Bull. Inst. Oceanogr. & Fish. ARE, 11 (1985): 119 - 129.

THE INDUCED SPAWNING OF THE GILTHEAD BREAM SPARUS AURATA AND THE REARING OF ITS LARVAE.

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ABSTRACT

The gilthead bream, **Sparus aurata** (L.), is one of the important cultured fish in Egypt. Recently, the catch of fingerlings from natural water has been dechined and a shortage of supply resulted. A programme to produce fingerlings by artificial propagation was started in 1981 at Alexandria Institute of Oceanography and Fisheries.

Significant success was achieved and gilthead bream could be induced to spawn by injection of human chorionic gonadotropin (HCG). Females caught before the breeding season (October),required about 11000 I.U. HCG, but those caught during the breeding season (December) required about 7500 I.U. HCG to release ripe eggs. Gonadotlopine requirement for spermation (in October about 6000) and about 2000 I.U. during breeding season (late December and beginning of January). Dose for spermation was less than that for ovulation but in the same manner.

Cultures of the hatched larvae were attempted in both running water and stagnant water systems. Survival in running water system with gentle water flow was found to be lower than that in the other system. On the 4^{th} day, the larvae started to feed on rotifers. Hence the rotifer, **Brachionus plicatilus** was cultured simultaneously on **Chlorilla** sp. and baker's yeast for feeding the larvae. On the 10^{th} day, larvae were fed on **Artemia** nauplii, and from the 15^{th} day onward they started to accept artificial powdered feeds.

INTRODUCTION

Gilthead bream, **Sparus aurata** (L.) is one of the important commercial marine fish in Egypt. This fish is highly esteemed also for brackishwater farming. Juveniles are collected from brackish shallows of lake Manzalah, along the Eastern Mediterranean Coast of Egypt, during the months of March to May.

In recent years, the fluctuating and capricious nature of the fry supply, made it difficult to find enough fry for stocking. Attempts to obtain fry

of gilthead bream by artificially induced spawning have been tried in various countries (Lumbar and Villani, 1971; Allesio and Bronzi, 1974; Arias, (1976). Although induced maturation has been achieved, the mass propagation results has not been good enough to initiate hatchery systems necessary to supplement juveniles to fish farms.

In Egypt, a programme of hypophysation experiments on gilthead bream was started in 1981 by the Institute of Oceanography and Fisheries at Alexandria. After repeated trials, the spawning of gilthead bream was induced successfuly by injection of human chorionic gonadotropin, but no fingerlings were produced (Zaki, 1984). Two main difficulties encountered in the mass propagation (1) the short period in which ripe fish specimens can be captured from the sea, usually from end of November to end of December and (2) mass mortality of the hatched larvae after the sixth day. To overcome these difficulties, it was necessary, therefore to try to prolong the breeding season of gilthead bream and to improve the methods of larval rearing.

This paper describes attempts to prolong the breeding season of gilthead bream by induction of ovarian maturation before spawning season with hormone injection and a successful method for the culture of its larvae.

MATERIAL AND METHODS

The experiments reported here were conducted during the period from July 1983 to April 1984, at El-Max Experimental Fish Farm, Alexandria, Egypt. This farm is situated about 3 km west of Alexandria harbour and is supplied with sea water and fresh water sources. The species **Sparus auratus** (L.), used in these experiments is known locally as "Denis". The specimens were caught alive by fishermen, they were collected and transported to the farm in thick plastic bags of 60 x 90 cm. One to three spawners were held in each bag, which was filled with 30 liters of sea water and then inflated with oxygen. The bags with fish were then taken ashore to the stock enclosures. Enclosures (6 x 2 x 1 m) were made of wooden frames lined with nylon net. These enclosures were fitted inside a brackish water fish pond. Salinity in the pond was ranging between 25 to 29‰ and temperature between 14° to 24°C during the experimental period. Ripe females varied in length from 28 to 34 cm, in weight from 290 to 530 g and fecundity from 17000 to 950000. These females are weighed, measured, injected and placed in the spawning tanks as early as possible in the morning. Eggs were collected from mature females by gentle stripping and the milt was obtained in a similar manner, then mixed in a plastic basin. Fertilization was carried out by the dry method. Incubtion and hatching of eggs were done in rectangular glass tanks of 20 l capacity and holdinng the eggs in one surface layer. Two systems were used, the flowing (running) water system and the static water system. In the flowing water system of hatching, small hatching nets were hung in the water, and a palstic pipe was used to supply a continuous upward

flow of fresh sea water so that the fertilized eggs roll calmly in the net. In the other system of hatching, the tanks wre aerated, and once the water became stale, it was changed. Sea water used for incubation and hatching of eggs had been pretreated by exposure to UV ligh, then inoculated with antibiotics (Penicillin G 4000 IV and Streptomycin sulphate 0.05 mg).

Rotifers were first obtained from the plankton of out-door brackish water ponds at El-Max Fish Farm, and then isolated in sterile water and fed on marine-type **Chlorella sp.** at a concentration of 4×10^4 cells/ml. Identification was made from Edmondson (1956) and kindly confirmed by Dr Amin Samaan.

Cultures were maintained in glass beakers (1 l cappacity) without aeration, at a temperature of 18 - 22°C. These cultures were used to inoculate 100 l gless tanks. Marine-type **Chlorella sp.,** and the flagellate **Chlamydomones coccides** Butch, cultured separately, were used to feed the rotifers at concentration of 2×10^6 and 3×10^4 respectively. Sometimes, when there was no sufficient amount of algal cultures, baker's yeast was used at a concentration of 1 g yeast per 1 million rotifers and prepared as described by Hirata (1980). When the rotifer density has reached 100 per ml, the cultures were harvested daily by removing 20 l and replacing with a fresh algal suspension. Temperatures varied between 16 and 20°C, salinities 13 and 15 %, and illumination was provided by sunlight entering through the laboratory windows. The rotifers were concentrated by means of a 60 um mesh net. **Artemia** nauplii were produced from eggs of San Francisco origin by a technique similar to that described by Riley (1966). The concentrated suspensions of nauplii were kept in 10 glass tanks, with vigorous aeration at room temperature until required.

RESULTS

Hormone Treatment

Two experiments were carried out to evaluate the effect of hormone injection on maturation of gilthead bream.

The first experiment was conducted as an attempt to prolong the breeding season of gilthead bream.

It is known that gilthead bream reach highest maturity during the period from beginning of December to middle of January (Wassef, 1979). Fish samples used in the first experiment were caught during the period from September 30th to October 5th when most of the females were found in the early maturity stage. After an acclimatization period of about two weeks in out-door ponds (Salinity 29-31 % and temperature 15 - 20°C), fish were divided into three groups : Group A (6 females + 4 males); Group B (4 females + 3 males); and Group C (4 females + 3 males).

Each female in group A was injected with comparatively low daily doses (1000 I.U.) of human chorionic gonadotropin (HCG). In Group B, each female was injected with initial high daily doses (5000 I.U.) of HCG for 3 days then followed by low daily dose (1000 I.U. HCG) until spawning. In Group C, each female was injected with daily dose of 1000 I.U. HCG + 50 mg vitamin E. In Group A, five females out of six ovulated after hormone treatment (Table 1) making a high percentage of 83 %. These females released ripe eggs after injections with cumulative doses varied between 10000 to 13000 I.U. HCG.

In Group B, two females released ripe eggs after one week during which each female was injected with cumulative doses ranged between 19000 20000 I.U. HCG. In Group C, two females released full ripe eggs after one week of daily injective with 1000 I.U. HCG. plus 50 mg vitamin E. The eggs of two females did not hatch, and cleavage in these eggs stopped after 24 h.

On the other hand, milt could be stripped easily from males after daily injection with 1000 I.U. HCG for 5 to 6 days. the spermation occurs after accumulative dose ranges from 5000-6000 I.U. HCG.

The results of this experiment showed that it is possible to obtain full ripe eggs from gilthead bream **Sparus aurata** during October by injection of fish with HCG, this means that the breeding season could be started two months earlier.

In the second experiment, females caught during the spawning season (end of December) were injected with either HCG only or combination of HCG and either pituitary gland extract or vitamin E (Table 2). In all cases it was noticed that at least 7500 I.U. of HCG in necessary to induce spawning in gilthead bream. The addition of either viatmin E or pituitary gland extract did not have any effect on the epawning of this fish. At least two injections were rquired, the first contained 2500 I.U. of HCG (about 30% of total dose) and the second 5000 I.U. of HCG.

It is clear that low daily does of HCG induced early mature females of gilthead bream to spawn after 10 to 12 days with hatching rate ranging between 30-50%. On the other hand initial high daily doses of HCG reduced the time required to complete ovulation to one week, but hatching rate was very low, varied between 5-15% and some of the eggs hatched were deformed.

The third experiment was done in the breeding season (End of December and beginning of January). Fishes were injected with HCG at least twice. The first contained 2500 I.U. HCG and the second 5000 I.U., HCG. In this experiment the temperature of water was raised, to about 24°C. All fishes, males and females, which were injected by the hormone did not give response and the gonadal resorbed.

TABLE (2)
Response of ovulated females of gilthead bream caught during breading season to hormone treatment, ovulation and fertilization. (First injection 24/12/1984).

Individual No	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	Total dosage	Date of Ovulation	Fertilization %	Remarks
					Injection						
1	1000 H	1000 H	1000 H	1000 H	1000 H	1000 H	1000 H	7000 H	31/12	70	
2	1500 H	1500 H	1500 H	1500 H	1500 H	-	-	7500 H	29/12	72	
3	2500 H	5000 H	-	-	-	-	-	7500 H	26/12	68	
4	1000 H	1000 H	1000 H	1000 H	1000 H	1000 H	1000 H	7000 H	31/12	74	
	+ 2p	+2p	+2p	+ 2p	+2p			+ 10p			
5	1500 H	1500 H	1500 H	1500 H	1500 H	-	-	7500 H	29/12	70	
	+ 2p	+ 3p						+ 6p			
6	1500 H	1500 H	1500 H	1500 H	-	-	-	7500 H	28/12	72	
			+ 2p					+ 2p			
7	2500 H	5000 H	-	-	-	-	-	7500 H	26/12	75	
8	3000 H	3000 H	1500 H	-	-	-	-	7500 H	27/12	77	
			+50V E					+ 50V E			
9	1500 H	3000 H	3000 H	-	-	-	-	7500 H	27/12	75	
	+ 50VE	+ 50VE	+ 50VE	-	-	-	-	+150VE	27/12	75	

H : HCG in I.U.

P : Pituitary gland in number, and VE: Vitamin E in mg.

TABLE (1)
Response of ovulated females of gilthead bream caught before breading season
to hormone treatment, ovulation and fertilization. (First injection 20 / 10 / 1984).

Individual	Number of injection	Single dosage	Total dosage	Date of ovulation	Fertilization %	Remarks
1 A	10	1000 H*	1000 H*	30/10/84	50	Hatched
2 A	11	1000 H	11000 H	31/10/84	53	Hatched
3 A	11	1000 H	11000 H	31/10/84	60	Hatched
4 A	12	1000 H	12000 H	1/11/84	56	Deformed embryos
5 A	13	1000 H	13000 H	2/11/84	75	Hatched
1 B	7	1 st 3X5000 H	19000 H	27/10/84	58	Low Hatching rate
		2 nd 4X1000 H				
2 B	8	1 st 3X5000 H	20000 H	28/10/84	66	Deformed embryos
		2 nd 5X1000 H				
1 C	7	1000 H	7000 H	27/10/84	65	Hatched
		50 VE**	350 VE			
2 C	7	1000 H	7000 H	27/10/84	68	Hatched
		50 VE	350 VE			

* H : Chorionic gonadotropin in I.U.

** VE : Vitamin E in mg.

This means that environmental factors such as temperature and light play an improtant role in the ovulation gonads of **Sparus aurata.**

Incubation and Hatching of Eggs :

The fertilized eggs by dry method were incubated and hatched in two types of hatching equipment: (1) flowing water type-very finely meshed hatching net hanging in the water was provided with a continuous slow flow of water and (2) non-flowing (static) water type-aquarium or round glass jars provided with adequate aeration. Hatching rate of eggs in the flowing water type was found to be higher (50 - 70 %) than that in the static water type (10 - 40 %). Incubation water salinity varied from 35 ‰ to 37 ‰ and temperature varied from 11°C to 20°C. The incubation time varied between 62 h and 30 h with an average of 36 h $\pm$ 2 h. Cleavage and embryonic development of the eggs were described in details in a previous paper (Zaki, M.I., 1984).

Rearing of the Larvae

The newly hatched larvae were planktonic, transparent and chromatophores were scattered throughout the whole body. Mouth and digestive tubule were not well-developed. They showed weak swimming activity with the posture of belly up and head down. The size of the hatched larvae varied between 2.01 - 2.08 mm. The hatched larvae did not like strong illumination but were attracted and tended to concentrate in places of low light intensity. The development of the larvae and their behaviour and feeds are shown in (Table 3). For rearing the larvae, both flowing water type and static water type were used. Survival rates for larvae reared in flowing water system was lower (5 - 10 %) than those for larvae reared in the static water system (20 - 50 %). The larvae should not be moved immediately after hatching, and sharp changes in water temperature and salinity should be avoided. Subsequently all rearing experiments of the larvae were carried out in stagnant water system.

"Green water", prepared by fertilizing natural sea water with 1 g of KNO_3, 0.01 g of $Na_2HPO_4 \cdot 12H_2O$, 0.005 g of $FeCl_3$ per litre and exposed to sunlight for 5 days was added to the larvae from the first day after hatching until the 6th day. This green water contained different kinds of dianoflagellates and diatoms at an estimated concentration of 20.000 cell/ml. Green water was used to keep the rearing water clean and it was observed also that the larvae feed upon these algae.

From the 4the day after hatching, the rotifer, **Brachionus plicatilis** cultured on marine-type **Chlorella** sp. and baker's yeast (Abdel Rahman & Zaki, 1985) was given to the larvae in addition to the green water. The density of the rotifers was maintained at a concentration of 200 to 250 organisms per litre. It was observed that the larvae fed on rofiers which were about 100 µ in length.

TABLE (3)
Food and development of larvae of gilthead bream.

Dayes after hatching	Total Length (mm)	Food size	Observation on the behaviour of the area
1	2.00-2.20	-	Week swimming ability with the posture of belly un and head down Mouth not developed.
2	2.10-2.22	G* (10-30 u)	More pigmentation was found on eyes and body Mouth was under development.
3-4	2.30-2.50	G.R.** (10-100 u)	Opening of mouth. Yolk was diminished, being 1/4 of its original size. Able to take food (greecn algae). 3 gill arch cen be seen.
5-7	2.70-2.90	G.R.	Digestive tuble was well developed. Moving up and down individually. Feeding was easy to be observed
8	2.80-3.01	R.	Complete disappearance of yalk. Critical period, the survival dropped suddenly. Gill filaments were well developmed.
10-15	3.20-3.80	R. A*** (100-350 u)	Finfold moved backmand. Strong phototoxic. Pigmentation were very clear on the body as black spots.
15	3.20	A.F. **** (460-500 u)	Starting swimming activity. Accept powdered food Strong phototoxis. Swimming in middle and lower levels.

* G: Green algae

** R: Rotifers

*** A: Artemia nauplii

**** F: Fish meal.

On the 10th day, newly hatched **Artemia naplii** were given to the larvae in addition to the rotifers. The larvae fed on **Aretmia naplii** after making on S-shape sudden movement. The quality, size and motility of the food were found to be important factors in this rearing technique. From the 15th day the larvae was able to feed an artificial powdered food (Fish meal).

DISCUSSION AND CONCLUSION

From our result it is clear that female **Sparus aurata** injected with low daily dose of HCG (1000 IU), after cumulative dose varied between 10000-13000 IU, Thus ovulotion commenced in about 83% of female. It is posible to obtain full ripe eggs from **Sparus aurata** during October (two month prior the breeding season) by injection with HCG.

According to colombo and Belvedere (1976), reported that reproduction is controlled by concerted interplay of neuroendocrine mechamism acting primarily along the hypothalamic-hypophysial gonadal axis and mediating both genetic factors and environmental influences. The hypothalmus appears to stimulat pituitary gonadotropes, since both androgens and gonadotropins induce spermation in male and regulate the last phase of oogenetic cycle in female. Crim et al (1973) have found that the sexual maturation in **Oncorhynechus tshawitsha** is associated with an increased secretion of gonadotropine which attains the maximum at the time of spawning. From the previous, evidet that our result is in agreement with colombo and Belveder (1976) with Crim et al (1976).

Then we concluded that the injection with low dose of HCG of early stage gonads of **Sparus aurata** accelerate the development and maturation of the gonad and after cumulative suitable dose, ovulation occur. In the males of **Sparus aurata** HCG requirment for spermation in October (two month earlier) was about 6000 IU and the dose for ovulation in the same time ranged between 11000-13000 IU. In the breeding season (Late December and beginning of January) the dose requirment for spermation was about 2500 IU and for ovulation was about 7500 IU.

Sundararaj et al (1972) reported that low doses of Salmon gonadetrepine maintain the ripe ovary and preventing follicular atresia, However single high dose induces ovulation at least stage yolky Oocytes. In gold fish, the gonadotropin requirement for vitellogenesis was greater than that for spermatogenes (Yamazaki and Donaldson, 1968). These results agree with our result that the dose for spermation was less than that for ovulation and the dose requirement for spermation or ovulation in the breeding season is less than that required two monthes earlier about the environmental condition for spermation or ovulation for **Sparus aurata.** It is clear that when temperature was raised above 20°C all gonads resorbed. Then from our absorvation we concluded that the most suitable temperature for spermation or ovulation ranged for 11-16°C.

"The green water" technique has been used successfully by Fujinura and Okamoto (1972) for the culture of **Macrobrachium rosenbergii,** and by several Japanese worker in the culture of Penaeid Prowns. According to Houde (1972) (Pelagic copepods), were probabaly ideal food for marine fish larvae.

From our result the most beneficial feeding regimes for **Sparus aurata** was as Follows: Green water containing different kinds of dinoflagellates and diatoms at an estimated concentration of 20000 cell/ml was used to feed early larval stage; and Rotifers at concentration of 200 to 250 organism/litre was used to feed the larvae at 6 days of age. On the 10th day of age, newly hatched Artemia was used to feed larvae. Fish meal powdered was used to feed larvae at 15th days old.
We conclude that the most successful method for larval culture are those using a mixed dietary regime together with the creation and stabilization of an ecological system.

ACKNOWLEDGEMENT

We express our sincere thanks to Professor A.A. Abu-El-Fotouh, Academy of Scientific Research, Cairo, who kindly supported this work. This work was supported by a grant from Academy of Scientific Research in Egypt.

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