*Bull. Inst. Oceanogr. & Fish. ARE, 12, 1986: 115 - 125.*

# REPRODUCTIVE BIOLOGY AND INDUCED SPAWNING OF *Solea solea*( (L.) IN EGYPT.

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## ABSTRACT

Experiments conoucted during 1985-1986 at Mex station, west of Alexandria to induce the flat fish **Solea solea** (L.) to breed under the effect of CGTH adiministered intramuscularly are described here. Hence sole is a multispawner fish, the spawning act takes place over a prolonged season extends from November to March with a peak on January - February and the emergence of eggs goes over a number of times.

Particulars of eggs laid, percentage of fertilization and hatching, the dose and number of injections are dealt with in this paper.

Successful spawning is a first record for the species in Egypt. It was achieved after the artificial propagation and the hatching of eggs thenafter the offsprings were reared.

The present work is also manifisting the optimum temperature of spawning which is 11° to 14°C. The incubation period lasted for four days and the complete transformation of larvae to fry stage took almost four weeks.

The success of induce breeding soles in laboratory is proposing a future work on the same line for economical purpose, in the field of aquaculture in Egypt.

## INTRODUCTION

The success of induced breeding of fishes in many countries initiated to breed some marine fishes of Egypt. The natural supply of fry is unpredictable and invariably comprises a mixture of different fish species. In order to get a steady supply of fish fry to new farms breeding experiments were conducted on **sparus aurata and Solea solea.** The first was successfully bred in the near past (Zaki, 1984) while the latter is the subject of this paper.

The importance of selecting **S. solea** for this work lies behind the following reasons:

1- The previous success in acclimatizing sole in different habitat (El-Zarka, 1963) and rearing of collected fry (Fabre-Domergue and Bietrix, 1905).

2- Being a bottom dwelling fish thrive on benthic organisms minimize the competition with other reared fishes for food or space.

3- High consumer acceptability which furnish a fairly well chance for producing sole on a large scale.

The present study aims at two main objectives which are:

a- Formulating a clear idea about the reproductive biology of **S. solea.**

b- Conducting induce breeding experiment on **S. solea** by hormone injection during the peak of the breeding season and to follow up the positive results.

The various aspects previously studied in the field of fish biology of **S. solea** in Egypt by El-Zarka, (1963); Ashour, (1971); El-Gharabawy, (1977) and Kerolus, (1977): helped in the preliminary knowledge required to conduct this work.

## MATERIAL AND METHODS

Fish material studied were collected and procured from lake Qarun, during the period from September 1984 to April 1986. This material, being breeders, ranged in total length between 180 to 280 mm; and 55 to 230 g in total weight. Fishes were transported in open tanks of 20 l capacity. The bottom of each tank was covered by a layer of sand about 5 cm. Lake water was then added in to the open tank to become half full. At most, ten fishes were allowed in each tank. A gentle flow of compressed oxygen was permitted to pass in the water via plastic hose inserted in the cover of the tank during the journey.

In the laboratary, fish material was recieved in fiberglass tank designed purposly for that experiment. The tank being 200 X 70 X 70 cm with continuous water flow arrangement. A layer of sand about 10 cm was kept on the bottom of the tank. The water column was maintained in the tank at 50 cm level. It was always regarded that accimatization tanks were provided with steady flow of water and air, in order to maintain suitable quality of water. The range of water temperature was 10 to 17°C in winter and 18 to 28°C in summer while the average salinity was about 35 ppt. The particulars of experimental technique adopted for induced breeding are explained later.

In nature, **S. Solea** feeds mainly on benthic organisms such as crustaceans and molluscs. However, during the acclimatization period in the tanks, one morning meal of minced shrimp was offered six times a week. The feeding rate was one tength of the total fish weight per tank per meal.

For biological analyses, about 60 fish were sampled monthly from the transported and acclimatized fish in the enclosures kept inside the ponds of Mex station. The following data were recorded: total length, total weight, weight of gonads, sex, maturity stages and egg diameter.

## OBSERVATIONS AND RESULTS

### 1- Biology of Solea Solea:

#### 1.a- Gonads Maturation;

The gonads of **S. soles** exhibit its maturation from May onward till October, thenafter, it goes ripening from November through December to January and ovulation takes place during January and February. The colour and size of gonads tend to indicate the maturation of **S. soles** which has a prolonged spawning season that extends annually from November to March.

Male fish has trianglar testes on both sides, upper and lower, testes are white yellowish in color and somewhat hard (Fig. 1). Female fish has ovaries on dorsal and ventral sides of the fish; ovaries are broad at the front tapering at the end. The ovaries increase in length and bridth with increasing size of fish (Fig. 2). The ovaries are golden yellow in colour, it turns from pinkish yellow to transparancy by the advancement of ripening.

Each ovary is sac like. Ovary membrane embodies the ovarian space and groups of ove with various diameters ranging from 0.25 mm to 1.00 mm. The ripe ova diameter during ovulation ranges from 0.80 to 1.08 mm while during maturation it ranged from 0.25 to 0.65 mm.

**Fig. (1)**
**Tests of Solea solea.**

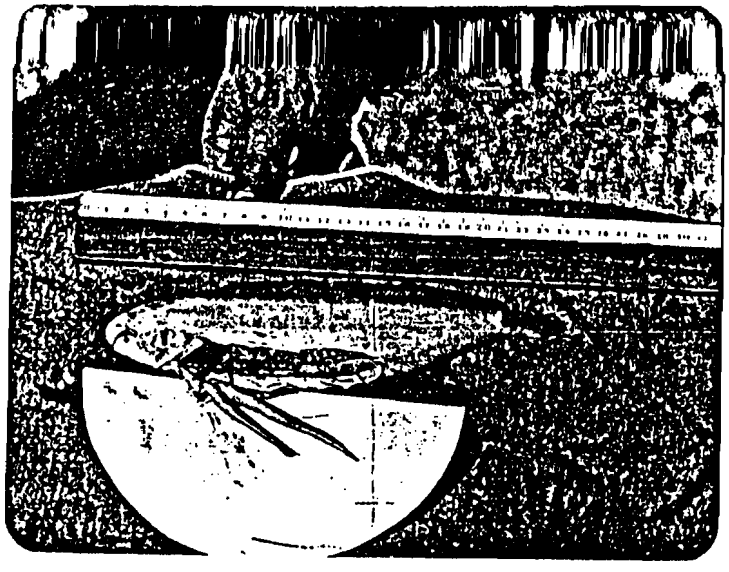

Fig. (2)
Ovaries of **Solea solea.**

**1. b – Gonadosomatic Index :**

Females of **S. solea** (153 specimen) were studied to relate the weight of ovaries to the total weight of the fish across successive months from September to April. The results are shown in Fig. 3. From this fig it is evident that GSI is higher during December (8.20) and lower during April (0.70). The first indecates the complete ripening of gonads while the latter reveals the final discharge of ovarian contents. It is also clear that **S. solea** has a prolonged spawning season extending from November to March with a peak during December / January.

**1. c – Fecundity :**

Table 1 shows the average length, weight and number of eggs for **S. solea.** The average total number of ripe eggs which studied during the breeding season were 54341 in average (33343 eggs for fishes of 190 mm length and 93786 eggs for 280 mm length). The number of ovulations or partial spawning varies from 6 times to 16 times according the total length and ovaries weight of the females. Apparently, there is a relation between the number of parts and the size of the female.

**1. d – Ova Diameter :**

The analysis of ova diameter was used in the present study (Fig. 4) to describe the frequency and the extent of spawning season of **S. solea.** The ovary at the maximum of the spawning act contains yolky ripe eggs with many oil globules. The ova vary in diameter from 0.8 to 1.08 mm. The eggs of 0.6 mm are unyolky and immature. The ferquency distribution of ova

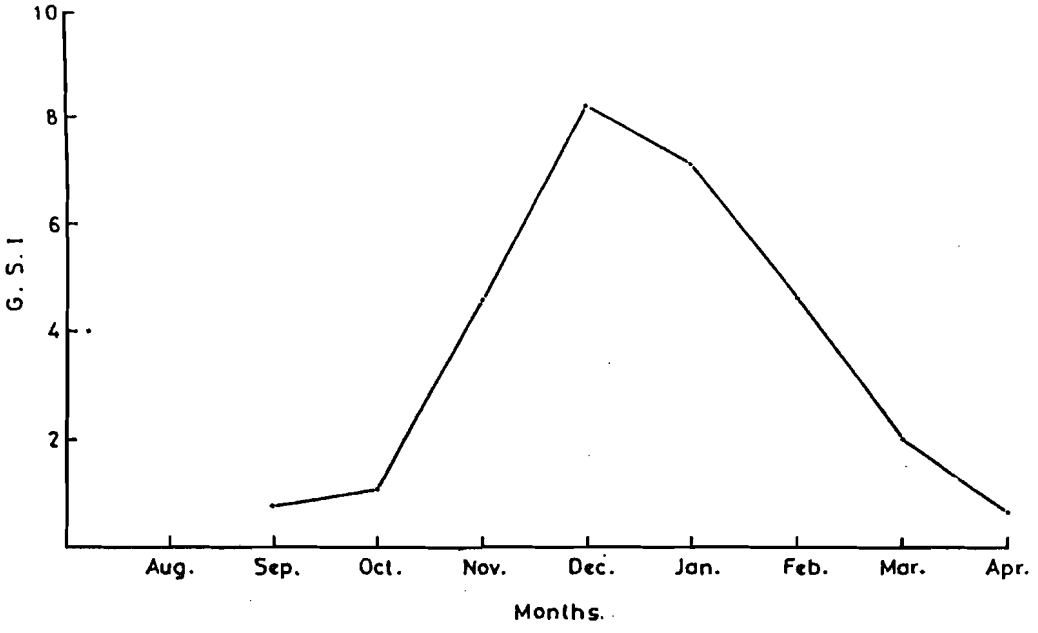


Fig. (3)
Gonado - somatic index of **Solea solea.**

TABLE 1
Average length, weight, number of eggs and number of egg layings for S. solea at Hex.

| Total length (mm) | Number of females | Average total weight (g) | Average number of egg layings | Average total number of eggs |
|---|---|---|---|---|
| 190 | 8 | 58 | 9 | 33343 |
| 200 | 15 | 73 | 14 | 46173 |
| 210 | 16 | 75 | 13 | 40969 |
| 220 | 17 | 89 | 14 | 54810 |
| 230 | 18 | 103 | 13 | 51308 |
| 240 | 14 | 126 | 14 | 65772 |
| 250 | 2 | 142 | 10 | 51243 |
| 260 | 4 | 165 | 7 | 45066 |
| 270 | 2 | 190 | 12 | 61940 |
| 280 | 4 | 230 | 16 | 93786 |

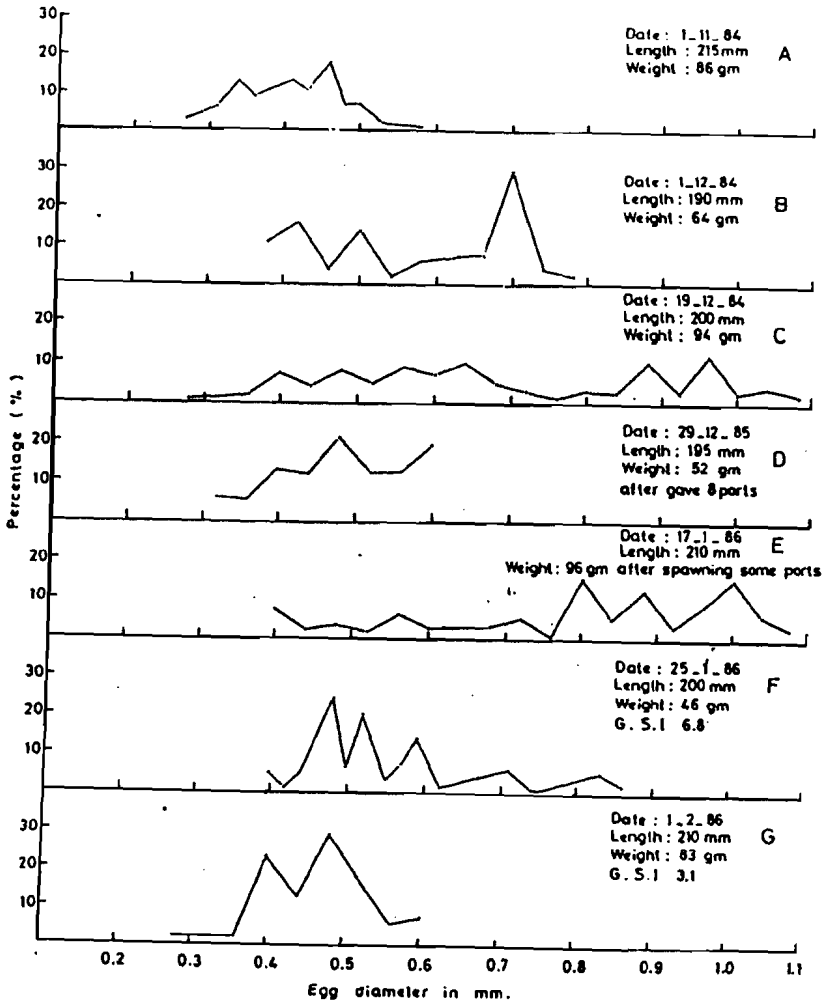


Fig. (4)
Ova diameter of **Solea solea**.

diameter for the fish under study during five successive months from October to February is shown in the figure. It is obvious that there is no sharp separation between the general egg stock and the ripe eggs but there is a continuous process of egg ripening. Moreover, the presence of more than two peaks or mode size of ova diameter points also toward the frectional spawning habit of **S. solea** within a prolonged spawning season which has been confirmed by the succession of maturity stages, ova diameter and gonadosomatic index. The spawning season is in coincidence with winter season during which lower values for temperature have been recorded.

2- Induced Breeding:

2.a- Induced Breeding Technique:

The total number of breeders used in these experiments was 72 males and 74 females ranging in total length from 190 to 280 mm and total weight from 50 to 240 g. A set of 70 fish of both sexes were kept as control to study their breeding without injection which proved to be negative. Two types of hormone were used for injection, the first is Synaborin 1500 IU combined with vitamin E (150 mg) this mixture was applied once only and the result was positive. The second is chorionic gonadotropin hormon (CG-T H) with potency of 1000 or 1500 IU. The total dose used of CG -T H varied from 2000 IU to 8500 IU. The frequency of injections applied to fishes varied between two to six times on weekly basis.

Ripe eggs were stripped by the delicate pressure of the thumb and forefinger on the abdomen of the female fish and the eggs were then recieved in enamel tray. The spawning takes place at the end of night time or at the break of day at the lowest temperature of the day. The dry method of artificial fertilization was followed. Water was added to cover the mixture of milt and riped eggs, then the sex products were thoroughly and lightly mixed together with the help of bird's feather. The mixture was rinsed with water number of times and sub-sample of fertilized eggs was taken to examine the success of insemination process. The fertilized eggs, bouyant and floating, were picked up and quickly transfered to glass basins of 40 X 30 X 30 cm supported by water flow. The water used was always biologically filtered and sterilized by UV unit in a tank from which a water flow system is running, with a salinity about 35 ppt.

In all cases, positive response was obtained in spite of the anatomical nature of tests in the males which sometimes called for adopting the so called Cesserian technique to collect the milt and sacrifice the fish while fishes kept as control with no injection did not spawn at all.

2.b- Dosages and Effect:

The experimental work has shown that all the fishes male and female have exhibited a positive response to hormone injection of different dosages either in number or in potency regardless of the fish size but depend on the ripening stage of gonad that the number of times in which eggs were laid increased with increasing size of fish and according size of ovaries. From table 2 the high number of eggs released may be a result of high dosages 8500 IU. resulted in (9470 eggs) which are used in case of the bigger fihes or may be attributed to the size of the fish.

The results show also that the optimum time for hormone injection is November-December which represent the peak of gonadal maturation, while the average dose is 4500 I.U. of CG T H and the average number of riped eggs is 50865.

**2.c- Incubation Period:**

The incubation period or hatching time in **S.solea** requires from 3 to 4 days. This differenc is attributed to the temperature of the medium or

the working range of temperature of the water in which eggs are incubated. Temperature, within limits affect the cleavage, in case of S. solea the working range is 11 to 17 $^{0}$C. Mostly the higher temperature leads to shorter hatching period.

**2.d- Viability of the Technique:**

The number of fretilized eggs has been calculated as subsamples after each experiment. The ratio of successful fertilization was 80% (as an average). Similarly the number of hatched eggs was counted and the ratio of survival among fertilized eggs was 90%. These figures express the high tendency of S. solea eggs to survive till hatching. Such observation may be supported by the fact that S. solea is a multispawner fish gives a fair chance to its progony to survive in nature.

**c- Devlopmental Stages:**

**c.1- Embryonic Stages:**

The cleavage process takes almost the first day. The first few steps takes much more time than other fishes. Balstodisc formation occurs during the first hour while cleavage of 2-cell stage, 4-cell stage and 8 to 16 cell stage takes place in the second hour.

Blastula stage, gastrulation and neurulation goes on during the first half of the first day while the complete C-shape embryo takes place by the end of the first day. During the second day, the head and tail become more differentiated, heart pulsation starts and the embryo develops more. On the third day the embryo becomes fully formed and the jerking and twicting movements are more apparent. The tail strokes increas and the embryo may emerge out by the end of third day or during the fourth day at most depending on the temperature. The hatching process is indicated by the heart and tail beats being more regular and frequent, the tail struck against egg's membrane causing its reupture. Head of embryo comes out first then shook off egg membrane by its jerking act till its complete freeness.

**c.2- Larval and post larval stages:**

Newly hatched larva has no mouth opening, has yolk sac, pigments and swim slowly. The hatchlings increase in size, while yolk decreases. Mouth opening starts to develop and the eye becomes shining and distinct. Yolk gradually disappeared and pigmentation regularly distributed. The larval stages take almost twenty days between hatching and the complete resemblence of adult. This period is quite enough for completion of different organs of the larval stage. S. solea larvae goes on its transformation to the be dorsoventrally flat fish during the 4th week of life.

## DISCUSSION

S. solea has a prolonged spawning season extends from November to March, with a peak during December and January. The fish is a multispawner shed its ripe eggs on instalments of 6 to 16 times according to the average size and fish weight. Sole in Egypt reach its maturity during the second year

of life and the average length of 19 cm was recorded by El-Gharabawy (1977) and Kerolus (1977) to be mature. The average number of riped egg per fish is almost 50.000 depending on the size of the female.

It is well known fact that most fishes do not breed in captivity that is why hypophysation is essential to overcome such difficulty. The hormone injections to some extent by pass the variables in the environment such as temperature, light and salinity; however the role of the above mentioned ecological factors is significant. The actual spawning is a reaction to some natural stimulus as defined by Nikolsky (1963). The fish discharges its ripe ova in batches during the spawning season and the with drawal of the eggs from the stocked egg to under go the maturation is a continuous process. The details of the spawning cycle and the embryological stages of **S. solea** are deseribed elsewhere (Zaki and Hamza, in press).

Various organic and nonorgonic genadotropins are in use to induce breed different fish species. Role of pituitary gland extract in the reproductive cycle has been reviewed by Atz (1957) and Atz and Pickford (1959). Collecting, preserving and applying, its extracts was also described by Chaudhuri (1976); Shehadeh (1976); Hamza (1980), Zaki (1980) and Zaki and Abdel Rahman (1986).

In the experiments described here, two types of injection were given to S. solea: Syndhorin with Vit. E. and Chorionic gonadotropin hormon (CGT H). The two methods gave a positive result. The first has a significant advantage for being used once only however, further study is needed for determining its viability, while chorionic gonadotropin hormone was used for the induced breeding of **S. solea** with different levels of potency. It was clear that neither the amount nor the number of doses has negative effect on the success of the breeding in the present case. Nevertheless, CGTH has been used effectively to induce spawning of the grey mullet (Kuo et al; 1973) but the estimation of the most suitable injection dose was very critical. This result hold true despite prolonged usage of hypophysation (Nash and Shehadeh, 1980).

Field and laboratory observations proved that spawning takes place at the end of night time or at the break of day during which temperature is recording its lowest values. This is character breeding of **Solea solea.**

The incubation period for eggs depends largly on temperature. Theoretically, there is a direct straight line relationship between temperature and incubation period (Apstein 1909). According to our observations, **S. solea** eggs hatched within 72 to 96 hr after fertilization. The temperature range was between 11° and 17°C.

From the present results it can be concluded that **S. solea** rearing should be carried out in salinity range of 32-35 ppt. and a sufficient supply of natural food of phytoplankton consist mainly of **Nitchia** spp. and **Navicula** and zooplankton of copepodes, cyclops and **Calanoid** spp.

According to Fabre-Domergue and Bietrix (1905) at Concarneau, France, could succeed in rearing the sole with food of cultured flagellates and plankton.

This result is in full agreement with our results that Pelagic marine larvae should feed on plankton, its size directely proportion with mouth size of larvae.

The successfull rearing of postlarvae till young or adult stage requires more attention toward the rearing conditions.

## ACKNOWLEDGEMENTS

The authors express their gratidude to Dr. A.F.A. Latif, vice president of Academy of Scientific Research and Technology, Egypt, and Dr. A.R. Bayumi, Director of Institute of Oceanography and Fisheries, for the opportunity accorded for this paper. The authors are deeply indebted to Prof. Dr. M.T. Hashem, Head of Fisheries Biology Department of IOF for scrutinizing the manuscript of this paper.

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