilineatum* (Thunberg, 1793), belonging to the order Acanthuriformes and family Haemulidae (subfamily Plectorhinchinae), inhabits the southern coastal waters of Korea and Japan, as well as the South China Sea [16]. This species is predominantly captured during the summer months when water temperatures are elevated and is currently cultured in Japan and China. Although the threeline grunt has not yet been cultured in Korea, rising water temperatures due to climate change have increased mortality among major aquaculture species, sparking interest in and demand for the threeline grunt as a viable alternative because it can tolerate rearing temperatures ranging from approximately 12–30 °C. Developing the threeline grunt as a new aquaculture species in Korea could not only address the current structural limitations of the industry (where production is concentrated on a few species) but also bolster the income of fish farmers by diversifying species suitable for high-temperature environments. Consequently, a thorough understanding of the reproductive ecology of this species and the potential for advancing the spawning season through environmental changes could provide strategic advantages for the aquaculture production cycle, including growth and wintering [17]. Nevertheless, studies on the aquaculture potential and reproductive biology of the threeline grunt are lacking in Korea.

This study aimed to elucidate the reproductive cycle of the threeline grunt and develop a safe and effective method for early spawning induction through the regulation of environmental factors, such as water temperature and photoperiod, to ultimately promote stable aquaculture production.

2. Materials and Methods

All threeline grunts used in the experiment (n = 470) were collected from the coastal waters of Jeju, Korea, using set nets and gillnets. Before the experiment, they were acclimated for over 6 months in indoor tanks at the Subtropical Fisheries Research Institute, National Institute of Fisheries Science (NIFS). All activities related to animal ethical considerations were approved by the Institutional Animal Care and Use Committee of the NIFS, Korea (2021-NIFS-IACUC-6) on 12 March 2021.

2.1. Experimental Design

2.1.1. Investigation of Reproductive Characteristics

A total of 250 threeline grunts (mean total length: 27.7 ± 1.6 cm, mean body weight: 302.2 ± 54.2 g) were reared in a 90-ton rectangular tank (6 × 6 × 2.5 m) for 1 year (January–December 2022) to monitor their reproductive cycle and spawning period. The experimental fish were reared under uncontrolled water temperature and photoperiod conditions throughout the experimental period. Daily water temperature was measured using a multi-parameter meter (ProDSS, YSI, Yellow Spring, OH, USA), and photoperiod data were obtained from the Korea Astronomy and Space Science Institute. The fish

were fed with commercial feed to satiation twice daily (Daebong L.S., Incheon, Republic of Korea). Monthly changes in plasma sex steroid hormones (females: estradiol-17β [E2]; males: 11-ketotestosterone [11-KT]) were analyzed by randomly sampling 15 females and males each between the 15th and 20th of each month. After anesthetization with 100 ppm of 2-phenoxyethanol (2-PE) (Sigma-Aldrich, Darmstadt, Germany), blood was drawn using heparin-treated syringes. Sampled fish were returned to the experimental tank post-recovery. Plasma was extracted through centrifugation (1500× *g* for 15 min, 4 °C) and stored at −80 °C until analysis. In addition, monthly gonadosomatic index (GSI) measurements and histological analysis were performed on five additional males and females each. After euthanizing the fish with the overdose of 2-PE (200 ppm), their gonads were extracted and weighed to calculate GSI (gonad weight/body weight × 100), and a transverse section of the middle portion of the gonads was fixed in Bouin's solution (Biognost, Zagreb, Croatia, EU) for further histological examination.

2.1.2. Spawning Response to Changes in Environmental Variables

A total of 220 threeline grunts (mean total length 27.3 ± 1.2 cm; mean body weight 321.4 ± 60.8 g) reared under natural temperature and photoperiod conditions were divided into two groups: environmental changes (experimental group) and natural conditions (control group), with 110 fish stocked in each 90-ton rectangular tank. The environmental changes experiment was conducted between 10 January and 19 April 2023 (100 days), with temperature and photoperiod adjusted based on reproductive cycle data (water temperature and photoperiod) from 2022 (Figure 1A). The initial water temperature was set at 16 °C for the experimental group and was gradually increased by 1 °C in 20-day intervals from 10 January until reaching 21 °C, which was maintained until the end of the experiment. The initial photoperiod was set at the natural photoperiod (10 h light: 14 h dark [10 L:14 D]) and was increased by 1 h in 20-day intervals until reaching 14 L:10 D. Threeline grunts in the control group were reared under natural water temperature and photoperiod conditions (Figure 1B). The water temperature in the tank was adjusted to the target temperature using a heat pump system (MCHV-P1800A1, Mitsubishi Electric, Tokyo, Japan), while the lighting was managed by installing overhead lights and programming a timer switch (HTS-24R, Han seung, Daegu, Republic of Korea) to operate at designated intervals.

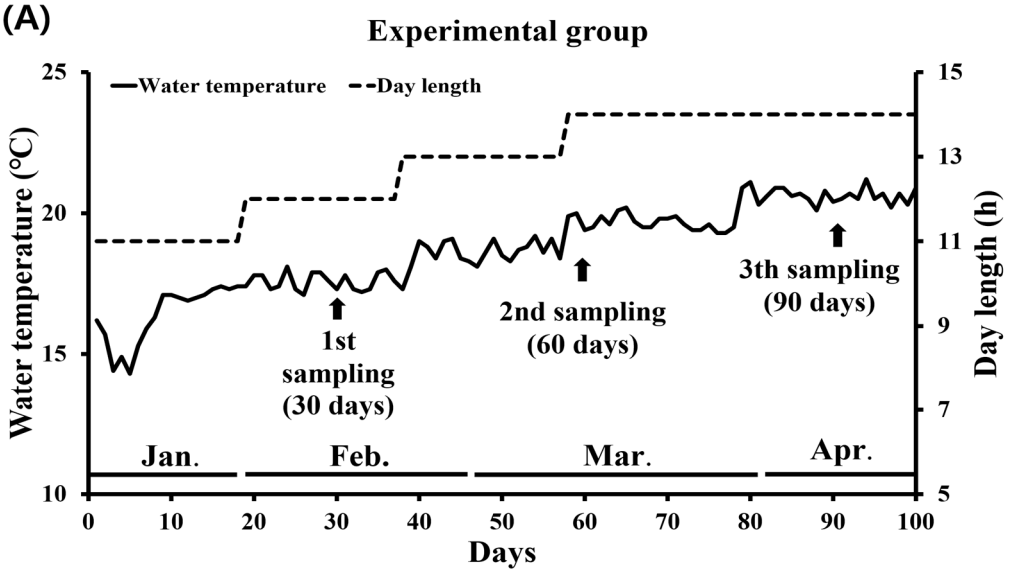

Figure 1. *Cont.*

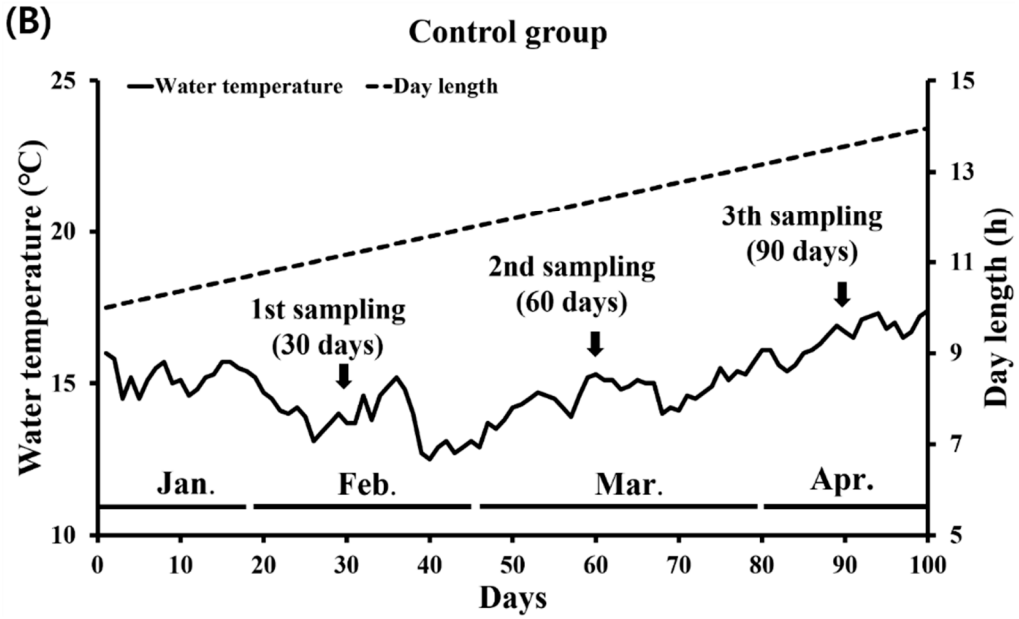

Figure 1. Environmental control program for spawning changes in the threeline grunt. The arrows in the figure indicate the sampling time. (**A**) Experimental group; (**B**) control group.

To evaluate the effect of environmental changes on sexual maturation induction, 15 males and females each were sampled from each group on days 30, 60, and 90 to measure sex steroid hormone levels (E2 and 11-KT). At the end of the experiment (day 100), five males and five females from each group were sampled for GSI measurements and further histological analysis.

2.2. Histological Analysis

All fixed gonadal tissues of the threeline grunt were processed using a tissue processor (TP1020, Leica Microsystems, Wetzlar, Germany) and embedded in paraffin with an embedding center (EG1150, Leica Microsystems). Serial sections were made at 5–6 µm thickness using a microtome (RM2235, Leica Microsystems), and the sections were stained with hematoxylin-eosin. Microscopic examination was conducted using an AXio Imager A2 microscope (Carl Zeiss, Oberkochen, Germany) and Zen imaging software v. 3.1 (Carl Zeiss, Oberkochen, Germany).

To characterize the reproductive cycle, all gonads collected over 1 year (January–December 2022) were examined, and the maturation stages for both females and males were categorized into four stages. Gonads of female threeline grunt were classified into four stages based on Palazón-Fernández [18]: stages I (immature, IM), II (early vitellogenesis, EV), III (late vitellogenesis, LV), and IV (post-spawning, PS) (Table 1). Male gonads were classified into four stages as defined by Palazón-Fernández and Schulz et al. [18,19]: stages I (immature, IM), II (early spermatogenesis, ES), III (late spermatogenesis, LS), and IV (post-spawning, PS) (Table 1). To determine the monthly distribution of gonadal development, all gonads were categorized based on these stages, and the frequency of each stage was recorded monthly. In the spawning changes experiment, the developmental state of gonads from both the experimental and control groups was compared according to the defined maturation stages.

Table 1. Characteristics of gonad development stages in female and male threeline grunt.

	Stage	Gonadal Maturity Classification	Germ Cell Condition
Female	I	Immature	An immature stage mostly composed of oocytes in the perinucleolar stage, and the cytoplasm is homogenized and strongly basophilic.
	II	Early vitellogenesis	The proportion of the nucleus is decreased compared to the previous stage, and a large nucleolus and several small nucleoli can be observed in the nucleus. In addition, it is an early vitellogenesis where oocytes at the red-stained yolk granules and vacuolar yolk vesicles are observed in the cytoplasm.
	III	Late vitellogenesis	The oocyte has increased in size compared to the previous stage, and the yolk sac in the cytoplasm is larger and filled with mature oocytes with developed zona radiate. In addition, some of the oocytes have hydrated oocytes before spawning.
	IV	Post-spawning	After ovulation, post-ovulatory follicle and α-atresia, which is a process of degeneration and resorption of oocytes, are observed. It is also a post-spawning stage where oocytes in the perinucleolar stage and early yolk formation stage are present at the same time.
Male	I	Immature	An immature stage composed of dividing and proliferating spermatogonia where spermatocytes and spermatids are rarely found.
	II	Early spermatogenesis	An early stage of spermatogenesis where all germ cells (spermatogonia, spermatocytes, and spermatids), except spermatozoa, are present and seminiferous tubules are filled with spermatocytes and spermatids.
	III	Late vitellogenesis	A late stage of spermatogenesis where some spermatocytes and spermatids are still present but at a much lower proportion compared to the previous stage, and the seminiferous tubules and main sperm ducts are mostly filled with sperm.
	IV	Post-spawning	A post-spawning period after ejaculation where residual spermatozoa are observed. Some sperm are degenerated and absorbed. The epithelium of the seminiferous tubules thickens, and a few spermatogonia appear.

2.3. Sex Steroid Quantification

Plasma levels of sex steroid hormones in all females and males collected during the experiment were measured using an enzyme-linked immunosorbent assay (ELISA) kit (E2 and 11-KT, Cayman Chemical, Ann Arbor, IM, USA). ELISA was performed according to the manufacturer's protocol. Absorbance was read with a microplate reader (Tecan, Männedorf, Switzerland), and results were calculated using Magellan v17.2 software (Tecan). All samples were measured in triplicate, and the intra-assay coefficients of variation for both E2 and 11-KT were below 9%.

2.4. Spawning Monitoring

Egg collectors were installed at the overflow outlets of all experimental tanks to monitor spawning events. The presence of eggs was checked daily between 9:00 and 10:00 AM. When eggs were detected, they were collected and separated into floating and sinking categories using a transparent measuring beaker without aeration, and the volumes (cc) of each category were recorded. Fertilized live eggs float, while dead eggs and unfertilized eggs sink. Therefore, the measured quantities of floating and sinking eggs were utilized to assess reproductive characteristics and evaluate offspring quality. The volumes of spawned eggs were measured using the same method for all experiments. The spawning period was defined as the duration from the first to the last observation of eggs.

2.5. Evaluation of Offspring Quality

In the spawning changes experiment, egg quality was compared between the experimental and control groups over a 1-month (30 days) spawning period. The collection period was in April (temperature 20.6 ± 0.1 °C, 14 L:10 D, 13 days after spawning initiation) for the experimental group and June (temperature 20.0 ± 0.2 °C, 14 L:10 D, 14 days after spawning initiation) for the control group. Eggs were collected for 17 and 18 days from the experimental and control groups, respectively. Egg diameter (μm, n = 100) was measured immediately after collection of the floating eggs, and hatching rate (hatching rate % = hatched larvae/fertilized eggs × 100) was calculated. Hatched larvae were sampled (n = 20) 3 days post-hatching (once the yolk sac and oil globule were fully absorbed) to measure notochord length (mm). Fertilized eggs were incubated in a multi-room low-temperature incubator (Hanbaek, Bucheon, Republic of Korea) at 20–21 °C under dark conditions, with over 70% water exchange daily and continuous aeration until sample collection. Egg diameter and notochord length were measured using an SMZ 754T stereo microscope (Nikon, Tokyo, Japan) and Optinity Optiview software v. 4.11 (Korealabtech, Seongnam, Republic of Korea), and the average values of the data collected throughout the sampling period were compared between the experimental and control groups.

2.6. Statistical Analysis

All data are expressed as mean ± standard error and analyzed using IBM SPSS 19 (SPSS Inc., Chicago, IL, USA). For the E2, 11-KT levels, and GSI changes obtained during the reproductive characteristic examination of threeline grunt obtained from January to December 2022, the homogeneity of variance was first evaluated using Levene's test; there was no violation of the assumption of equal variance ($p > 0.05$). Subsequently, analysis of variance was performed, followed by Tukey's test for post hoc analysis to determine statistical significance at a 95% confidence level ($p > 0.05$).

The E2, 11-KT, and GSI data from the 2023 spawning changes experiment were analyzed using a t-test to compare the means between the experimental and control groups at 30, 60, and 90 days. In addition, t-tests were used to assess the significance of differences in egg diameter and notochord length between the groups across all samples. All data were evaluated for normality and homogeneity using the Shapiro–Wilk and Levene's tests, and no violations of these assumptions were detected ($p > 0.05$).

3. Results

3.1. Reproductive Cycle Under Natural Conditions

3.1.1. Female

The ovaries of the female threeline grunt were classified into four stages (Table 1), and their development is illustrated in Figure 2. Stage I (IM; Figure 2A) consists mainly of oocytes in the perinucleolar stage. Stage II (EV; Figure 2B) shows the initial accumulation of yolk granules and the growth of oocytes. Stage III (LV; Figure 2C) is an advanced maturation stage that features a high number of yolk granules and oocytes surrounded by well-developed ovarian follicle cells, representing advanced maturation. Stage IV (PS; Figure 2D) is when post-ovulatory follicles and degeneration of oocytes are observed. The monthly ovarian development of females (n = 60) from January to December 2022 is shown in Figure 3. No spawning-capable ovaries were observed between January and April, with only IM and EV stages present. LV-stage ovaries were first observed in May, with these stages peaking in June and July. Between June and August, ovaries in the PS stage also appeared. No spawning-capable ovaries were observed from September to December.

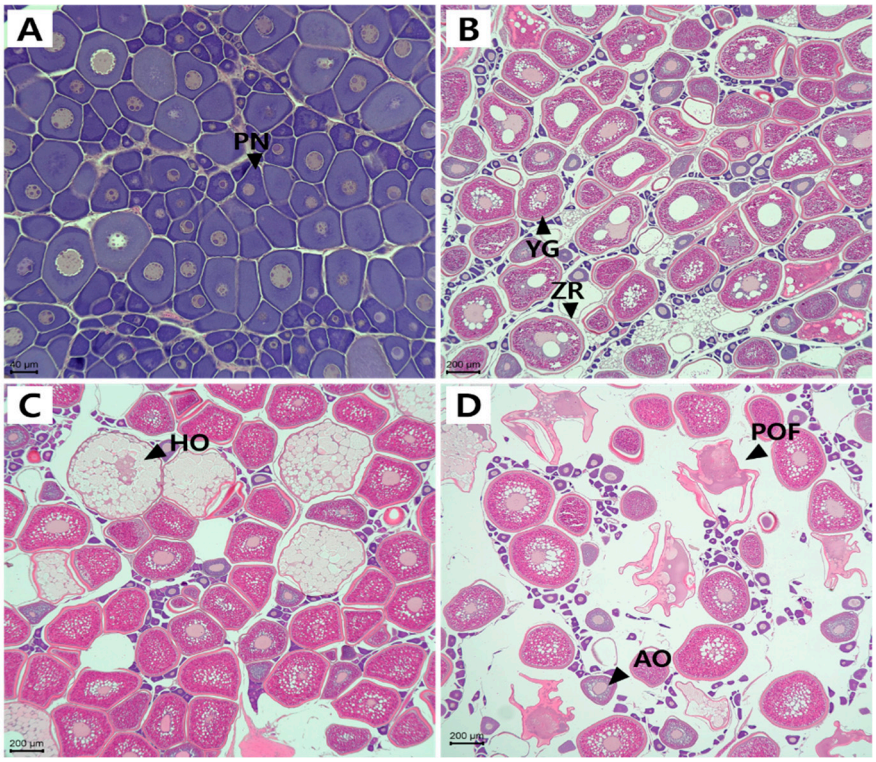

Figure 2. Histological sections of oocytes at different stages in threeline grunts. (**A**) Immature; (**B**) early vitellogenesis; (**C**) late vitellogenesis; (**D**) post-spawning. PN, perinucleolar; YG, yolk globule; ZR, zona radiata; HO, hydrated oocytes; AO, atretic oocytes; POF, post-ovulatory follicles. Scale bars: (**A**), 50 μm; (**B**–**D**), 200 μm.

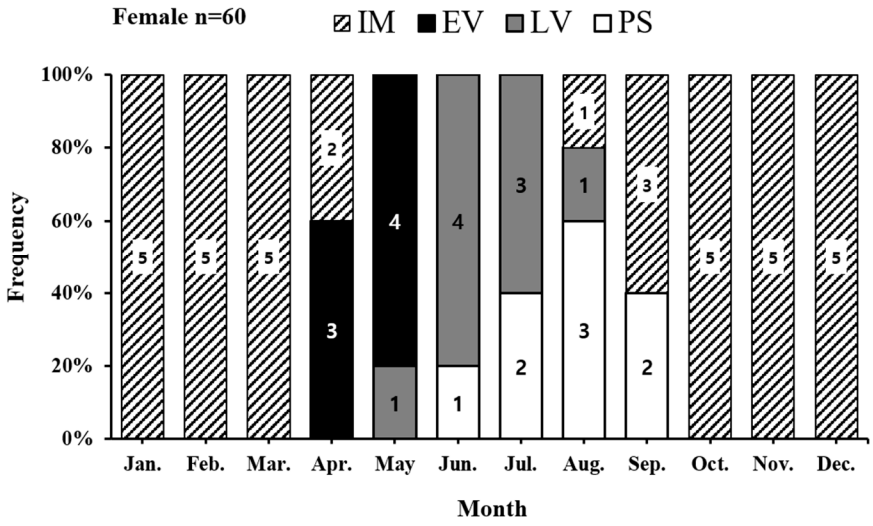

Figure 3. Monthly distribution of gonads of female threeline grunt according to oocytes development stages. IM, immature; EV, early vitellogenesis; LV, late vitellogenesis; PS, post-spawning. The numbers inside the bars represent the number of individuals at each stage of ovarian development.

The annual changes in E2 and GSI of females are presented in Figure 4. Both E2 and GSI increased gradually from April, peaked in June and July, and then decreased from August onward.

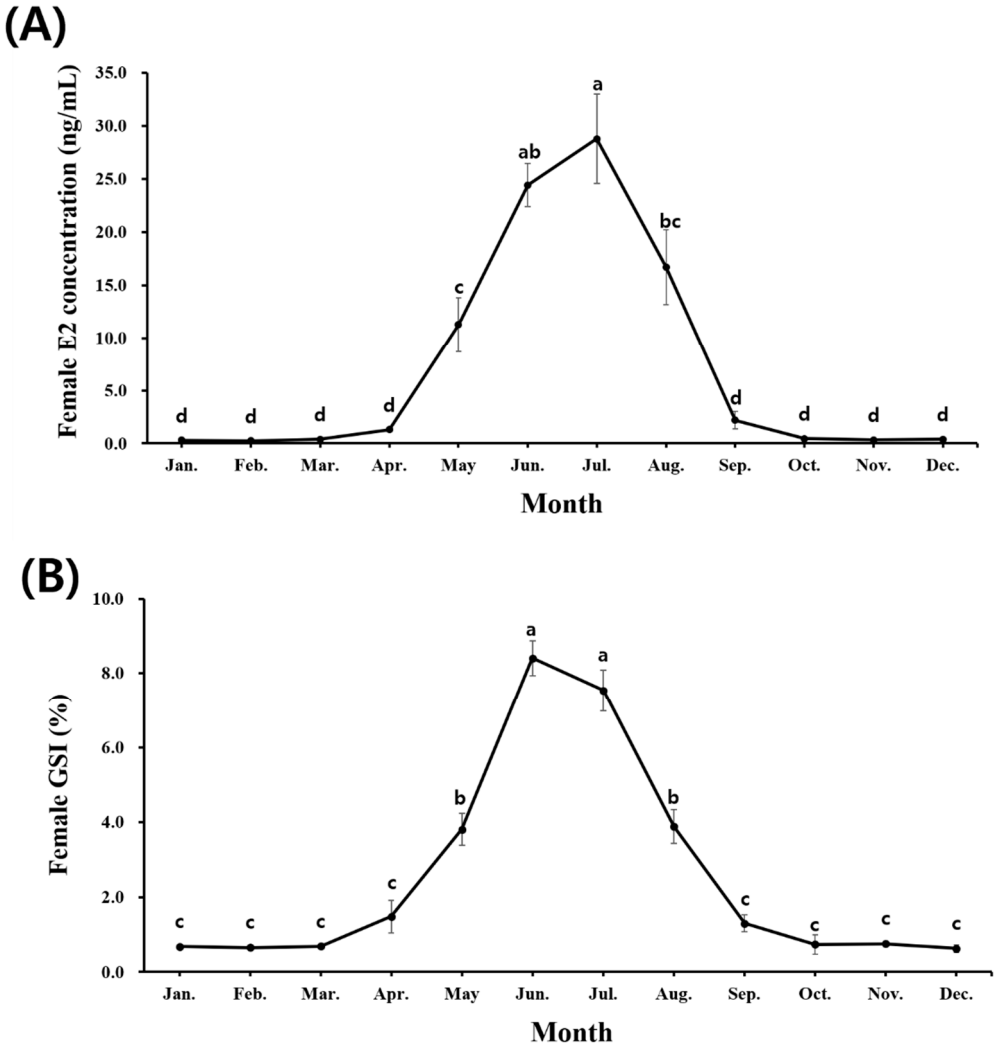

Figure 4. Monthly changes in (**A**) estradiol-17β (E2) level and (**B**) GSI of female threeline grunt. Results are expressed as mean ± SE. Different letters indicate significant differences ($p < 0.05$). GSI, gonadosomatic index.

3.1.2. Male

The testes of males were also categorized into four stages (Table 1). Stage I (IM; Figure 5A) primarily shows spermatogonia within the testicular tubules. Stage II (ES; Figure 5B) marks the growth stages, where developed spermatocytes and spermatids begin to appear. Stage III (LS; Figure 5C) represents full maturation, characterized by spermatozoa-filled tubules. Stage IV (PS; Figure 5D) shows thickened cytoplasm post-spawning. The monthly testicular development of males ($n = 60$) between January and December 2022 is shown in Figure 6. Most males were in the IM stage between January and March; however, most were in the ES stage with developed testes in April. LS stage was observed between April and August (April, $n = 1$), peaking in May and June. Males in the PS stage appeared in June and peaked in August. Between September and December, only PS or IM stages were observed. The annual changes in 11-KT and GSI of males are presented in Figure 7, showing a gradual increase from April, peaking in June, and then declining afterward ($p < 0.05$).

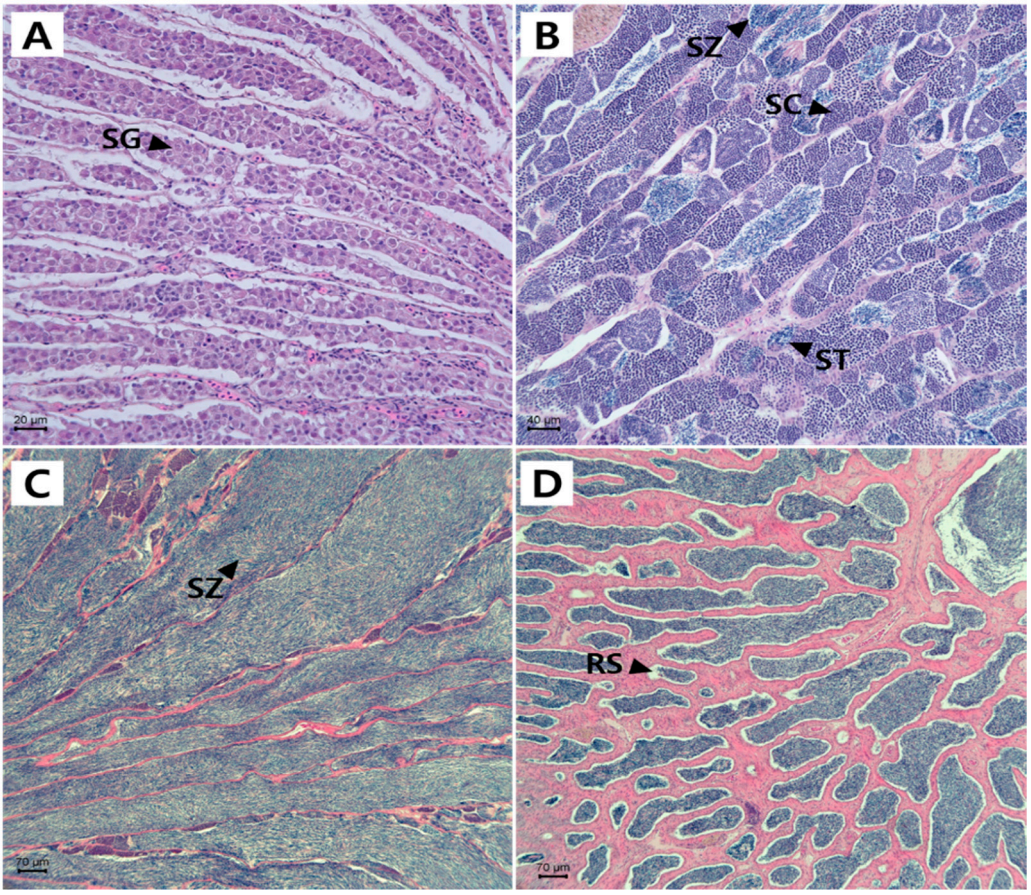

Figure 5. Histological sections of testes at different stages in threeline grunt. (**A**) Immature; (**B**) early spermatogenesis; (**C**) late spermatogenesis; (**D**) post-spawning. SG, spermatogonia; SC, spermatocytes; ST, spermatids; SZ, spermatozoa; RS, residual spermatozoa. Scale bars: (**A**), 20 μm; (**B**), 40 μm, and (**C**,**D**), 70 μm.

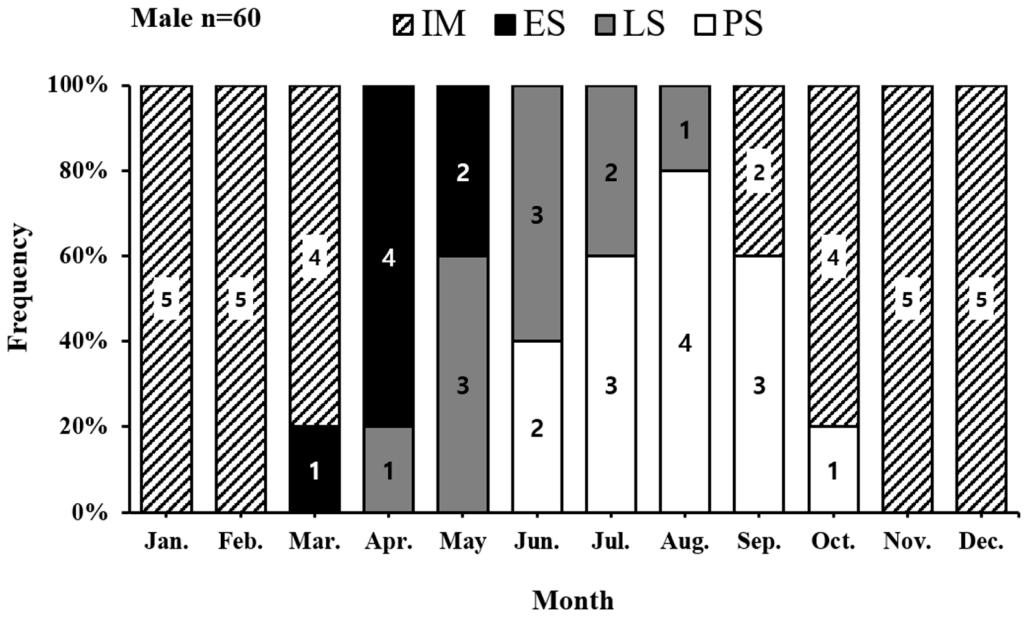

Figure 6. Monthly distribution of gonads of male threeline grunt according to testes development stages. IM, immature; ES, early spermatogenesis; LS, late spermatogenesis; PS, post-spawning. The numbers inside the bars represent the number of individuals at each stage of testes development.

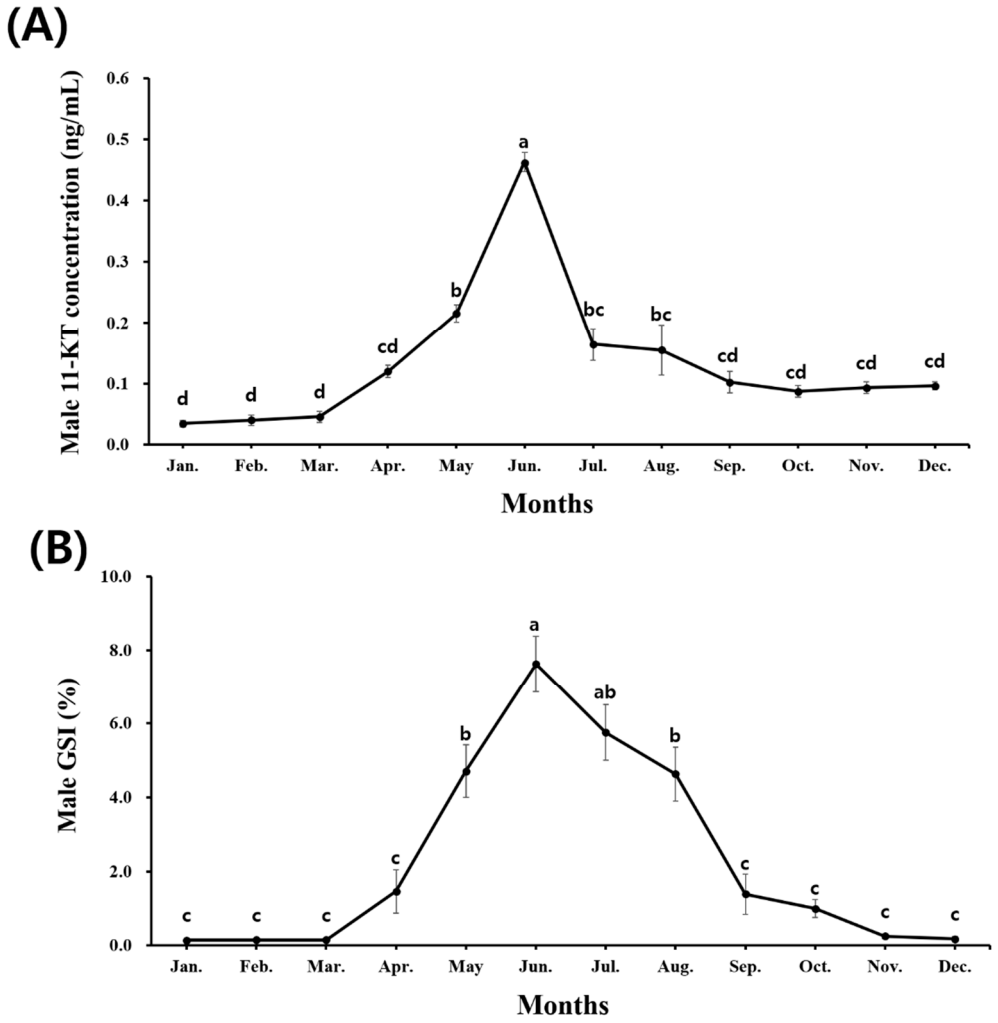

Figure 7. Monthly changes in (**A**) 11-ketotestosterone (11-KT) level and (**B**) GSI of male threeline grunt. Results are expressed as mean ± SE. Different letters indicate significant differences ($p < 0.05$). GSI, gonadosomatic index.

3.1.3. Spawning Characteristics

Environmental changes (water temperature and photoperiod) and results of spawning monitoring throughout the experimental period (2022) are illustrated in Figure 8. The highest water temperature was recorded in August (average 26.3 ± 0.6 °C), peaking at 27.3 °C on 19 August. The lowest was in February (average 13.9 ± 0.7 °C), with the lowest being 12.5 °C on 18 February. The longest day length was in June (14 L:10 D), and the shortest was in December (10 L:14 D) (Figure 8A).

Spawning occurred for 61 days from 4 June to 3 August, with 41 spawning days identified (Figure 8B). The total volume of spawned eggs was 13,881 cc, with 9335 cc and 4546 cc of floating and sinking eggs, respectively (Figure 8B).

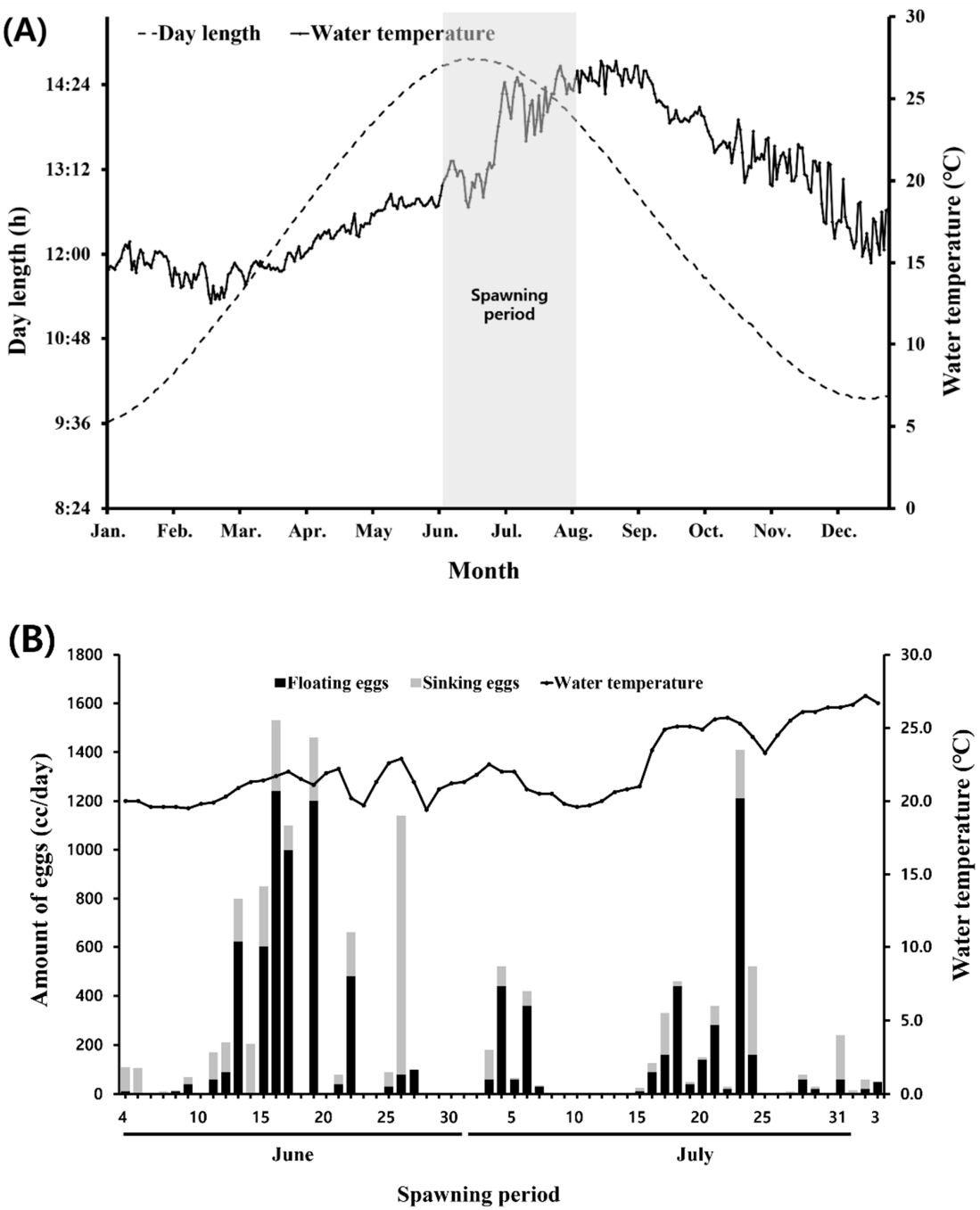

Figure 8. (**A**) Annual environmental changes during the reproductive cycle study period (2022); (**B**) Daily spawning amounts during the spawning period of 2022. The black solid line in graph (**A**) represents the daily water temperature, and the dotted line represents the day length. The vertical gray bars in graph (**A**) represent the spawning period of this study. The black line on the top of the graph (**B**) indicates the daily water temperature during the spawning period. The black and gray columns in graph (**B**) represent floating and sinking eggs, respectively.

3.2. Spawning Response to Environmental Changes

3.2.1. Steroid Hormone Levels, GSI, and Gonadal Development

The 11-KT and E2 levels in the experimental and control groups on days 30, 60, and 90 of the spawning changes experiment are shown in Figure 9A,B. No significant differences were observed in 11-KT for males and E2 for females between the groups on day 30; however, the levels were significantly higher in the experimental group than in the control group

on days 60 and 90 ($p < 0.05$). GSI values at the end of the experiment (day 100) were significantly higher in the experimental group for both sexes than in the control group ($p < 0.05$, Figure 9C). Histological analysis indicated that all males and females in the control group had immature gonads unsuitable for spawning, and the average water temperature for the control group throughout the experiment was 14.9 ± 1.0 °C, with an average photoperiod of 10 L:14 D (January) to 13 L:11 D (April). In contrast, the experimental group exhibited gonads in mature (male: LS; female: LV) or PS stages (Figure 10).

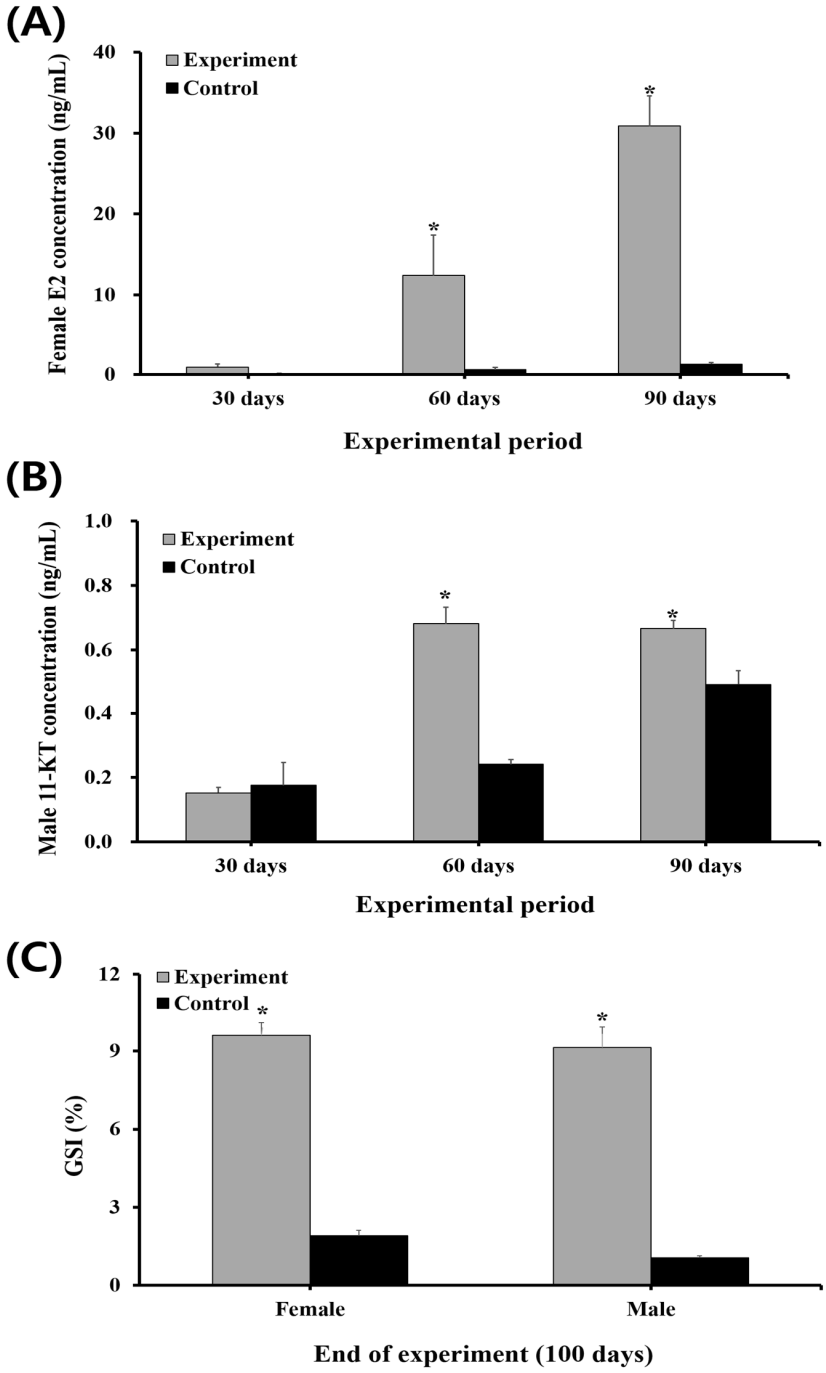

Figure 9. Comparison of (**A**) male 11-KT level and (**B**) female E2 level between the control and experimental groups on days 30, 60, and 90 of the spawning changes experiment; (**C**) comparison of GSI between males and females in the control and experimental groups on day 100 of the experiment. Results are expressed as mean ± SE. * Asterisk indicates a significant difference between experimental and control groups ($p < 0.05$). GSI, gonadosomatic index.

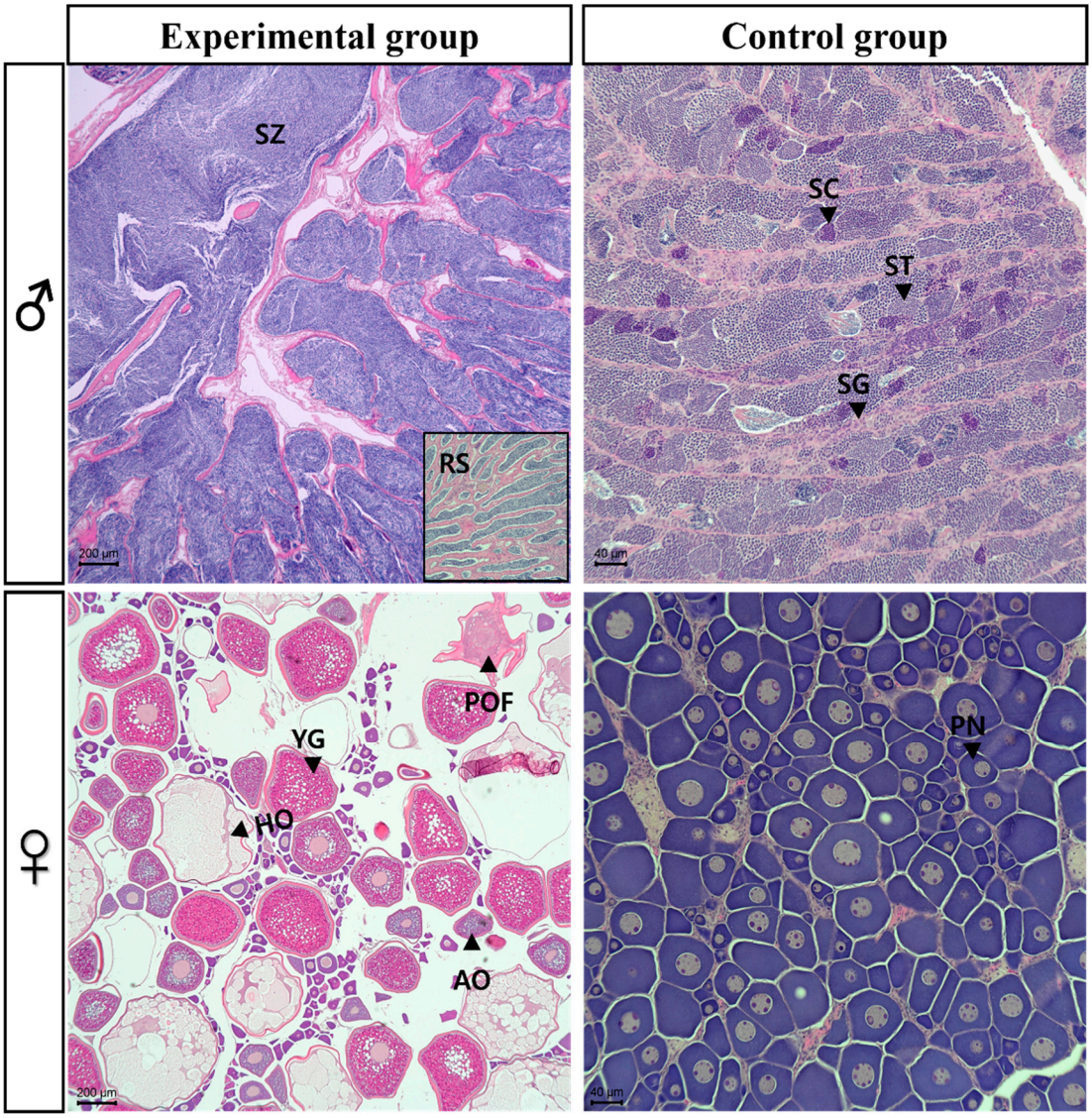

Figure 10. Histological results of male and female gonads in the experimental and control groups on day 100 of the spawning changes experiment. SG, spermatogonia; SC, spermatocytes; ST, spermatids; SZ, spermatozoa; RS, residual spermatozoa; PN, perinucleolar; YG, yolk globule; HO, hydrated oocytes; AO, atretic oocytes; POF, post-ovulatory follicles. Scale bars: control group, 40 μm; experimental group, 200 μm.

3.2.2. Spawning Monitoring and Egg Quality

The results of spawning monitoring in response to experimental conditions are shown in Figures 11 and 12. The experimental group began spawning on 20 March at a water temperature of 19.8 °C, with the last spawning occurring on 28 June. The total spawning period was 101 days, with 57 spawning days. The control group started spawning 2 months later, on 18 May at 19.0 °C, lasting 99 days with 52 spawning days. Although the control group had a higher total spawning volume (18,195 cc vs. 9092 cc), the floating rate of spawned eggs was higher in the experimental group (39.1% vs. 32.3%). No significant differences were observed regarding egg diameter, hatching rate, or larval notochord length between the groups ($p > 0.05$).

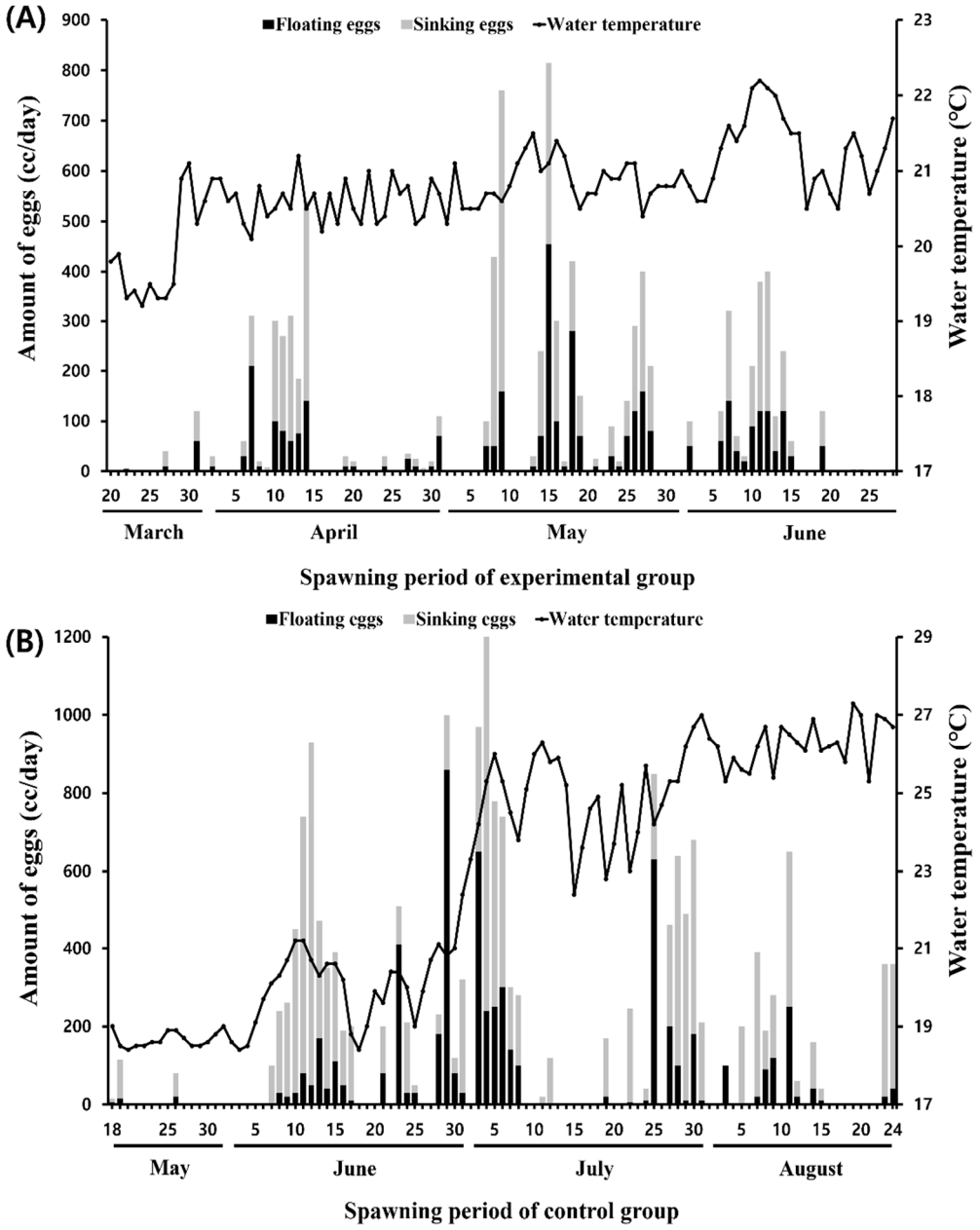

Figure 11. Spawning records of the (**A**) experimental and (**B**) control groups in the spawning changes experiment. The black solid line in the graph represents the daily water temperature. The black and gray columns in the graph represent floating and sinking eggs, respectively.

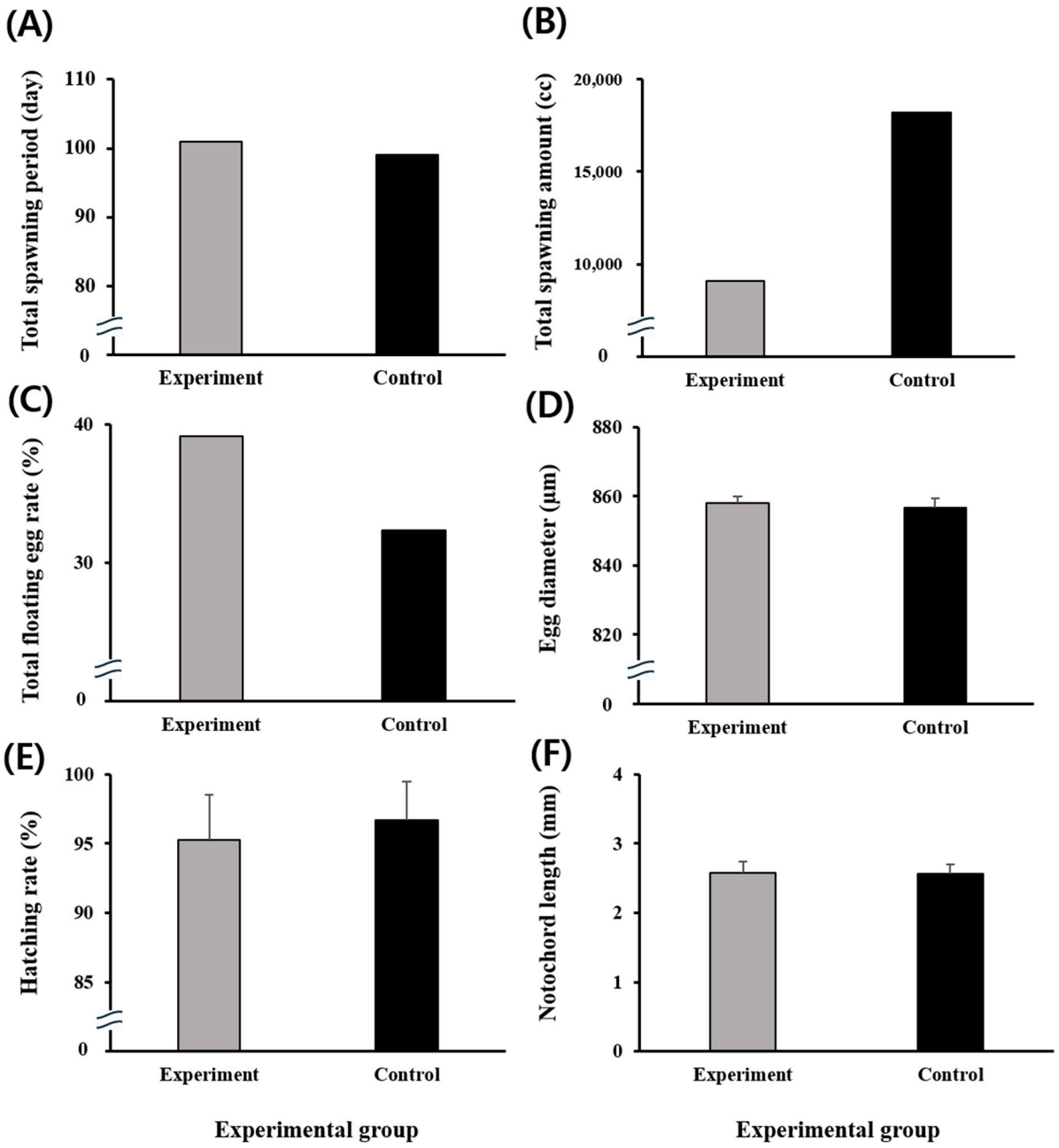

Figure 12. Comparison of spawning characteristics between the experimental and control groups of threeline grunt in the spawning changes experiment (**A**–**F**).

4. Discussion

This study confirmed that the threeline grunt, a newly introduced aquaculture species in Korea, achieves sexual maturity and optimal spawning conditions in late spring/summer when the water temperature rises, and the photoperiod extends. The findings demonstrate that artificial environmental changes can advance maturation and spawning by approximately 2 months.

In this study, the reproductive cycle of the threeline grunt was characterized by four distinct stages in both males and females. Similar to other temperate fish species, threeline grunt showed a clear seasonal breeding pattern influenced by environmental changes [20]. Sexual maturation of the threeline grunt occurred between May and July, marked by increased levels of 11-KT, E2, and GSI. Water temperature plays a crucial role in regulating final maturation stages and reproductive cycles in fish [21]. Similarly, photoperiod affects

the reproductive activities of teleosts [7], with most teleosts developing gonads under longer photoperiods and halting development under shorter ones [22,23]. Conversely, the initiation of sexual maturation and reproduction could be delayed if photoperiod is extended in some species, such as Atlantic cod (*Gadus morhua*), Atlantic salmon (*Salmo salar*), and rainbow trout (*Oncorhynchus mykiss*) [24–26]. Bromage et al. [7] reported that several marine fish species synchronize reproduction with warm water temperatures and long daylight periods, and this trend was also observed in the threeline grunt. The first spawning occurred on 12 June and lasted until late July. The fact that spawning occurred when water temperature increased by at least 20 °C and photoperiod extended to 14 L:10 D suggests that increased temperature and photoperiod stimulate reproductive hormones and induce sexual maturation. Upon analyzing the water temperature and photoperiod conditions during the spawning period of the threeline grunt, we found that the water temperature from early August to September, when spawning ceased, remained within the range observed during the spawning period in July (22–27 °C). In contrast, the day length shortened to below 14 L after early August. Although changes in both water temperature and photoperiod are crucial for regulating reproductive events, their significance varies among fish species [27]. In temperate teleosts, the photoperiod is the primary environmental factor governing reproductive events, while temperature is often regarded as a secondary cue [20,28,29]. Additionally, fish that spawn in spring or early summer, such as the threeline grunt, are often triggered by changes in photoperiod length [30]. Taken together, these findings indicate that variations in photoperiod may exert a greater influence on the spawning of threeline grunt than water temperature.

In our experiment, gradually raising the water temperature to 21 °C and extending the photoperiod to 14 h per day successfully advanced spawning by approximately two months compared to the control group. The experimental group exhibited significantly higher levels of E2, 11-KT, and GSI than the control group, resulting in a greater number of mature gonads by the end of the experiment. These findings align with previous studies demonstrating the effectiveness of environmental control in fish reproduction and provide valuable data for the industrialization of threeline grunt aquaculture [7].

Sexual maturation and spawning can reduce feed intake and thus cause weight loss [31]; thus, unintended sexual maturation and spawning lengthen the time required for fish to reach harvest size. Therefore, delaying sexual maturation and spawning through photothermal changes could be an effective technique for enhancing growth in the threeline grunt. Studies have shown the benefits of delaying spawning on growth in various aquaculture species, including gilthead seabream (*Spaus aurata*), Atlantic cod (*Gadus morhua*), and Atlantic halibut (*Hippoglossus hippoglossus*) [32–34]. In regions with significant seasonal temperature variations, such as Korea, wintering is a major issue in aquaculture production. Most teleost fish are ectothermic; thus, ambient temperature directly affects their metabolic rate, reproduction, somatic growth, and mortality, ultimately influencing individual productivity [20,35,36]. While fish in natural environments migrate to more favorable habitats, fish reared in aquaculture settings, such as net cages, are directly exposed to harsh conditions. In aquaculture settings, enhanced growth rates allow fish to reach a suitable size for wintering more quickly, reducing mortality from harsh conditions such as low winter temperatures [37]. Hence, advancing the spawning season through environmental changes could extend the period available for larval growth before the temperature drops, which can be beneficial for wintering.

In aquaculture, the quality of eggs and offspring is paramount for successful reproduction [38–40]. The quality of offspring produced through artificial maturation and spawning control is influenced by an array of inter/intra effects or in vivo/in vitro effects [38–41]. In our study, no remarkable differences were observed between the experimental and control groups in terms of egg diameter, hatching rate, or larval notochord length despite advancing maturation and spawning through environmental changes. This suggests that the controlled environment did not negatively impact the quality of the threeline grunt offspring. Several studies investigating the correlations between artificial induction of

reproduction and offspring quality have been reported. In which burbot (*Lota lota*) was reared under photothermal control to induce out-of-season spawning, no significant differences were observed in egg size, fertilization rate, larval survival, or growth between the manipulated and natural spawning conditions [42]. Similarly, in Atlantic cod (*Gadus morhua*), photoperiod changes successfully delayed or advanced the spawning season, and larvae produced under these conditions developed normally beyond metamorphosis [43]. However, some studies have reported adverse effects of artificial environmental changes or hormone treatments on gamete quality [44,45]. According to Bromage et al. and Zohar et al. [7,8], an appropriate level of environmental changes does not significantly affect gamete or offspring quality in aquaculture species [7,8]. Thus, the spawning induction technique using controlled environmental conditions established in this study proves to be effective for optimizing the reproduction of the threeline grunt.

Despite the comparable egg quality between groups, the total spawning amount in the experimental group was lower than that in the control group. Early induction of maturation and spawning could increase physiological stress, leading to higher energy expenditure and reduced total spawning volume [7]. Thus, it is possible that physiological changes induced by environmental adjustments could have impacted spawning volume in the threeline grunt.

While this study provides fundamental insights into the reproductive characteristics of the Threeline grunt and valuable techniques for its aquaculture management, certain limitations remain concerning the detailed physiological and endocrine responses to various environmental factors and their effects on aquaculture productivity. Therefore, further research should aim to refine the intensity and duration of environmental changes to optimize spawning volume and reproductive efficiency.

5. Conclusions

We demonstrated the feasibility of advancing sexual maturation and spawning by manipulating water temperature and photoperiod, which could significantly boost the productivity of threeline grunts in aquaculture. Importantly, the quality of eggs obtained from early spawning remained comparable to those produced under natural conditions, indicating that advancing the spawning period does not compromise egg quality. Given the high mortality of major aquaculture species in Korea due to high water temperatures, threeline grunt presents a viable alternative. These findings can provide valuable insights into the reproductive biology of threeline grunts and demonstrate the potential for improving aquaculture productivity through environmental changes. Controlling the reproductive environment to regulate the spawning period and enhance spawning efficiency is crucial for aquaculture. This is especially significant for regions like South Korea, where climate change and rising water temperatures are influencing traditional aquaculture species.

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