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INDUCE SPAWNING AND LARVAL REARING OF GILTHEAD SEA BREAM *(SPARUS AURATA)* COLLECTED FROM FISH FARMS, EGYPT

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ABSTRACT

Sparus aurata females in captivity were injected with human chorionic gonadotropin hormone (HCG), pituitary extraction and both them during the spawning season. First hormonal treatment was applied as cumulative doses required for successful ovulation ranged from 4500 to 7500 IU\fish, the maximum fertilization percent was 71. The ovulation period under the influence of this treatment extended up to 14 days. In the second experiment, the total dose requirement for successful ovulation ranged from 2 to 4 pituitary extractions. /fish and the maximum fertilization percent recorded was 81, while the longest ovulation period obtained was 15 days. In the third experiment the total doses required for successful ovulation ranged from 2 pituitary extractions and 4500 IU to 3 pituitary extraction and 7500 IU/fish. The maximum fertilization percent recorded was 88, while the ovulation period was extended up to 23 days. The combined treatment gave the highest value of fertilization percent and the longest ovulation period. Moreover, the results from the combined treatment showed the highest value of hatching percent of 83.5 when compared to those of HCG was 70.5 % or pituitary extraction was 80%. The maximum ovulation and fertilization percent was recorded at temperature ranging between 10 and 16°C. The survival percent of *Sparus auratus* larvae up to 10 days old after hatching were greatly affected by both temperature and photoperiod. The proper temperature ranged between 17 – 22 °C recording the highest survival percent 29 at natural photoperiod (8 h. light), while it increased to35% under continues illumination. The larvae starts feeding with rotifer at third day old were measured 0.2 mm. At 11th day old the post larvae measure 3.1 mm. and being reared to accept next feeding regime represented by *Artemia nauplii*.

1. INTRODUCTION

Gilthead sea bream (*Sparus aurata*) is amongst the most important fish species cultured in the Mediterranean region (Oliva, 2000). In natural environment of the eastern Mediterranean, *Sparus aurata* breeds once a year during a six-week period from the middle of December till the end of January (Zaki, 1984). In captivity this species does not spawn spontaneously as reported by Arias (1976) and Villani (1976). The success of reproduction largely depends upon the complex interactions taking place along the hypothalamus-pituitary gonads axis. (Hassin, 1991). Zaki and Abdel Rahman (1985) found that, when water temperature was raised above 20°C gonads were resorbed in *Sparus aurata*.

The hormonal approach is presently more feasible for the induction and synchronization of ovulation and spawning in commercial hatcheries as reported by Zaki (1984) and Zohar (1989). The use of pituitary hormones for the induction of spawning in fish has been

established for many years (Donaldson and Hunter, 1983). Zaki (1984) reported that the exogenous gonadotropin administration to female *Sparus aurata* in captivity induced maturation, ovulation and spawning of oocytes, therefore enabled the onset of a daily spawning rhythm. Ovulation and spawning in *S. aurata* have been induced by means of HCG treatments (Lumare and Villani, 1973; Zaki, 1984 and Zaki *et al.*,1985). Rowland (1988) reported that, a combination of the pituitary gland extract of common carp and human chorionic gonadotropin HCG, induce ovulation in some females of Murray cod and Pecli. Zaki and Abdel Rahman (1985) concluded that, it is possible to obtain full ripe eggs from *S. aurata* during October (two months prior to the breeding season) by the injection with HCG. As for many cultured species, the successful culture of Gilthead sea bream, *S. aurata* depends on the improvement of both larval survival and growth. High losses at early larval stages for the first three weeks (6-7 mm S.L) may be attributed mainly to nutritional and environmental factors (Tandler and Mason, 1983). Johnson and Katavic (1986) reported that the aquaculture potential of many species is limited by their live-food requirements as larvae.

Freddi *et al*. (1981) studied the optimum salinity-temperature combination for the early life stages of *S. aurata* and found that, the optimum salinity range for egg incubation was 30-40‰ at 23°C while, that for the yolk-sac larvae remained at 15-25‰ at all test temperatures.

2. MATERIALS AND METHODS

Work was applied in marine hatchery unit of National Institute of Oceanography and Fisheries, Alexandria, Egypt. *S. aurata* were collected from fish farms at Dammiatta, Egypt by means of surround nets. Only good individuals were selected. Circular holding tanks with a maximum capacity of 3,000 liters of sea water were used for the brood stock. Each tank had both fresh and sea water inlets, with regulators and independent large drainage system. The sea water used was filtered with sandy filters and steriled using ultraviolet units. The sea water parameters were controlled: temperature of 10-16°C using an air conditioner; salinity of 35-38 ‰; pH of 7-8; dissolved oxygen from 0.8 to 1.0 ppm by using an air blower and water flow rate of one liter per hour by using inlet-outlet system. The experiment was preceded in a natural photoperiod. The sex ratio was two females to one male. A suitable food (shrimps) was supplied to the holding tanks

Spawning Process: Two hormones were used to induce spawning; the first was human chorionic gonadotropin hormone (HCG) and the second was carp pituitary extract. The fish were divided into different groups to evaluate the variations taking place under the effect of the previous treatment at two different temperatures. The hormones were injected either independent or in combination with each other to determine the effect of each hormone and the effect of both when injected together. Fish were divided into three groups:

Group (1): Fish were reserved as control group at temperature of 22 – 23°C.

Group (2): Fish specimens were reserved at temperature of 10-16°C.

Group (3): Fish were treated with the hormones according to the following seasonal periods in which:

Males were treated only with 1500 IU daily dose of HCG hormone for 1-2 days at temperature 10-16°C.

Females were treated as follows:

Pre-spawning season (November**):** Females were treated with 1500 IU daily dose of HCG for 5-7 days. Another group of females were treated with one carp pituitary extracted in one ml distilled water daily dose for 4-5 days. The previous two treatments were done at temperature of 10 -16°C.

Spawning season (December and January**):** In this season various treatments were applied:

HCG treatment: in which HCG hormonal dosage was applied in three treatments; the first was of 1,500 IU daily dose for 3-5 days while

the second was of 2,500 IU daily dose for 3-5 days. While the third was 5,000 IU daily dose of HCG was applied for 2-3 days.

Carp pituitary extract treatment: A group of fish received doses of pituitary extract in two treatments; the first was of 1 ml pituitary as daily dose for 2-4 days while the second treatment was 1 ml of pituitary extract for 4-6 days.

Combined treatment of HCG and pituitary extract: The combined dose of 1 ml pituitary extract and 1500 IU HCG was injected as daily dose. The total dose was 2-4 ml pituitary extract and 4500-7500 IU of HCG.

Post-spawning season (February**):** A group of fish received 1,500 IU daily dose of HCG for 5-7 days. Another group of fish was injected with 1 ml pituitary extract for 5-6 days.

Incubation and Hatching: After the ovulation and natural fertilization occurred, aeration of the spawning tanks was stopped and fertile buoyant eggs rise to the surface. Those were transferred by water outflow directly into the incubator units. Cylindrical incubators with a cone-shaped bottom were used. They have a minimal depth of 1.5 m to provide a long water column and a diameter of 1 m allows maximum circulation. Incubators were provided with running water system to supply a continuous flow of fresh sea water. These incubators were aerated by using air pumps through air-stones placed in the base of the cone producing small bubbles to prevent the egg damage. The outlet opening was provided with a phytoplankton net to prevent the eggs loss. Water temperature was regulated within a range of 22-24°C. Dissolved oxygen was maintained at saturation point. After 2-3 days of the incubation under the previous conditions, the hatching of fertilized eggs takes place.

Larval Rearing: The rearing unit consists of five rectangular tanks with capacity of two m^2. Rearing conditions were preserved at temperature of 11-28°C, salinity of 38 ‰ and photoperiod of 10-24 hours light. The tanks were supplied with air pumps and fresh sea water as a running system. Feeding of *S. aurata* larvae required phytoplanktons, zooplanktons and *Artemia salina* nauplii, which were collected and cultured under laboratory conditions.

Phytoplankton: The Chlorophyte *Chlorolla salina* was obtained from Seafadec Aquaculture department (Philippines).

Rotifer *Brachionus plicatilis* Muller (L. and S. type): were obtained from Aquaculture department, Philippine. *Brachionus plicatilis* were collected by sieving them through plankton net with mesh size more than 350 µm and were added to the larval tank directly.

***Artemia salina* :** Dry cysts of good strain of *Artemia salina* were obtained from Japan. This quality reached 95 hatching rate and the nauplii were very small. Each gram of cysts should yield 200,000 to 300,000 hatched nauplii. The nutritional value of the newly hatched nauplii is thought to be influenced by their lipid level and fatty acid composition. To increase the nutritional value of *Artemia* nauplii, they were fed on *Chlorella salina* before feeding larvae by 1 hr.

3. RESULTS AND DISCUSSION

Induced spawning of female *Sparus aurata* was studied under the condition of hormonal treatment and controlled temperature.

3.1. Human Chorionic Gonadotropin Hormone (HCG) Treatment:

Human chorionic gonadotropin hormone (HCG) is one of the most important means used to induce spawning of *Sparus aurata*. In the spawning season, *Sparus aurata* in captivity was treated by low dose (first and second HCG treatments) and another group of fishes were treated by high dose of HCG hormone at controlled temperature, each treatment gives a positive result. Lee *et al*. (1986) reported that the injection of exogenous gonadotropin has been traditionally used to induce the final maturation of captive female fish at the completion of vitellogenesis. The obtained results in each case were represented as shown in Table (1).

First treatment at spawning season with low dose (in cumulative dose of 4,500 - 7,500 IU) of HCG at temperature of 10-16°C recorded a fertilization percentage of 66-71 while, at temperature of 18-20°C the fertilization percentage was 25 - 55 (for a cumulative dose of 7,500 - 8,000 IU). Moreover, the second treatment gave fertilization percentage ranging from 19 - 66 at temperature of 10-16°C with cumulative dose of (7,500 - 10,000 IU). The ovulation process under the effect of first treatment started 1-2 days after the last injection and prolonged for 9-14 days under the temperature of 10-16°C. On the other hand the ovulation began 2 days after the last injection and prolonged for 7-9 days at temperature of 18-20°C. The ovulation in case of second treatment with low daily doses of HCG treatment at temperature of 10-16°C started 1-3 days after the last injection and continued for 4-7 days. Furthermore, when the *Sparus aurata* treated with high doses of HCG hormone the fertilization percentage was 43-61 (for cumulative dose of 10,000 IU) at temperature of 10-16°C. The ovulation started after 2-3 days from the last injection and prolonged for 5-11 days. In the pre-spawning and post-spawning seasons, low daily doses (1500 IU) of HCG hormone were applied under temperature of 10 – 16°C. The fertilization percentage obtained was 35-42 for pre-spawning and 30-35 for post-spawning. Moreover, the ovulation started after 2-4 days from the last injection and continued for 3-5 days.

From the previous results it is noticed that, the higher fertilization percentage was recorded at the spawning season of first treated groups when compared with groups of other seasons under the same temperature and injected doses. Gordin and Zohar (1978) and Zohar and Gordin (1979) reported that low doses of human chorionic gonadotropin (HCG), administrated to females *Sparus aurata*, in which oocytes were in the stage of vitellogenesis, resulted in the natural spawning of fertilizable eggs for a long period.

During the spawning season, the fertilization percentage of low daily doses treated groups was higher than those treated with high daily doses in the first treatment process at temperature of 10-16°C. Rowland (1983, 1984 and 1988) reported that the lowest species specific dosage of HCG required to induce all females to ovulate or spawn resulted in the highest hatchability of eggs, and the use of higher dosages resulted in lower hatchabilities and presumably less viable eggs. Also Ramos (1986) observed low egg fertilization rates in common sole induced to spawn with high doses of human chorionic gonadotropin (HCG), fertilization was higher with lower hormone doses used. Zohar and Gordin (1979) found that *Sparus aurata* treated with higher doses of HCG either did not spawn at all or spawned for 2 days only and gave poor quality of eggs. Also, in the first treatment process fertilization percentage recorded higher values at temperature of 10-16°C when compared with those at temperature of 18-20°C. Crim *et al*. (1983) reported that spawning of rainbow trout held at low rearing temperature was advanced by one month after pelleted LHRHa treatment. Also, Fovche *et al*. (1985) reported that by manipulation of temperature and day light period during late winter, it would be possible to induce spawning of *Cyprinus carpio* during this period. Zaki and Abdel Rahman (1985) found that when temperature was raised above 20°C all gonads were reabsorbed in *Sparus aurata*. Legendre (1986) demonstrated that, the largest egg diameter of *H. longifilis* was noticed when the water was coolest, and a rapid decline in oocyte diameters was observed as water temperature increases.

Table (1): Fertilization percentage of ovulated female *Sparus aurata* under the influence of Human chorionic gonadotropin hormone (HCG).

Treatment date	No. of fish		Hormonal treatment			Ovulation date	Fertilization percent	Temperature
	Female	Male	Daily dose	No. of doses	Total doses			
Pre-spawning season:								
06/11/1993	8	4	1,500 IU	5	7,500 IU	14/11/1993	35%	10-16 °C
13/11/1993	10	5	1,500 IU	6	9,500 IU	22/11/1993	37%	10-16 °C
20/11/1993	4	2	1,500 IU	7	10,500 IU	29/11/1993	42%	10-16 °C
Spawning season (low dose): 1st treatment:								
05/12/1993	18	9	1,500 IU	4	6,000 IU	10/12/1993	70%	10-16 °C
11/12/1993			1,500 IU	5	7,500 IU	18/12/1993	71%	10-16 °C
11/12/1993	12	6	1,500 IU	5	7,500 IU	17/12/1993	70%	10-16 °C
18/12/1993	24	12	1,500 IU	3	4,500 IU	22/12/1993	66%	10-16 °C
21/12/1993	22	11	1,500 IU	5	7,500 IU	28/12/1993	55%	18-20 °C
25/12/1993	8	4	1,500 IU	5	7,500 IU	31/12/1993	25%	10-16 °C
01/01/1994	16	8	2,000 IU	4	8,000 IU	06/01/1994	50%	18-20 °C
09/01/1994	14	7	2,000 IU	4	8,000 IU	15/01/1994	52%	18-20 °C
2nd treatment:								
04/12/1993	20	10	2,500 IU	3	7,500 IU	09/12/1993	66%	10-16 °C
26/12/1993	6	3	2,000 IU	4	8,000 IU	01/01/1994	21%	10-16 °C
17/01/1994	10	5	2,500 IU	4	10,000 IU	24/01/1994	19%	10-16 °C
Spawning season (high dose):								
03/01/1994	8	4	5,000 IU	2	10,000 IU	09/01/1994	61%	10-16 °C
18/01/1994	14	7	5,000 IU	2	10,000 IU	23/01/1994	59%	10-16 °C
22/01/1994	18	9	5,000 IU	2	10,000 IU	28/01/1994	43%	10-16 °C
25/01/1994	12	6	5,000 IU	2	10,000 IU	29/01/1994	58%	10-16 °C
Post-spawning season:								
05/02/1994	8	4	1,500 IU	6	9,000 IU	14/02/1994	33%	10-16 °C
12/02/1994	14	7	1,500 IU	5	7,500 IU	21/02/1994	30%	10-16 °C
19/02/1994	10	5	1,500 IU	7	10,500 IU	28/02/1994	35%	10-16 °C

On the other hand, the fertilization percentage was greatly reduced after the second treatment, while the total doses required in this treatment was greatly increased up to 10,000 IU /fish. But the longest ovulation period (21 days) was recorded under the influence of the first and second treatments at spawning season. The present findings confirm the previous results obtained by the some investigators interpreted the above statements such as Anon. (1977a and b) who reported that a resistance to HCG was developed in some Chinese carp species when spawned for several consecutive years. Also, Legendre (1986) stated that, mammalian high molecular weight substance (HCG) could provoke an immune reaction in some species, making it inefficient after repeated use.

It was found that the spawning season of *Sparus aurata* could be extended for a period of six months. This result was obtained under the influence of hormonal injection with HCG during pre-spawning, spawning and post-spawning seasons. The hormonal injection was applied at temperature range 10-16°C as cumulative low daily doses. Zaki and Abdel Rahman (1985) concluded that the injection with low dose of HCG in *Sparus aurata* having early stage gonads accelerates the development and maturation of the gonads and after cumulative suitable doses, ovulation occurs. So it is possible to obtain full ripe eggs from *Sparus aurata* during October (two months prior to the breeding season) by injection with HCG. Zohar *et al*. (1984) reported that the hormonal treatments and manipulation of environmental factors may advance or delay the reproductive season of sea bream and sea bass. Also, Legendre (1986) reported that the ovulation was induced in all *H. longifilis* females treated with HCG, regardless of season.

3.2. Pituitary Extraction Treatment:

In the present work, fish was induced for spawning by hormonal injection using pituitary extraction. Rowland (1988) reported that, different dosages of the pituitary gland preparation of common carp induced ovulation of all injected *Maccullochella peeli* females.

The results demonstrated in Table (2) showed that, the recording fertilization percentage of ripe ova during the spawning season ranged between 69 and 81 for the first treatment with cumulative dose ranged from 2 to 4 pituitary extraction and the maximum fertilization percentage recorded was 81, while the long ovulation period obtained was 15 days at temperature between 10 – 16 °C. In the second treatment with pituitary extraction, the percentage of fertilization ranged between 40 and 48 with cumulative dose ranged from 4 to 6 pituitary extractions.

Furthermore, it is clear that, the total ovulation period extension recorded the maximum value of 20 days under the influence of the first and second treatments. In the pre-spawning season the fertilization percentage ranged from 39-45 at temperature of 10-16°C. and the ovulation started after 1-3 days from the last injection. In the post-spawning season, the fertilization percentage ranged from 32 to 36 at temperature 10-16^{o}c and the ovulation began 1-3 days after the last injection and continued for 4-5 days.

From the previous results, it is noticed that, the fertilization percentage recorded maximum values under the influence of lower doses of pituitary extraction during the spawning season as first treatment, when compared with the two other seasons. It is confirmed by the previous results of Jalobert *et al*. (1978) in *Macquana ambigua*; Khalil (1988) in *Clarias lazera*; Suzuki *et al*. (1988) in goldfish and Rowland (1983) in *Macquaria ambigua* by using extract of dry pituitary gland.

Table (2): Fertilization percentage of female *Sparus aurata* under the influence of pituitary extraction.

Treatment date	No. of fish		Hormonal treatment			Ovulation date	Fertilization percent	Temperature
	Female	Male	Daily dose of Pit.extract	No. of doses	Total dose of Pit.extract			
Pre-spawning:								
21/11/1992	10	5	1 ml	4	4 ml	27/11/1992	39%	10-16 °C
22/11/1992	16	8	1 ml	5	5 ml	30/11/1992	45%	10-16 °C
Spawning:								
1st treat:								
01/12/1992	22	11	1 ml	2	2 ml	05/12/1992	75%	10-16 °C
02/12/1992	16	8	1 ml	3	3 ml	07/12/1992	81%	10-16 °C
05/12/1992	18	9	1 ml	3	3 ml	09/12/1992	78%	10-16 °C
11/01/1993	10	5	1 ml	3	3 ml	16/01/1993	69%	10-16 °C
03/01/1993	20	10	1 ml	3	3 ml	08/01/1993	61%	18-20 °C
10/01/1993	18	9	1 ml	4	4 ml	15/01/1993	70%	18-20 °C
2nd treat:								
02/01/1993	10	5	1 ml	4	4 ml	08/01/1993	48%	10-16 °C
09/01/1993	14	7	1 ml	5	5 ml	15/01/1993	40%	10-16 °C
30/01/1993	12	6	1 ml	6	6 ml	07/02/1993	42%	10-16 °C
Post-spawning:								
06/02/1993	12	6	1 ml	6	6 ml	14/02/1993	33%	10-16 °C
07/02/1993	8	4	1 ml	5	5 ml	15/02/1993	32%	10-16 °C
13/02/1993	14	7	1 ml	6	6 ml	20/02/1993	36%	10-16 °C

The second treatment during the spawning season gave minimal values of fertilization percentage when compared with the first treatment. Tamaru *et al*. (1989) who reported that, a small percentage of *Mugil cephalus* females which became mature earlier than others can be induced to become mature once more again. Such females can be spawned a second time within the same season by hormonal administration.

In the first treated group, the higher values of fertilization percentage were obtained at temperature of 10-16°C when compared to the values obtained at temperature of 18-20°C during the spawning season. Alvaraza *et al*. (1991) reported that the highest fertilization rate in female *Mugil laza*, injected with pituitary extraction was 60. Only 9 of the fertilized eggs hatched after incubation at temperature of 24°C. It is clear that, higher total doses needed at elevated temperatures could be due to the rapid clearance of inducing hormone from the blood after injection. Cook and Peter (1979) interpreted this statements and reported that the poor success in the hormonal induction of a number of carp species, may in part, be attributed to the rapid clearance of hormone from the blood after intraperitoneal administration into fish at warm temperatures.

Treated groups of *Sparus aurata* at temperature of 10-16°C were induced to spawn with higher total doses during the period from October to November (2 months before the spawning season) and during the period from February to March (2 months after the spawning seasons). Lee *et al*. (1986) concluded that the maturation and spawning of milk fish in tanks can be induced and accelerated 1-2 months earlier than the beginning of the normal spawning season by hormonal implantation. Zaki and Abdel Rahman (1985), who reported that the dose requirement for spermiation or ovulation in the breeding season of *Sparus aurata* is less than that required two months earlier.

3.3. Treatment with HCG Hormone and Pituitary Extraction:

The female *Sparus aurata* was treated with both HCG hormone (1,500 - 2,500 I.U.) and pituitary extraction (1 ml) as a daily dose during the spawning season as recorded in Table (3). The total cumulative dose of HCG hormone ranged between 4,500 - 7,500 IU and pituitary extraction ranged between 2-4 ml. The fertilization percentage values ranged from 67-88 which represent the higher fertilization percentage compared with those obtained from the injection with either HCG or pituitary extraction individually. The ovulation began after 1 day from the last injection and prolonged for 10-23 days. It is considered to be the longest period of ovulation that recorded in the present work. These results showed the effect of both HCG hormone and pituitary extraction treatment on the fertilization percentage and ovulation period of *Sparus aurata* in the spawning season at temperature (10-16°C). Clemens and Sneed (1959) and Sneed and Clemens (1959) reported that the induced spawning in hatchery fish by the pituitary has many advantages and the use of human chorionic gonadotropin enhances these advantages. Similar findings obtained by Rowland (1988) who found that the combination of HCG and a preparation of pituitary gland of common carp induced ovulation of some Murray cod females. Kulikova and Gnatchenko, (1987) reported that the injection of 10,000 to 80,000 IU of chorionic gonadotropin induced only minor pre-maturation changes in the vitelline oocytes. However full maturation occurred in striped mullet females treated with pituitary extract before injection of gonadotropin.

Table (3): Fertilization percentageof female *S. aurataunder* under the influence of both Pituitary extraction and human chorionic gonadotropin hormone at temp. (10-16).

Treatment date	No. of fish		Hormonal treatment						Ovulation date	Fertilization percent
			Daily dose		No. of doses		Total cumulative dose			
	Female	Male	Pit. ex.	HCG	Pit. ex.	HCG	Pit. ex.	HCG		
Spawning:										
11/12/1992	22	11	1 ml	1,500 IU	3	3	3 ml	4,500 IU	20/12/1992	85%
12/12/1992	16	8	1 ml	1,500 IU	2	4	2 ml	6,000 IU	18/12/1992	67%
19/12/1992	8	4	1 ml	1,500 IU	2	5	2 ml	7,500 IU	26/12/1992	78%
16/01/1993	20	10	1 ml	1,500 IU	4	3	4 ml	4,500 IU	24/01/1993	81%
22/01/1993	14	7	1 ml	1,500 IU	2	5	2 ml	7,500 IU	30/01/1993	83%
24/01/1993	10	5	1 ml	2,500 IU	2	3	2 ml	7,500 IU	30/01/1993	88%

3.4. Hatching and survival percentage of fertilized eggs and larval growth of *Sparus aurata*:

The hatching percentage of fertilized eggs was studied under the influence of treatment of spawners with HCG, pituitary extraction and combined treatment of both HCG and pituitary at 23°C as incubation temperature during the spawning season. From Table (4), the results showed that, hatching percentage recorded an average of 83.5 for treatment with pituitary extract and HCG. This percentage was considered the highest value recorded when compared to HCG treatment that gave 70.5 and to the pituitary treatment that gave 73. The present study shows that, the embryo hatched in about 3 days after fertilization, and measured about 2 mm. Eye pigmentation appeared on the fourth day after hatching and the mouth opening appeared one day after that. This is in agreement with Barnabé (1976), Zaki (1984) and Zaki and Abdel Rahman (1985). The survival percentage was studied under the influence of three different temperatures, 11-16°C, 17-22°C and 23-28°C as recorded in Table (5). After 10 days from hatching it was obvious that, the survival percentage values varied owing to the change of temperature. The results showed that, the higher survival percentage 29 was recorded at temperature of 17-22°C as shown in Table (5), where it was 20.5 at temperature of 11-16°C, and 10 at temperature of 23-28°C under constant photoperiod (8 hours light). When photoperiod increased into 24 hours the survival percentage increased to 35 as shown in Table (5). The present study demonstrated that the survival

percentage of *Sparus aurata* larvae up to 10 days post-hatching was greatly affected by temperature variation. The proper temperature ranged between 17 to 22°C recorded the highest survival percentage 29 under natural photo-period (8 hrs). This is supported by rearing procedures for gilthead sea bream described by Barnabé (1976), who recommended the rearing temperature ranging from 16 to 18°C for first 10 days after hatching and 18-21°C for the later period. Person-Le-Ruyet and Verillaud (1980), in Laboratory-scale experiments with gilthead sea bream, used a temperature of 19 ±1°C for entire rearing period up to 25 days, the mean survival percentage in these experiments was 11 with a maximum of 67. Ortega *et al*. (1983) used a temperature range of 17-19°C for first 30 days. The survival percentage in these trials was 27-30. The present results concerning the effect of photoperiod on larval rearing of *Sparus aurata* up to 10 days old; have shown that better survival percentage 35 was obtained under continuous illumination than with 8 hrs photoperiod. This is in agreement with the results of Laurence (1977), who proposed a direct relationship between the duration of day light and survival of *Pseudopleuronectes americanus* larvae. A similar positive effect of a long photoperiod on survival of marine fish larvae *Nautichthys oculofasciatus* was also reported by Marliave (1977). According to these findings, Tandler and Mason (1983, 1984) suggested a causative relation between improved chances of successful hunting for food by these larvae and their survival. Concerning the larval growth, in the third day after hatching, the larvae measured 2.0 mm total length, the mouth is formed and the larvae ready to feed. The external feeding started before the complete resorption of yolk sac. These results were in agreement with those of Zaki and Abdel Rahman (1985) and Kissil (1991). The yolk sac was completely absorbed at the 5th day after hatching. Similar results have been reported for *Solea* species (Ramos and Roures, 1983; Dendrinos, 1986; Zaki and Hamza, 1986 and Dinis, 1992). The larvae start feeding with Rotifer *Brachionus plicatilis* by adding density of 2-3 organism/ml of water. The density is increased from 3-5 organism /ml at the end of third day to 5-10 organism/ml at the tenth day old, in this age post larvae increased in length into 3.0 mm. In order to serve as a food of rotifer, the microalgae *chlorella salina* is added to the rearing tanks. These algae increased the oxygen concentration of water and decreasing the concentration of ammonia in these tanks. By the 11 Th day old, the post larvae measured 3.1 mm as total length and being ready to accept the next stage of feeding regime represented by *Artemia nauplii*. The supplying rate of this organism as a food is less than 0.2 organism /ml of post larvae whose length measured 4.4 mm at the 15Th day old to 0.5 organism/ml at 30 days old post larvae with total length of 9.0 mm. by increasing the density of organism to 1.0 organism/ml the fry length increased from 10 mm at 35 days old to 12 mm at 45 days old as reported in Fig (1).

Table (4): Hatching percentage of Sparus aurata in relation to different types of hormonal treatment during spawning season.

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Hormonal treatment	Hatching percent		
	Minimum	Maximum	Mean
HCG	68%	73%	70.50%
Pituitary	68%	78%	73.00%
Pituitary + HCG	80%	87%	83.50%

Table (5): Survival percentage of Sparus aurata larvae at different temperatures and photoperiod.

Period after hatching in days	Average survival percent.			
	11 - 16 °C	17 - 22 °C		23 - 28 oC
	8 hrs light	8 hrs light	24 hrs light	8 hrs light
1	100%	100%	100%	100%
2	90.50%	95.00%	95.00%	90.80%
3	85.00%	89.30%	89.50%	80.00%
4	81.00%	85.00%	86.50%	72.20%
5	70.40%	75.40%	76.00%	63.00%
6	64.00%	70.00%	69.00%	49.00%
7	48.00%	62.10%	63.00%	32.10%
8	42.30%	53.20%	56.50%	25.00%
9	30.00%	45.50%	49.70%	18.00%
10	20.50%	29.00%	35.00%	10.00%

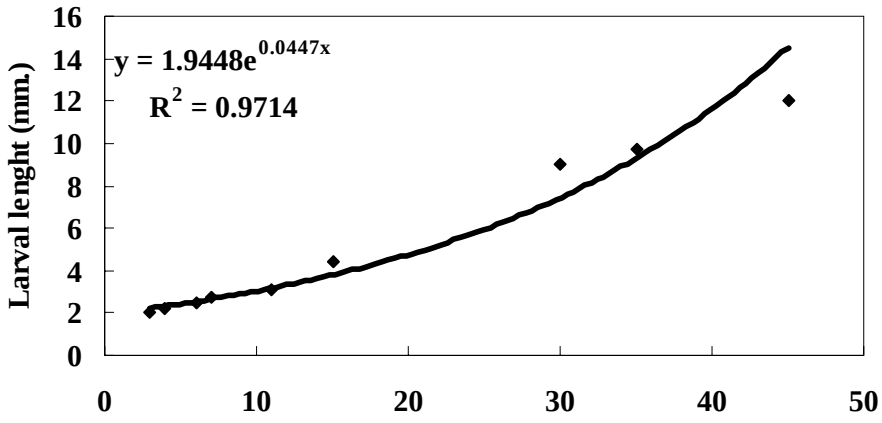

Fig. (1): Relationship between larval age and larval length in Sparus aurata.

4. CONCLUSION

It is obvious that the results obtained from the combined treatment represent the highest value of fertilization percentage and the longest ovulation period. Moreover, showed the highest value of hatching percentage when compared to those of HCG or pituitary extraction only. The larvae start feeding with rotifer at third day old after hating. At 11th day old, the post larvae measured 3.1 mm and being ready to accept the next feeding regime represented by *Artemia nauplii* with density less than 0.2 org./ml.

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REFERENCES

Alvareza, L.L.; Hernandez, M.O.G. and Fcrez, S.L.: 1991, Production de juveniles de la Lisa, *Mugil liza*, Valenciennes, 1836, por reproduction controlada en Cuba. *Ciencias-Marinas.* **17**(2): 47-56.

Anon.: (The Fukien-Kiangsu-Chekiang-Shanghai Cooperative Group for Artificial Reproduction of Fresh water Economic Fishes) 1977a, A further investigation on the stimulatory effect of a synthetic analogue of hypothalamic Luteinizing hormone releasing hormone (LRH-A) on spawning in domestic fishes. *Acta Biochem. Biophys. Sin.,* **9**: 12-25.

Anon.: (Cooperative Team for Hormonal Applicatin in Piscicuture) 1977b, A new highly effective ovulating agent for fish reproduction. Practical application of LH-RH analogue for the induction of spawning of farm fishes. *Sci. Sin.,* **20**: 469-474.

Arias, A.M.: 1976, Rerproduction artificielle de la daurada *Sparus aurata* (L.) Etud. Rev. Cons. Gén Péches Mediterr. *FAO,* **55**: 159-173.

Barnabé, G.: 1976, Rapport technique sur la ponte induite et l'èlèvage des larves du loup *Dicentrarchus labrax* (L.). Et de la dorade *Sparus aurata* (L.). Stud. Rev., *GFCM,* **55**: 63-116.

Clemens, H.P. and Sneed, K.E.: 1959, Spawning channel of catfish *Ictalurus punctatus* in aquaria by injection of fish pituitaries. V.S.D.I., Fish and Wildl. Serv.,

Fish Bull., **20**(3): 119-125.

Cook, A.F. and Peter, R.E.: 1979, The effect of temperature on the clearance of intraperitoneally injected gonadotropin in the goldfish, *Carassius auratus*, *Aquaculture*, **19**: 275-285.

Crim, L.W.; Sutterlin, A.M.; Evans, D.M. and Weil, C.: 1983, Accelerated ovulation by pelleted LHRH analogue treatment of spring spawning rainbow trout (*Salmo gairdneri*) held at low temperature. *Aquaculture,* **35**: 299-307.

Dendrinos, P.: 1986, Effects of temperature and salinities on yolk conversation efficiency and growth in larvae of Dover Solea, *Solea solea* (L.) *Thalassographica,* **9**: 7-29.

Dinis, M.T.: 1992, Aspects of potential of *Solea senegalensis* for aquaculture, larval rearing and artificial diet. *Aquaculture fish manag.,* **23**(4): 515-520.

Donaldson, E. M. and Hunter, G.: 1983, Induced final maturation, ovulation and spermiation in cultured fish. In: W.S.Hoar; D.J.Randall and E.M.Donaldson (Ed.), Fish physiology vol. IX: Reproduction, Part B, Behaviour and Fecundity control Academic Press, New York chap. 7, pp. 351-403.

Fouche, CH.; Vermaak,IE.; Vuren, JHJ. Van; Schoonbee, HJ. and Van-Vuren, JHJ.: 1985, The female reproductive cycle of the European common carp, *Cyprinus carpio* at a Tranvaa fish farm: gonadal merphometric development. *South African Journal of Zoology,* **20**(3): 172-176.

Freddi, A.; Berg, L. and Bilio, M.: 1981, Optimal salinity-temperature combinations for the early life stages of gilthead bream, *Sparus aurata* L. J. *World Mari. Soc.* **12**: 2, 130 - 136.

Gordin, H. and Zohar, Y.: 1978, Induced spawning of *sparus aurata* (L.) By means of hormonal treatments Ann. Bio. *Anim. Bioch. Biophys.,* **18**(4): 985-990.

Hassin, S.: 1991, The gonadal development in captive European seabass, *Dicentrarchus labrax*, and its hormonal control. M.Sc. Tel-Aviv University, George, S. Wise Faculty of Science, Zool. Dep. Israel.

Jalabert, B.; Goelz, F.W.; Breton, B.; Fostier, A. and Donaldson, E.M.: 1978, Precocious induction of oocyte maturation and ovulation in coho salmon, *Onchorhynchus kisutch*. *J. Fish Res. Bd. Canada,* **35**: 1423-1429.

Johnson Donald, W. and Ivan Katavic.: 1986, Survival and growth of seabass (*Dicentrarchus labrax*) larvae as influenced by temperature, salinity and delayed initial feeding. *Aquaculture,* 2 pp. 11 - 19.

Khalil, A. A.: 1988, Reproductive biology of *Clarias lazera* in Lake Manzalah. M.Sc. Thesis, Faculty of Science, Alexandria University.

Kissil, G.W.: 1991, Gilthead seabream, *Sparus aurata* In: Handbook of Nutrient Requirements of Finfish. R.R. Wilson (ed.). CRC Press., pp. 210-215.

Kulikova, N.I. and Gnatchenko, L.G.: 1987, Response of pre-spawning female Black Sea striped mullet, *Mugil cephalus*, to chorionic gonadotropin. *Journal of Ichthyology,* **27**(3): 44-53.

Laurence, G.C.: 1977, A bioenergetic model for the analysis of feeding and survival potential of winter flounder, *Pseudopleuronectes americanus*, Larvae during the period from hatching to metamorphosis. *Fish Bull.* **75**: 529-546.

Legendre, M.: 1986, Seasonal changes in several maturity and fecundity and HCG-induced breeding of the catfish, *Heterobranchus longifilis* val. (Clariidae), reared in Ebriè Lagoon (Ivory coast). *Aquaculture,* **55**: 20-213.

Lee, C.S.; Tamaru, C.S.; Banno, J. E.; Kelley, C.D.; Bocek, A. and Wyban, J.A.: 1986, Induced maturation and spawning of milt-fish, *Chanoschanos forsskal*, by hormone implantation. *Aquaculture,* **52**: 199-205.

Lumare, F. and Villani, P.: 1973, Maturita sessuale in dotta èfocondazione artificiale in *Sparus aurata* (L.) *Invest. Pesq. Parc.,* **37**(1): 57-71.

Marliave, I.B.: 1977, Effects of three artificial lighting regimes on survival of laboratory

reared larvae of the sailfin sculpin. Prog. *Fish Cult.,* **39**: 117-118.

Oliva-Teles A.: 2000, Recent advances in European sea bass and gilthead sea bream nutrition. *Aqua. Int.* V. (**8**) 477-492

Ortega, A.; Santaclla, E.; Carica, A.; Olmedo, M. and Peleteiro, I.B.: 1983, Cultivo de dorada, *Sparus aurata* L. En elcentro costero del mar menor duranta la temporada 1978-79. *Irf. Tec. Inst. Esp. Oceanogr.,* 5: 29.

Person-Le Rvyet, J. and Verillaud, P.: 1980, Techniques d'èlevage intensif de la daurade dorée *Sparus aurata* (L.) De la naissance a l'age de deux meis. *Aquaculture,* **20**: 351-370.

Ramos, J.: 1986, Induction of spawning in common sole (*Solea solea* L.) with human chorionic gonadotropin (HCG). *Aquaculture*, **56**: 239-242.

Ramos, J. and Roures, S.: 1983, Estudio del estado larvario post larvario del lenguado, *Solea solea* (Linn, 1758) (Pisces, Soleidae) Inf. *Tecn. Inst. Inv. Pesq.,* **110**: 3-14.

Rowland, S.J.: 1983, The hormone induced ovulation and spawning of the Australian freshwater fish golden perch, *Macquaria ambigua* (Richardson) (Percichlhyidae). *Aquaculture*, **35**: 221-238.

Rowland, S.J.: 1984, The hormone induced spawning of silver perch, *Bidyanus bidyanus* (Mitchell) (Teraponidae). *Aquaculture,* **42**: 83-86.

Rowland, S. J.: 1988, Hormone-Induced spawning of the Australian freshwater fish Murray cod, *Maccullochella peeli* (Mitchell) (Perichthyidae). *Aquaculture,* **70**: 371-389.

Sneed, K.E. and Clemens H.P.: 1959, The use of human chorionic gonadotropin to spawn warm-water fishes. *The progressive Fish-Culturist,* **21**(3): 117-120.

Suzuki, Y.; Kobayashi, M.; Nokamura, O.; Aida, K. and Hanyu, I.: 1988, Induced ovulation of the goldfish by oral-administration of salmon pituitary extract. *Aquaculture,* **74**: 379-384.

Tamaru, C.S.; Kelley, C.D.; Lee, C.S.; Aida, K. and Hanyu, I.: 1989, Effects of choronic LHRH-α plus 17-methyltestosterone or LHRH-α plus testosterone therapy on oocyte growth in the striped mullet (*Mugil cephalus*). Gen. and Comp. *Endocrin.* **76**(1): 114-127.

Tandler, A. and Mason, C.: 1983, Light and food density effect on growth and survival of larval gilthead seabream (*Sparus aurata* L., Sparidae) Proceeding of the Warmwater fish culture Workshop. Word Mariculture.. Soc., Spec., *Publ. Ser.,* **3**: 103 - 116.

Tandler, A. and Mason, C.: 1984, The use of 14C labelled rotyers (*Brachionus plicatilis*) in the larvae of gilthead seabream (*Sparus aurata)* measurements of the effect of rotifer concentration, the lighting regime and seabream's larval age on their rate of retifer ingestion. German-Israeli cooperation in aquaculture research. 2nd status seminar. *European Maricult. Soc. Spec. Publ.,* **8**: 241-259.

Villani, P.: 1976, Ponte indite et al evacye des Larves des poissons marins dans las conditions de Laboratorire. Etud. Rev. Cons. Gen. Peches Mediterr., *FAO,* **55**: 117-132.

Zaki, M.I.: 1984, Artificial breeding and embryonic development of *Sparus aurata* L. in Mediterranean Sea, *Egypt Problems Ichtyology. USSR.* **4**(3): 438-445, Russian.

Zaki, M.I. and Abdel-Rahman, S.H.: 1985, The induced spawning of the gilthead bream *Sparus aurata* and the rearing of its larvae. *Bull. Inst. Oceanogr. and Fish. A.R.E.,* **11**: 119-129.

Zaki, M.I.; Dowidar, M.N. and Abdala: 1985, Reproductive biology of *Clarias gariepinus* (Syn. Lazera) Burchell (Claridae) in lake Manzalah, Egypt. II. Structure of testes,FOLIA Morph., **33**(3): 307-313.

Zaki, M.I. and Hamza, A.K.: 1986, Reproductive biology and induced spawning of *Solea solea* (L.) In Egypt *Bull. Nat. Inst. Oceanogr. and Fish. A.R.E.,* **12**: 115-125.

Zohar, Y.: 1989, Endocrinology and fish farming aspects in reproduction growth and

smoltification *Fish Phys. Biochem.,* **7**: 395-405.

Zohar, Y. and Gordin, H.: 1979, Spawning kinetic in the gilthead seabrem *Sparus aurata* L. After low doses of human chorionic gonadotropin. *J. Fish Biol.,* **15**: 665-670.

Zohar, Y.; Billard, R. and Weil, C.: 1984, La reproduction de la daurada (*Sparus aurata*) et du bar (*Dicentrarchus labrax*): Connaissance du cycle sexual et controle de la gametogenese et de la ponte. Biolographic Citation, L'Aquaculture Du-Bar-Et-Des-Sparides. Barnabe, G.; Billard, R., eds, 1984, pp. 3-24.