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Research Article

The Effect of Artificial Photoperiod and Water Temperature Program on the Reproductive Performance, Hormonal Profile and Maturation Stages of Male Gilthead Sea Bream (*Sparus aurata*)

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Abstract

The Gilthead Sea bream (*Sparus aurata*) is an important commercial species in the Egyptian coasts of Mediterranean Sea and the most relevant marine species in Mediterranean aquaculture. Water temperature and photoperiod are the most important factors driving sexual maturation in fish. This study investigates the effect of artificial photoperiod and water temperature programs on reproductive performance, gonadal maturation and the hormonal profile of male gilthead sea bream. 102 Gilthead Sea bream (males) which used in the present study were collected from Manzala Lake in Damietta governorate and Suez Canal Company for Fish Farming and Aquaculture at Ismailia Governorate, Egypt. This study shows the possibility of Gilthead Sea bream induced maturation in captivity by exposing to a program of water temperature (from 21.5 - 18°C) and artificial photoperiod for 4 months to complete the maturation and spawning. The results showed that biological measurements, sex hormone levels and histological sections estimate the maturation stages. The highest value of the male gonadal weight and GSI and the peak levels of FSH, LH, Total and Free Testosterone in males at T2 at TP4. It could be concluded that exposure to artificial photoperiod and water temperature programs can be used to control the spawning season of gilthead sea bream to start before or after the normal season of spawning. Also, it could be possible to induce maturation in captivity when the water temperature is at 18 and 19 °C through the artificial photoperiod and water temperature programs to reach the final gonadal maturation and release sperm.

Keywords: Gilthead Sea Bream; Gonadal Maturation; Testis, Water Temperature; Artificial Photoperiod; *Sparus aurata*

Introduction

Fish are a key component of marine biotic communities. For millennia mankind has had especially close bond with fish because they provide people with food. Around 43 million people worldwide make their living directly from fishing or fish farming, but people are careless with this natural resource. Over thousands of years too many fish have been caught. Many fishing grounds have been overfished [1].

The Gilthead Sea bream (*Sparus aurata*) is an important commercial species in the Egyptian coasts of Mediterranean Sea and the most relevant marine species in Mediterranean aquaculture. In

Egypt, it is known as "Denees" [2]. Although sea bream production reached about 35,880 tons, about 2.19 percent of total aquaculture production in 2019 [3]. During the last twenty years, all the previous studies agreed that the stock of sea bream shows a serious decline in Bardawil lagoon [4-6].

Gilthead Sea bream, *Sparus aurata* is a perciform fish, belonging to the family Sparidae and the genus Sparus. The seabream (*S. aurata*) occurs naturally in the Mediterranean and in the Eastern Atlantic, from the British Isles, Strait of Gibraltar to Cape Verde and around the Canary Islands [7,8].

The Sea bream was found in a variety wild of marine habitats, seagrass beds and from rocky to sandy bottoms as well as the surf zone, commonly to depths of about 30 m, but adults may occur at 150 m depth [9]. It is a euryhaline species and moves in early spring towards protected coastal waters in search of abundant food and sustainable milder temperatures (trophic migration). It returns to the open sea in late autumn for breeding purposes, being very sensitive to low temperatures (the lower lethal limit is 2°C). It is mainly carnivorous (shellfish, including mussels and oysters), and accessorily herbivorous [7]. In the wild, it spawns in the winter months but in aquaculture farms, it is conditioned to spawn all year round under controlled methods. In recent years, it has been widely cultured in many countries including Egypt [10,11].

Reproduction is an essential characteristic of life on this planet. During the last few decades, the number of aquatic species is rising under domestication efforts. The sustainable aquaculture industry is the capacity to control the reproduction processes of fish in captivity and to acquire high-quality seed in the future, aquaculture production and development is based on the control of aquaculture reproduction. Reproduction in fishes is regulated by the external environmental factors that trigger internal mechanisms into action [12].

Unfortunately, some dysfunctional form of reproduction was exhibited in most fish when reared in captivity condition. Many species of captive fish are able to reach reproduction maturity in aquaculture conditions and gonadal growth occurs normally [13]. However, some of female species often fail to undergo FOM stage and do not spawn, while males exhibit reduced production or low quality of milt [12,13]. Fish Reproduction in captivity can be controlled by environmental manipulations, such as temperature, photoperiod, salinity, tank volume, depth, substrate vegetation and spawning substrate, etc. can often improve the reliability of spawning [14-16].

Spawning in sea bream and sea bass similar to other vertebrates, the reproductive cycle is controlled by several interacting factors. Environmental stimulation is reception and the brain translated it into neural signals which trigger to release of GnRH or inhibition of the release of GnRIF causing the pituitary to secrete GTH [17-19]. When a certain GTH level is reached, vitellogenic oocytes undergo the process of final oocyte maturation: the germinal vesicle migrates to the periphery, theca and granulosa cells of the follicle are stimulated to secrete a MIS which induces GVBD [16,20-22].

Similar to the most animals from wild held in captivity, many commercial fish which interest to the aquaculture industry display reproductive dysfunctions. These dysfunctions are a result of the fact that fish in captivity don't experience the conditions of the spawning grounds, and as a result there is a defeat of the pituitary to release the maturational gonadotropin, LH (GTH II). These dysfunctions may be resulted from the combination of captivity-induced stress and the lack of the suitable "natural" spawning environment. Problems of Reproduction often diminish over the years, after many generations of fish are reared and produced in culture [16].

To the best of our knowledge, there are lack of information on the maturation, gonadal development and hormonal profile, therefore we plan to investigate the effect of artificial photoperiod and water temperature program on gonadal maturation and the spawning induction of marine gilthead sea bream brood stock (males) related to fish reproduction through determination of: I Biological measurements (body weight, total length, Gonadosomatic index and K factor) in male gilthead sea bream *Sparus aurata*, II Hormones levels in male gilthead sea bream, III Testes development and gonadal histological sections in male gilthead sea bream.

Materials and Methods

Fish collection

A total number of 102 males of Gilthead Sea Bream *S. aurata* broodstocks were caught from Manzala Lake, Damiatta Governorate and Suez Canal Company for Fish Farming and Aquaculture at Ismailia Governorate, Egypt. Average body weight was 290.84 ± 75g. Average total length was 24.56 ± 3 cm. The prevailing weather condition during the period of fish collection was showed in table 1.

The collected fish were transported by a vehicle prepared by tanks 1 m^3 (1 × 1 × 1m) and supplied with oxygen by using portable air pump at stocking density 51 fish/m^3 to Fish Farming and

	Item	Measurements
Manzala Lake, Damiatta governorate	Avg. Temperature (° C)	24.8
	Day Time (hours)	12:33
Suez Canal Company for Fish Farming and Aquaculture at Ismailia governorate	Avg. Temperature (° C)	26.2
	Day Time	12:40

Table 1: Weather condition during the period of fish collection.

*According to Egyptian Meteorological Authority - EMA.

Technology institute, Suez Canal University, Ismailia governorate, Egypt. The transportation water parameters were measured and controlled as following: salinity 28 - 32 ppt, pH 7.5 - 8 and DO ≤ 6 mg/l.

Male maintenance

Fish were acclimated to laboratory conditions for about two weeks prior to the beginning of the experiment. Because Sea bream have no apparent external dimorphism, identification of sex, male and female were separated and determined by total length which reported 20 cm for males, and sex inversion at lengths between 15 - 27 cm according to [23,24]. Fish were anaesthetized with tricaine methane sulfonate (Ms-222, dose:100 mg/L, Argent Lab. Inc. Philippines) [25].

Fish were kept in indoor fiber circular holding tanks with maximum capacity of 3 m^3 (1.7 m diameter and 1.4 m high) filled with salt water, under controlled photoperiods and temperature. The salt water used was filtered with sandy filters and sterilized using ultraviolet units (Fujan Newland Entech Co., Ltd, China).

Vigorous aeration was provided by air blower (Rotary Blower, SWR, China) through diffuser stones.

Water Physico-chemical parameters

The water parameters were measured before the experiment beginning as shown in (Table 2). The daily water parameters were measured and controlled at the indoor holding tanks in the hatchery unit to be as follow: temperature of 24 - 18° C, salinity of 30-35 ppt, pH 7- 8, dissolved oxygen from 6 - 8 mg/L by using an air blower provided through diffuser stones and water flow rate of one liter per min by using inlet-outlet system. Water parameters were daily measured by: Thermometer apparatus, DO meter (E x Stik II D-0600, FLIR systems, Inc., USA), pH meter (Milwaukee MW-100) and Digital refractometer (DRBS-300).

	Parameters	Value
1	T.D.S (mg/l)	46480
2	Alkalinity (mg/l)	144.02
3	pH value at 20° C	7.7
4	Total Hardness (mg/l)	500
5	Ammonia (mg/l)	0
6	Salinity, ppt	33.39
7	DO (mg/l)	6.8

Table 2: Water Physico-chemical analysis before the beginning.

*Water quality lab., Fish farming and technology institute.

Feeding

The fish was manually fed once daily (3%) of body weight and fed to satiation from the third month with minced pieces of (squad, mackerel, sardine, shrimp and small fish). Broodstock were prevented from feeding 24 hours before sampling.

The experimental design of fish

Fish were exposed to temperatures program by using chiller systems (A chiller is a machine that removes heat from a water a vapor-compression or absorption refrigeration cycle. This liquid was then circulated through a heat exchanger to cool water or equipment as required. Chilled water temperatures ranged between 25 and 16 °C, the chilled water moves directly from the chiller to the holding tanks) and photoperiod program was applied by using neon lamps. Natural photoperiod and water temperature were used in control to reach final maturation.

Fish were exposed to photoperiod program and light intensity in the range of 600 lux (Table 3) and measured by Light meter (YK-10LX, Taiwan), which was changed every 15 days and the temperature (Table 4) was changed every 15 days at a rate of half degree

until it reaches the target temperature. Large windows should be avoided to prevent direct sunlight rolling on the tanks.

102 were divided into 2 groups (three replicates/group). Each replicate consists of 17 males

- The first group (control - T_1) of broodstocks were exposed to water temperature of 22 ± 1˚C, natural photoperiod and natural light intensity. The group tanks were near large windows to let direct sunlight rolling on the tanks.
- The second group (T_2) Brood stocks were stocked at water temperature program from 21.5 to 18˚ C and exposed to artificial photoperiod program (Table 3,4).

Day No.	Lighting hours	Dark hours
1 - 15	11	13
16 - 30	10:45	13:15
31 - 45	10:30	13:30
46 - 60	10:15	13:45
61 - 75	10:30	13:30
76 - 90	10:45	13:15
91 - 105	11	13
106 - 120	12	12

Table 3: Photoperiod program used in treatments for the induce spawning of *S. aurata*.

Period (Days)	Control T_1Natural Light	T_3
1 - 15	22 ± 1	21.5
16 - 30	22 ± 1	21
31 - 45	22 ± 1	20.5
46 - 60	22 ± 1	20
61 - 75	22 ± 1	19.5
76 - 90	22 ± 1	19
91 - 105	22 ± 1	18.5
106 - 120	22 ± 1	18

Table 4: Temperature (˚C) program used in treatments for the induce spawning of *S. aurata*.

At the end of every month (Time periods), fish Total length, body weight and gonads weight were measured. Blood samples and histological sections of ovaries and testis were taken. The age-length-sex–weight relationships were compared and the sex inversions were observed as a result of population effects of fish.

Biological measurements

The reproductive activities and maturity were determined from the temporal development of the Fulton's condition factor (K) and gonadosomatic index (GSI).

The Fulton's condition factor (k), which relates body length of the fish to the body weight, was calculated according to [26]

$K = \frac{BW \times 100}{L^3}$ (BW: Body weight, L: Total Length)

GSI is the method of studying the spawning season by following the seasonal changes in the gonadal weight in relation to the body weight [27].

$GSI = \frac{Gonad\ weight}{Total\ Body\ weight} \times 100$

Blood sampling

At the end of every month, one male from each replicate were anesthetized in diluted MS-222 at a concentration of 100 mg/l for sampling. Blood samples were quickly withdrawn by heparinize syringe (5000 IU, Amoun Pharmaceutical Co.) from heart puncture and then transferred to heparinize tubes immediately and shaken gently in order to avoid hemolysis, and thereafter centrifuged at 2500 rpm at 4 °C for 10 min. Plasma was separated and then stored at −80 °C for subsequent analysis of hormonal profile. It should be mentioned that blood sampling of fish was executed in the morning around 8:00 a.m. before providing food [28].

Determination of hormonal levels

FSH, LH, Testosterone (Total and Free), Estradiol, Progesterone levels were determined by using commercial assay ELISA kits as manufactories instructions.

Histological sampling and sections

Testis of fish in treated and control groups were collected from freshly dead fish and fixed in formal solution (8.5 g of sodium chloride, 100 ml of 40% Formalin and 900 ml of distilled water). Clearing and paraffin embedding were performed using standard histological techniques**,** after one week they were dehydrated in Ascending

graded alcohol series, from alcohol 70%,80%,90%,95%then absolute alcohol, exposed to xylene and embedded in paraffin wax. Sections of 6 µm thickness were prepared and stained with harris haematoxylin and eosin, then mounted with DPX [29-31]. Histological photos were taken using binocular light microscope equipped with camera (Olympus DP25, U-CMAD3).

Statistical analysis

Statistical analysis was performed using the 2 x 4 factorial design according to the following model:

$Y_{ijk} = \mu + P_i + T_j + P_iT_j + e_{ijk}$

Where

µ is the overall mean,

P_i is the fixed effect of time period (I = 1…4),

T_j is the fixed effect of treatment (j = 1….2),

P_iT_j is the interaction between effect of the time period and treatment,

e_{ijk} is random effect.

Values were expressed as Mean ± SE. Mean was tested for significant differences at P-values ≤ 0.05 [32] to determine the effects of time period, and treatments on biological and physiological parameters. Some other values were subjected to one-way ANOVA. All the statistical analyses were calculated using SPSS program version 20 (SPSS, Richmond, USA) as described by [33].

Results

The effect of time period and treatments on body weight, total length and condition factors in male gilthead sea bream *S. aurata* specimens

Data presented in table 5 showed that the average live body weight of males *S. aurata* ranged from 230.07 to 349.82 g while total length ranged from 24.40 to 27.33 cm. Non significant differences were observed between the experimental groups for live body weight and total length indicated the homogeneity of fish body weight and length.

The condition factor (k) was non significantly difference by time periods, while by treatments and by the interaction of time periods and treatments it showed a significant (P ≤ 0.05) difference. In the present investigations, the highest value of k factor of male

		Body weight (g)	Total length (cm)	k factor
Time period				
TP_1		282.64 ± 24.43	25.97 ± 0.70	1.59 ± 0.10
TP_2		327.66 ± 15.18	26.66 ± 0.26	1.74 ± 0.09
TP_3		303.54 ± 30.25	26.30 ± 0.86	1.63 ± 0.06
TP_4		257.79 ± 14.07	25.28 ± 0.50	1.59 ± 0.05
Treatments				
T_1 (Control)		283.21 ± 15.87	26.51 ± 0.60	1.51^{b} ± 0.04
T_2		279.61 ± 21.35	25.10 ± 0.36	1.73^{a} ± 0.09
Interaction effect of time period and treatments				
T_1	TP_1	296.73 ± 29.09	27.17 ± 1.36	1.48^{ab} ± 0.09
	TP_2	300.43 ± 34.47	27.33 ± 0.33	1.46^{b} ± 0.11
	TP_3	305.60 ± 35.36	27.03 ± 1.29	1.54^{ab} ± 0.04
	TP_4	230.07 ± 18.87	24.50 ± 1.26	1.57^{ab} ± 0.11
T_2	TP_1	250.93 ± 73.83	24.40 ± 1.20	1.64^{ab} ± 0.32
	TP_2	332.73 ± 27.72	25.80 ± 0.21	1.93^{a} ± 0.12
	TP_3	261.37 ± 21.52	24.53 ± 0.29	1.76^{ab} ± 0.08
	TP_4	273.40 ± 36.53	25.67 ± 0.67	1.60^{ab} ± 0.09

Table 5: The effect of time period and treatments and their interactions on body weight, total length and condition factors in male gilthead sea bream *S. aurata* specimen.

(TP: Time Period, T_1: control treatment, T_2: Artificial photoperiod and water temperature treatment).

Mean value in the same column with different superscript are significantly different (Duncan's multiple range test at $P \leq 0.05$).

fish specimens was 1.93% at the second time period (TP_2) in T_2, while, the lowest value was recorded at second time period in T_1 (Table 5).

The effect of time period and treatments on gonadal weight and GSI in male Gilthead Sea bream S. aurata

In the current studies, significant variations ($P \leq 0.05$) of gonad weight in male *S. aurata* were documented for different time periods and in different treatments. Where, the highest gonad weight was recorded in TP_4 (15.33g) regardless the treatments, while, significant differences ($P \leq 0.05$) of gonad weight was observed in T_2 regardless the time periods.

Concerning the combined effects of time periods and treatments, the highest value of male gonad weight was documented in TP_4 at T_2 (44.57g) as shown in table 6.

		Gonadal weight (g)	GSI (%)
Time period			
TP_0		$0.49^b \pm 0.11$	$0.16^c \pm 0.03$
TP_1		$1.33^b \pm 0.33$	$0.45^c \pm 0.08$
TP_2		$2.90^b \pm 0.73$	$0.88^c \pm 0.24$
TP_3		$6.90^b \pm 3.48$	$2.46^b \pm 1.20$
TP_4		$15.33^a \pm 7.72$	$5.62^a \pm 2.76$
Treatments			
Before the beginning		$0.49^b \pm 0.11$	$0.16^b \pm 0.03$
T_1 (Control)		$0.51^b \pm 0.04$	$0.18^b \pm 0.01$
T_2		$17.48^a \pm 5.59$	$6.30^a \pm 1.99$
Interaction effect of time period and treatments			
T_1	TP_1	$0.58^c \pm 0.09$	$0.19^c \pm 0.04$
	TP_2	$0.44^c \pm 0.06$	$0.15^c \pm 0.00$
	TP_3	$0.62^c \pm 0.10$	$0.20^c \pm 0.02$
	TP_4	$0.44^c \pm 0.04$	$0.19^c \pm 0.02$
T_2	TP_1	$2.08^c \pm 0.83$	$0.70^c \pm 0.01$
	TP_2	$5.06^c \pm 0.73$	$1.58^c \pm 0.37$
	TP_3	$18.20^b \pm 6.97$	$6.63^b \pm 2.07$
	TP_4	$44.57^a \pm 8.62$	$16.30^a \pm 2.39$

Table 6: The effect of time period and treatments and their interactions on gonadal weight and GSI % in male Gilthead Sea bream *S. aurata*.

(TP: Time Period, T_1: control treatment, T_2: Artificial photoperiod and water temperature treatment).

Mean value in the same column with different superscript are significantly different (Duncan's multiple range test at $P \leq 0.05$).

The effect of time period and treatments on FSH, LH and, Total and Free testosterone levels of male Gilthead Sea bream S. aurata

Before the beginning (TP_0) of the experiment, plasma hormonal levels FSH, LH, Total Testosterone and Free Testosterone were 0.16, 0.08 miu/ml, 0.34 ng/ml and 0.18 Pg/ml respectively.

Data presented in table (7) showed significant ($P \leq 0.05$) differences between time periods. The peak value of FSH was recorded at TP_4 (0.68 miu/ml) and the lowest level at TP_1 (0.31). The LH highest level was recorded in TP_2 and TP_4 (0.23 ± 0.06 and 0.23 ± 0.04 miu/ml). The peak of Total and free Testosterone was ob-

served at TP_4. There was significant different between treatments in FSH, Total and free testosterone which recorded the highest levels were recorded in T_2 while, LH values showed no significant difference by treatments.

About the merged effects of time period and treatments, the highest level of FSH, LH and, total and free testosterone were observed at TP_4 in T_2 (1.20 miu/ml) (0.38 miu/ml) (2.30 ng/ml) (0.79 pg/ml) respectively (Table 7).

		FSH (miu/ml)	LH (miu/ml)	T. Testosterone (ng/ml)	F. Testosterone (pg/ml)
Time period					
TP_0		$0.16^{e} \pm 0.07$	$0.08^{b} \pm 0.01$	$0.34^{d} \pm 0.28$	$0.18^{d} \pm 0.09$
TP1		$0.31^{d} \pm 0.03$	$0.10^{b} \pm 0.01$	$0.88^{c} \pm 0.19$	$0.28^{b} \pm 0.08$
TP2		$0.42^{c} \pm 0.05$	$0.23^{a} \pm 0.06$	$1.18^{bc} \pm 0.09$	$0.46^{ab} \pm 0.05$
TP3		$0.53^{b} \pm 0.06$	$0.19^{ab} \pm 0.02$	$1.34^{ab} \pm 0.15$	$0.58^{a} \pm 0.09$
TP4		$0.68^{a} \pm 0.14$	$0.23^{a} \pm 0.04$	$1.66^{a} \pm 0.23$	$0.63^{a} \pm 0.09$
Treatments					
Before the beginning		$0.16^{c} \pm 0.07$	$0.08^{b} \pm 0.01$	$0.34^{c} \pm 0.28$	$0.18^{d} \pm 0.09$
T1 (Control)		$0.27^{b} \pm 0.02$	$0.15^{a} \pm 0.05$	$0.83^{b} \pm 0.15$	$0.30^{b} \pm 0.08$
T2		$0.72^{a} \pm 0.10$	$0.24^{a} \pm 0.03$	$1.63^{a} \pm 0.18$	$0.59^{a} \pm 0.07$
Interaction effect of time period and treatments					
T1	TP1	$0.22^{f} \pm 0.02$	$0.10^{b} \pm 0.02$	$0.17^{d} \pm 0.09$	$0.08^{d} \pm 0.04$
	TP2	$0.26^{ef} \pm 0.03$	$0.28^{ab} \pm 0.21$	$0.93^{c} \pm 0.09$	$0.31^{bcd} \pm 0.06$
	TP3	$0.34^{def} \pm 0.03$	$0.13^{b} \pm 0.00$	$0.85^{c} \pm 0.02$	$0.29^{cd} \pm 0.12$
	TP4	$0.27^{ef} \pm 0.02$	$0.10^{b} \pm 0.01$	$1.37^{bc} \pm 0.30$	$0.54^{abc} \pm 0.23$
T2	TP1	$0.38^{de} \pm 0.06$	$0.10^{b} \pm 0.00$	$1.20^{bc} \pm 0.20$	$0.31^{bcd} \pm 0.05$
	TP2	$0.56^{c} \pm 0.04$	$0.22^{ab} \pm 0.02$	$1.39^{bc} \pm 0.14$	$0.50^{abc} \pm 0.11$
	TP3	$0.72^{b} \pm 0.08$	$0.25^{ab} \pm 0.02$	$1.65^{b} \pm 0.28$	$0.77^{a} \pm 0.04$
	TP4	$1.20^{a} \pm 0.11$	$0.38^{a} \pm 0.03$	$2.30^{a} \pm 0.46$	$0.79^{a} \pm 0.06$

Table 7: The effect of time period and treatments and their interactions on FSH, LH and, Total and Free testosterone levels in male gilthead sea bream *S. aurata*.

(TP: Time Period, T_1: control treatment, T_2: Artificial photoperiod and water temperature treatment).

Mean value in the same column with different superscript are significantly different (Duncan's multiple range test at $P \leq 0.05$).

The histological sections.

Histological section of *S. aurata* testis before the beginning of the experiment. Testis showed many spermatogonia, sperm ducts not visible and few spermatocytes (Figure 1).

The effect of natural light and constant water temperatures on testis maturity stages (T_1 - Control)

During the experiment, this group got exposed to natural light and constant water temperature. The histological sections of *S. aurata* testis sampled throughout the experiment four times showed many spermatogonia, sperm duct not visible and few spermato-

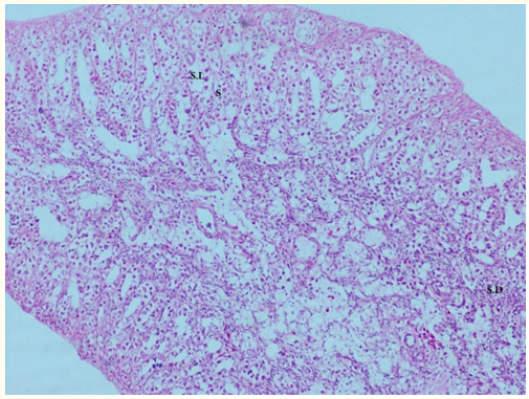

Figure 1: Histological section of *S. aurata* testis in immature stage (I). S.L: Seminiferous Tubules, S: Spermatogonia, S.D: Spermatic duct. H and E Stain. 4X.

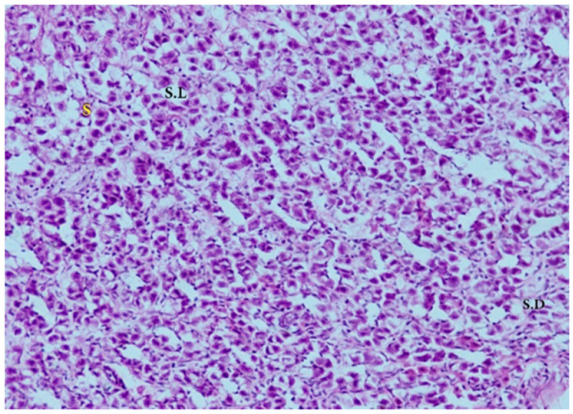

Figure 2: Histological section of *S. aurata* testis (Control) in immature stage (I). S.L: Seminiferous Labules, S: Spermatogonia, S.D: Spermatic duct. H and E Stain. 4X.

cytes in immature stage (I) (Figure 2). Some changes started from the third month when ovary tissues begin to formed and sex inversion was observed in one male after the third month and two males after the fourth month (Figure 3).

The effect of artificial photoperiod and water temperatures program on testis maturity stages (T_2)

Throughout the experiment this group exposed to artificial photoperiod and water temperature programs. The histological sections of *S. aurata* testis were sampled during the experiment four

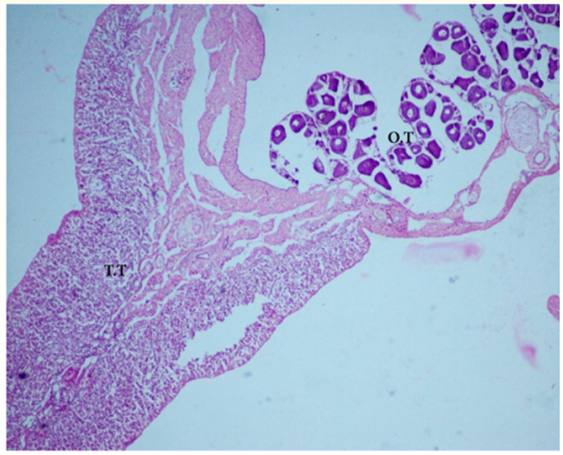

Figure 3: Histological section of *S. aurata* testis (Control) in immature stage (I) were ovary tissues begin to formed and sex inversion was observed. T.T: Testis tissues and O.T: Ovary tissues. H and E Stain. 4X.

times, the first histological section was in maturing stage (II) after 1 month (TP_1) when the water temperature reached 21 °C, showed spermatocytes increased in number with the spermatogonia and sperm ducts more visible than in immature stage (I) (Figure 4). The second histological section was in maturing stage (III) after 2 months (TP_2) when the water temperature reached 20 °C, showed primary and secondary spermatocytes more abundance and few spermatogonia and spermatids (Figure 5).

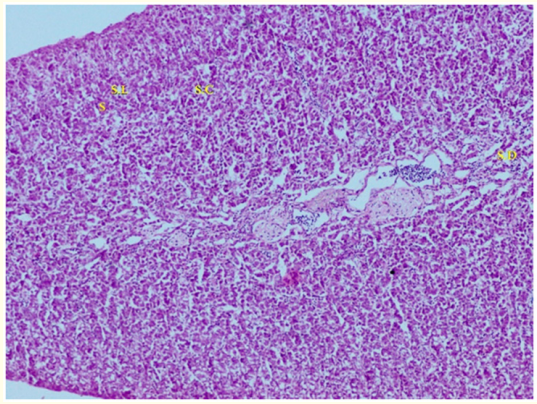

Figure 4: Histological section of *S. aurata* testis in maturing stage (II) at 21° C. S.L: Seminiferous Tubules, S: Spermatogonia, S.D: Spermatic duct, S.C: spermato cytes. H and E Stain. 4X.

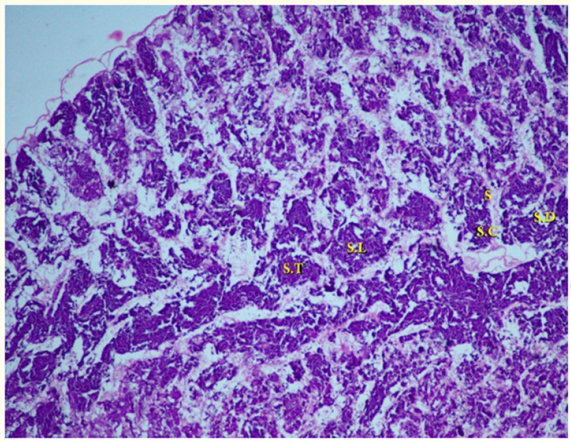

Figure 5: Histological section of *S. aurata* testis in maturing stage (III) at 20° C. S.L: Seminiferous Tubules, S: Spermatogonia, S.D: Spermatic duct, S.C: spermato cytes, S.T: spermatids. H and E Stain. 4X.

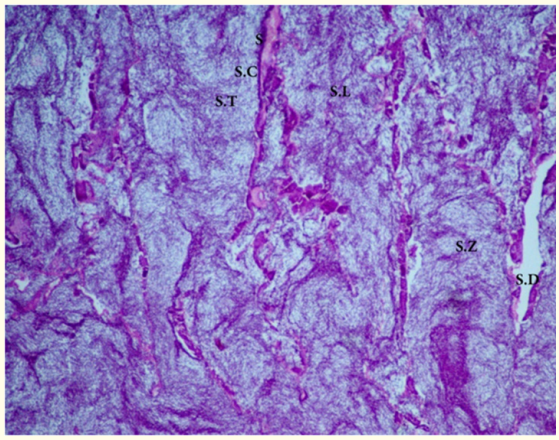

Figure 7: Histological section of *S. aurata* testis in maturing stage (V) at 18° C. S.L: Seminiferous Tubules, S: Spermatogonia, S.D: Spermatic duct, S.C: spermato cytes, S.T: spermatids, S.Z: spermatozoa. H and E Stain. 4X.

The third histological section was in maturing stage (IV) after 3 months (TP_3) when the water temperature reached 19 °C, it showed more spermatids than before and few spermatozoa and fewer spermatocytes (Figure 6). The fourth histological section was in maturing stage (V) after 4 months (TP_4) when the water temperature reached 18 °C, it showed more spermatozoa in the seminiferous tubules and spermatic duct than before, few spermatids and fewer spermatocytes present than before (Figure 7).

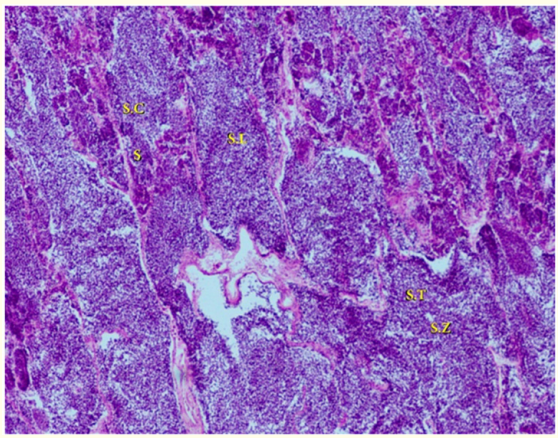

Figure 6: Histological section of *S. aurata* testis in maturing stage (IV) at 19° C. S.L: Seminiferous Tubules, S: Spermatogonia, S.D: Spermatic duct, S.C: spermatocytes, S.T: spermatids, S.Z: spermarozoa. H and E Stain. 4X.

Discussion

Controlled hatchery production of juveniles and fry of economically important finfish ensures a steady supply of quality fry for aquaculture operations. The control of spawning in captivity allows a better monitoring of the quantity and quality of eggs collected during or outside the normal spawning periods [34]. Induced spawning of fish in captivity can be approached by two methods, the control of environmental conditions and hormonal treatment [35]. There is lack of information on the spawning, gonadal development, hormonal levels and steps of oocyte maturation on gilthead sea bream, therefore we plan this study to investigate the effect of photoperiod, water temperature on gonadal maturation and the spawning induction of marine gilthead sea bream brood stock related to fish reproduction.

Fisheries science and management are quantitative description and relationship between length and weight of individuals in a fish population as basic tool for assessing the natural population known as Fulton condition or K factor [36,37]. K coefficient is affected by maturing of genital product. It is an index reflecting interaction between biotic and abiotic factors in the physiological condition of fish. It shows population welfare during the varies stages of the life cycle [38].

In the present study K factor was peaked in T_2 at TP_2 in male when spawning season approaching that related with high body weight (332.73 ± 27.72 g) and short total length (25.80 cm). This increasing in K factor may be attributed to the development of gonads that become mature. These results are agreed with [39,40].

There is normally a gradual increase in the condition factor during the reproductive period (T_2 at TP_2 in male). Condition factor doesn't relate the feeding condition of the adult stage but includes the state of gonadal development based on fat reserves during the spawning period [41].

Looking to table (6) it was observed that gonadal weight (g) and GSI (%) were peak at T_2 at TP_4. These were in agreement with [24] who showed monthly variation in GSI values and the distribution of different matury stages revealed that the spawning activity was continued from month September till month January when both light and temperature programs are used in our study. It is familiar that GSI increases with the maturation of fish being maximum during the period of peak maturity (January) then decline after that. These results are in agreement with [42].

Gonadal development, maturation stages and the changes in reproductive cycle in gilthead sea bream brood stocks may be attributed to the controlled artificial photoperiod and water temperature programs which triggered HPG axis to start and complete the reproductive cycle [43,44]. Increasing in gonadal weight and GSI observed in this study may be attributed to increasing the level of measured FSH and LH after the fourth month. Photoperiod has been the primary hint to HPG axis to start the reproductive cycle but it can't trigger this axis to complete the maturation, spermiation where the role and importance of temperature in the success of maturation and spermiation is very clear. These data demonstrated that water temperature program has the greater effect on maturation. These results are largely supported by the findings of [45] who reported that under artificial photoperiod and water temperature program for 4 months gilthead sea bream it reached to final maturation. The best result was recorded in fish group which exposed to 18° C then to 19° C and the lowest results in fish group exposed to 20° C. Relative interaction between environmental signs and endocrine control of reproduction were settled. The endocrine control cannot continue without the favorable environmental cues required to stimulate reproduction [46].

The gonadal development failure indicated by decreased gonadal weight and GSI which didn't differ from the start of the experiment till reach the fourth month in control group. These decreasing of these levels indicating the immature stage for testis. Histological sections confirmed the status of immaturity stages where many spermatogonia and few spermatocytes. Reproductive failure in fish is often the result of a block at specific level of hormonal cascada. The results are in agreement with [47] who reviewed that these gonadal steroid hormones drive the development and maturation of male that observed after the fourth month.

In male of artificial photoperiod and water temperature treatment, there was a gradual significant ($P \leq 0.05$) increase in FSH starting from the first month (TP_1) at the spermatogenesis stage to the fourth month (TP_4) and reached the peak 1.20 miu/ml. Increasing FSH levels accompanied with a gradual significant increasing on free and Total testosterone from the first month (TP_1) till reach peak level in (TP_4) 2.30 ng/ml. Total testosterone has an effect on primary spermatogenesis like spermatogonia and spermatocyte formation. The present results were in agreement with [48-51] who reported that in males, FSH levels are high at early spermatogenesis, increase to maximum levels during the rapid testicular growth phase and then decline after spawning. FSH exert more complex functions in the male testes, stimulating androgen production from the Leydig cells, as well, but also regulating Sertoli cell activity during spermatogenesis [52].

LH levels in male at T_2 (artificial photoperiod and water temperature treatment) increased significantly before spermiation from the second month (TP_2) to reach the peak in the fourth month (TP_4) 0.38 mIu/ml, which affect directly on final gametes maturation. LH affect directly free testosterone releasing where it reached the peak in the fourth month (TP_4) 0.79 Pg/ml which may have an important role in the development of secondary sexual characteristics and attaining with sex-specific reproductive behaviors. The results are agreed with [50,51] who noted that LH was low during early spermatogenesis, increases during spermiation and peaks during the spawning season, when LH induces a shift in the steroiodogenic pathway of the testes leading to the production of the MIS [52]. LH is mainly involved in the stimulation of androgen production in Leydig cells and stimulates the development of secondary sex characteristics in male [53].

The effect of hormonal administration in males under artificial photoperiod and water temperature programs were clearly affected on gonadal weight, GSI and histological sections. The first histological section of testis in maturing stage (II) when the water temperature reached 21° C, showed more spermatocytes in number with the spermatogonia and sperm ducts more visible than in immature stage (I). The second histological section in maturing stage (III) at water temperature 20 °C, showed more primary and secondary spermatocytes in abundance and few spermatogonia and spermatids. Our results are confirmed by the observation of [54]. The changes in gonadal development in the first and second sections affected by the level of FSH which is responsible for spermatogenesis. The third histological section of testis in maturing stage (IV) when the water temperature was 19 °C, showed more spermatids than before with few spermatozoa and spermatocytes. Sperm was extruded when light pressure was applied. The fourth histological section in matured stage (V) when the water temperature reached 18° C, showed more spermatozoa in the seminiferous tubules and spermatic duct than before. Our results are confirmed by the observation of [54]. Few spermatids and fewer spermatocytes present may due to the effect of high levels of LH and, total and free Testosterone as well as due to highest gonadal weight and GSI at TP_3 and TP_4.

In the present experiment, the controlled temperature of salt water of *S. aurata* ranged between 22 ± 1 for T_1 (control) and T_2, and 21.5 – 18°C by chiller system for T_3 and T_4 which are considered as the most suitable temperature for *S. aurata* reproduction. These range of water temperature was completely agreed with [10,11,45] who noticed the range of reproduction temperature was 16-19°C for *S. aurata*.

In the present experiment values of all water quality parameters were within the acceptable limits for reproduction of *S. aurata* as stated by [45,55], which mean that water parameters did not influence by the experimental treatments.

Conclusion

In the present study on male of Gilthead Sea bream, it could be concluded that exposing to artificial photoperiod and water temperature programs can be used to control the spawning season of gilthead sea bream to start before or after the normal season of spawning. Also, it could be possible to induce maturation in captivity when the water temperature is at 18 and 19 °C through the artificial photoperiod and water temperature programs to reach the final gonadal maturation and release sperm. Therefore, this is an important step towards the domestication and culture of *S. aurata*, which are good aquaculture potential species.

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