Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(6): 2175 – 2193 (2024)
www.ejabf.journals.ekb.eg

Aspects of the Potential Artificial Spawning and Reproductive Performance of *Solea aegyptiaca*

Safaa Aljilaney*, Esam Aiaad
National Institute of Oceanography and Fisheries, NIOF, Cairo, Egypt

***Corresponding Author: Elgelani2003@yahoo.com**

ARTICLE INFO

Article History:
Received: Dec. 4, 2024
Accepted: Dec. 23, 2024
Online: Dec. 28, 2024

Keywords:
Solea aegyptiaca,
Induced spawning,
Human chorionic gonadotropin,
Carp pituitary homogenate,
Larval production

ABSTRACT

The present experiments focused on induced spawning in *Solea aegyptiaca* using different hormones at varying doses compared to non-injected fish. Broodstock were collected from the 3rd depression of Wadi El-Rayan from December 2022 to March 2023. The experiments were divided into three groups. The first group of fish was intramuscularly injected with 4000, 5000, and 5200 IU HCG per kg of body weight, split into three trials. The second group was injected with CPH at 3.5, 4.0, and 4.5mg/ kg BW. The third group served as the control, receiving 0.9% saline water injections. This study compared the effectiveness of hormones on egg diameter, ovulation, fertilization, hatchability rate, and larval production. A total of 108 sole fish, with females weighing 55 ± 5g and males weighing 90.33 ± 1.25g, were used. Prior to primary injection, the oocytes were in the ripening period, with an average diameter of 600 ± 50µm. After hormone injections, egg diameters increased to a range of 927 ± 5.7 to 933 ± 12.2µm. The study showed that the longest spawning duration was 70 days for 28 batches of fish injected with 5000 IU HCG, while the shortest duration was 42 days for 8 batches of non-injected fish. The highest total number of fertilized eggs was 94,122 from fish injected with 5000 IU HCG, while the highest value from the CPH-treated fish was 30,506 at 4.0 mg CPH. The highest hatchability rate, 89%, was observed in fish injected with 5000 IU HCG, and the survival rate of metamorphosed larvae reached 71% at 30 days post-hatch (dph), decreasing to 25% at 60 dph. A significant difference ($P< 0.05$) was found during larval rearing. Based on these results, the best doses for induced spawning were determined to be 5000 IU of HCG and 4.0mg of CPH per kg of body weight.

INTRODUCTION

The demand for fish has increased in recent years due to population growth and the constant search for a healthy diet. On the other hand, natural fish populations have declined during the last several decades because of environmental degradation and over-fishing. Aquaculture in Egypt has been growing strongly in all regions of the country, mainly due to advances in the technical management of fish cultivation to cope with the increasing worldwide demand for fish as well as the economic and environmental sustainability of fish farming. This has resulted in an increased effort in the development

of techniques for hatchery production of fish. In addition to breeding other desirable fish species, there are a number of fish species that either do not reproduce spontaneously in captivity or have limited reproductive success in aquaculture settings. Reproductive biology plays a crucial role in determining productivity and, consequently, a population's resilience to exploitation by fisheries or to disturbances caused by other human activities **(Morgan, 2008)**. Hormones in aquaculture are used for artificial reproduction and sex reversal. The first sustains the production chain with the constant production of seeds. The second is used when the growth rate and/or gain weight are different between the male and female. This difference between genders is very common in teleost fish and usually occurs during puberty. According to **Rottmann *et al.* (1991)** and **Taranger *et al.* (2010)**, hormone-induced spawning is the only reliable method to induce reproduction in these fish. However, induced spawning can also be used to synchronize the reproduction of large numbers of fish for simultaneous spawning, thereby simplifying production and marketing, produce fry outside the normal spawning season to maximize hatchery production and to meet peak market demand, in addition to enhancing fry survival under controlled hatchery conditions. Additionally, hormones can be used during and outside the spawning season to improve reproduction **(Cejko *et al.*, 2009)**. Injected pregnancy hormone mimics the natural GtH produced. HCG may be more suitable because it acts much faster, by direct stimulation of the gonads, in stimulating final oocyte maturation, sperm, and ovulation. HCG is the common protocol to induce spawning. On the other hand, **Szedlmayer (1987)** reported an unsuccessful attempt to induce spawning with HCG outside the normal spawning season of weak fish, despite changing all conditions to mimic a natural spawning season. Many authors have reported that carp pituitary extract stimulates fish reproduction, resulting in improved fertilization rates, hatching rates, and larval growth and survival. Examples include *Cyprinus carpio* **(Abdel Hakim, 1998)**, *Liza ramada* **(Fahmy & El-Greisey, 2014)**, *Orthopristis chrysoptera* **(Natea *et al.*, 2017)**, *Sparus aurata* **(Badran *et al.*, 2019)**, and a hybrid of *Solea aegyptiaca* from Qarun Lake and *Solea vulgaris* from Bardawil **(Wafeek *et al.*, 2020)**.

Egyptian sole, *Solea aegyptiaca*, is a common type in the Egyptian coasts of the Mediterranean Sea. It is a flatfish (Order: Pleuronectiformes; Family: Soleidae). It has a high percentage of fat, which provides a lot of taste and nutritional value **(Vachon *et al.*, 2008; Bukovala *et al.*, 2012; Gabr, 2015)**. It is considered a promising species for economic aquaculture in Egypt. In the first decade of the 21st century, due to increasing salinity (up to 20%) in the third depression of Wadi El-Rayan, El-Fayoum, Egypt **(Konsowa & Abd Ellah, 2002a, 2002b)**, *S. aegyptiaca* was transplanted to the Lower Lake of El-Rayan. The production yield in the new environment was recorded at about 1.5 metric tons in 2021, as reported in the **Fish Statistics Yearbook (2021)**. Additionally, the reproductive characteristics during the natural spawning season of *Solea vulgaris* were studied by **Ashour (1971)**, while studies on *Solea senegalensi*s by **García-Lopez *et al.* (2006, 2007)** and **Guzmán *et al.* (2009)** provided valuable information on gonad

development and spawning. The reproductive characteristics of *Solea* species in Lake Qarun during the natural spawning season were studied by **Ashour (1971)** and **El-Hussiney (2001)**, as well as those of *Solea senegalensis* **(García-Lopez *et al.*, 2006a, 2007; Guzmán *et al.*, 2009)**. Their research provided valuable information on gonad development and spawning, focusing on the induction of final oocyte maturation (FOM), ovulation, and spawning processes that are often incomplete in captivity. Human chorionic gonadotropin (HCG) is used to stimulate spawning in fish. Several species have been reported to successfully undergo induced ovulation and spawning with HCG, including *Solea* species **(Salvatori *et al.*, 1985)**, *Solea vulgaris* **(Zaki & Ramzi, 1986)**, *Solea vulgaris* **(Abdelkawi, 1995)**, and *Solea solea* **(Assem, 1995)**. Additionally, treatment with GnRHa has successfully induced spawning in several flatfish species, improving spawning quality compared to naturally spawning females **(Mañanos *et al.*, 2008)**. Induced ovulation via GnRHa implants has been reported in *Paralichthys lethostigma* **(Berlinsky *et al.*, 1996)**, *Paralichthys dentatus* **(Berlinsky *et al.*, 1997)**, *Pleuronectes ferrugineus* **(Larsson *et al.*, 1997)**, and *Scophthalmus maximus* **(Watanabe *et al.*, 1998)**. Hormonal protocols, including the use of gonadotropins or GnRHa, may also enhance oocyte maturation and spermatogenesis to facilitate hatching operations. **Agulleiro *et al.* (2006)**, **Mañanos *et al.* (2008)**, **Mylonas and Zohar (2008)** and **Mylonas *et al.* (2010)** successfully increased ovulation rates, fecundity, fertilization, and hatching in Senegalese sole.

In the last decade, the total catch of *Sole* species in Egypt has decreased **(MOA, 2021)**. This decline, coupled with the scarcity of freshwater in Egypt, has created an urgent need to establish fish farms. There is also an increasing demand for marine fish farms and the establishment of marine hatcheries to supply these farms with fish fingerlings. This study aimed to stimulate the spawning of *Solea aegyptiaca* using various hormonal treatments during the natural spawning season. The goal was to identify the most effective hormones and their optimal doses, and assess their impact on reproductive performance. By increasing egg and larval production during the reproductive period, this research is crucial for developing marine hatcheries that can produce the Egyptian sole fry, meeting the growing demand from fish farms and boosting economic returns.

MATERIALS AND METHODS

1. Study site

This study was conducted from November 30, 2022, to May 25, 2023, in the hatchery and aquaculture laboratory at the Aquatic Research Station, Shakshouk, the National Institute of Oceanography and Fisheries in Fayoum, Egypt.

2. Collection and acclimatization of broodstoks

Wild *Solea aegyptiaca*, were collected from the 3rd depression of Wadi El Rayan Lake, Fayoum, Egypt. These fish were caught by professional fishermen and were

immediately placed in a plastic tank with a capacity of 1.0m³. The tank was filled with lake water salinity (18‰) and was equipped with an oxygen supply. The fish were then transported safely to the aquaculture laboratory in Research Station of (NIOF) within 45 minutes.

When the *S. aegyptiaca* spawner fish arrived at the laboratory, they were placed in a small circular plastic tank with a capacity of 60 liters. They were leaved in the same water for 30 minutes to regain their vitality. After the initial acclimatization, the broodfish were further acclimatized indoors and were placed in aerated, circular fiberglass tanks with a capacity of 1.5m³. These tanks were supplied with saline lake water at a rate of 5 L/min for two weeks, while gradually increasing the salinity from 20 to 33 ± 1.5‰ to match the salinity of Lake Qarun. The bottom of the tanks was covered with a 5cm thick layer of sand to provide shelter for the fish **(Fuchs, 1979; Abd El-Kawi, 1995)**. During the acclimatization period, the broodfish were fed live red worms, constituting 1.5% of their total biomass. Throughout the experimental period, the water temperature was maintained at 14 ± 3°C; the average dissolved oxygen was 5.5 ± 1.2 mg/L, and the pH ranged from 8.00 to 8.21

3. Experimental design and injection with hormones

S. aegyptiaca spawners were distributed into two divisions and control trails (in 7 circular fiberglass tanks). Mature fish samples were carefully selected to identify the optimal time to begin hormone therapy. The maturity of the female fish was assessed based on their external appearance by a swollen abdomen and a distinct orange coloration along the ventral side. Furthermore, histological examination of the ovaries (Fig. 1A) indicated that a mature female is ready for hormone injection when the oocytes are at least 600 ± 50µm in diameter.

3.1. Injection with different hormones

About 108 mature adult Egyptian sole were used in the study, with females weighing 55 ± 5g and males weighing 90.33 ± 1.25g. The total length (TL) of females ranged from 18 to 28cm, while males ranged from 20.9 to 29.5cm. These experiments were conducted during the natural spawning season in the last week of December 2022. The fish were injected intramuscularly on the dorsal side, toward the head, using an insulin syringe at a 45-degree angle. The *S. aegyptiaca* broodstock spawners were distributed in each tank at a sex ratio of 1 female to 1 male, with 16 fish per tank in the hormone treatment trials and 12 fish in the control experiment. The experiments were divided into three groups as follows:-

The 1st group experiments: It was divided into 3 tanks. Fish were injected with Human chorionic gonadotropin (HCG) “Pregnyl” (Nile Co. for Pharmaceuticals, Cairo, ARE) of 4000, 5000 and 5200IU/ kg of body weight of fish in trial 1, 2, and 3, respectively.

- **The 2nd group**: It was divided into 3 tanks. Fish were injected with carp pituitary homogenate (CPH) which were obtained from sexually mature donor fish of 3.5, 4.0, and 4.5mg/ kg of B W. of fish in trial 4, 5, and 6, respectively.

- **Control (non-injected):** About 12 fish in tank. Fish were injected with the appropriate dosage of 0.9% saline water injection. They were subjected in the same rearing conditions of the pervious injected experiments.

3.2. Spawning performance

Spawning quality throughout the spawning period (from December 25th to March 5th) was evaluated by measuring the fecundity of the fish volumetrically. Fertilized eggs were collected using a 150-mesh phytoplankton net in a cylinder filled with lake water at 34‰ salinity. The number of eggs was estimated based on the assumption that 1ml contains approximately 1000 fertilized eggs. Hatchability was determined by calculating the number of fertilized eggs and the newly hatched larvae. From each spawn, a sample of 50 fertilized eggs was examined under a binocular microscope equipped with an eyepiece micrometer to measure egg diameter. Daily relative fecundity was calculated using the weight of the females at the start of the trial. The spawning duration was defined as the period between the first and last spawn.

3.3. Hatchability rate and larvae production

Three ± 1 days after injection, the fish naturally spawned surface-fertilized eggs. At an average temperature of 15 ±1°C, the larvae hatched three days after fertilization. The newly hatched larvae were collected from the water surface of each tank using phytoplankton nets with a mesh size of 150µm and were counted. The collected larvae were then incubated in plastic tanks (capacity = 120 liters) with an average density of 60 ± 12 individuals per liter. The number of spawns and the number of fertilized eggs in each trial were recorded. Hatchability rates and the total number of surviving fry produced during the spawning period were subsequently calculated.

3.4. Histological examination of gonad

The gonads were removed and fixed in Bouin's fluid for 24h at 4°C. The fixed gonads were thereafter dehydrated through series concentration of ethanol solution, cleared and embedded in paraffin (M.P: 57 °C). Transverse sections of the gonads (5-6µm thickness) were stained with Harris's alum hematoxylin and aqueous solution of eosin **(Abd El-Kawi, 2002)**.

4. Method of larval feeding

The larvae were transferred to the larval rearing tanks at a density of 60 ± 12 larvae per liter. *Solea aegyptiaca* larvae were reared from the absorbed yolk sac stage

until completing metamorphosis. From the 3rd day post-hatching (dpH) until the 12th dpH, the larvae were fed rotifers (Brachionus plicatilis) at a density of 10 individuals per ml. From the 14th dpH, they were fed Artemia nauplii at a density of 4 ± 1 individuals per ml. At 24 ± 2 dpH, the larvae were fed Artemia metanauplii at a density of 10 individuals per ml. A weaning trial was conducted on juveniles at 27 ± 3 dpH. These juveniles were fed live benthic plankton from the lake, along with Artemia, until they reached 60 dpH.

RESULTS

1. Ovarian biopsy

In these experiments, a comparison was made between different doses of HCG and CPE, along with a control group. At the beginning of the experiments, an ovarian biopsy was performed on *Solea aegyptiaca* females before they were injected with different hormones (HCG and CPE). The oocytes were in the vitellogenic stage, specifically the tertiary yolk stage, as shown in Fig. (1A, B), with the nucleus (N) centrally located. Before the primary injection, the oocytes were in the tertiary yolk deposition and ripening stage, measuring about 600 ± 50µm in diameter. Three ± 1 days post-injection, the nucleus migrated to the periphery of the oocyte. The oocytes then developed to a hydrated stage, with egg diameters increasing to over 927 µm (Fig. 1C). Ovulation occurred from late December to the first week of March. After the spawning period, the ovaries entered the apoptotic stage, characterized by atretic oocytes and the presence of primary oocytes for the next season (Fig. 1D, E).

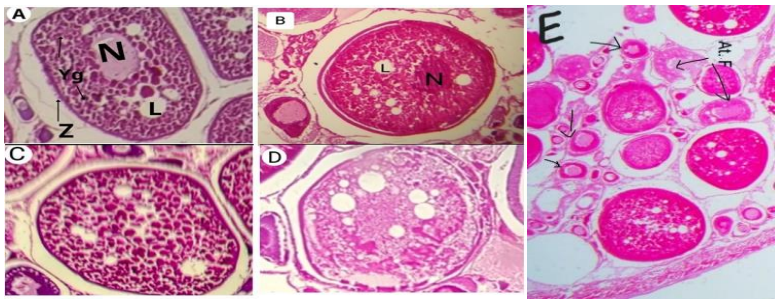


Fig. 1. Histological sections of *Solea aegyptiaca* oocytes obtained from ovarian biopsies displaying: **(A)** Vitellogenic oocyte with yolk globules (Yg), a central nucleus (N), and lipid droplet (L); **(B)** Post-vitellogenesis oocyte with the nucleus (N) migrated to the periphery and lipid droplets beginning to coalesce into a large mass; **(C)** Large lipid droplet and yolk globules still in a dispersed form; **(D)** Atretic oocyte (apoptotic oocyte) at 200x magnification; **(E)** Late in the spawning season, showing atresia (apoptotic oocyte) and different stages of oocyte development (arrows) at 40x magnification (HE stain)

2. Induction of oocyte maturation and spawning

As shown in Table (1), the spawning characteristics of *Solea aegyptiaca* treated with different doses of HCG injection (4000, 5000, and 5200IU/kg BW) were compared

with treatments using different doses of CPH (3.5, 4.0, and 4.5mg/kg BW) and a control group (0.9% saline water injection). Spawning was recorded from the 3rd ± 1 day of treatment (December 25th) until the end of the natural spawning season (March 5th). All three HCG and CPH treatments improved spawning performance compared to the control group.

2.1. 1st trials using different doses of HCG hormone

From data in Table (1), the fish weights are 520 ± 24, 440 ± 20, and 469 ± 12g per tank for those injected with 4000, 5000, and 5200 IU HCG/kg BW, respectively. Table (1) also indicates that the number of fertilized eggs per female varied with the different doses of hormones. The number of fertilized eggs recorded was 5671.25, 11,765.5, and 7171.5 per female for fish injected with 4000, 5000, and 5200 IU HCG/kg BW, respectively. Therefore, the total number of fertilized eggs produced per tank was 45,370, 94,122, and 57,372 for fish injected with 4000, 5000, and 5200 IU HCG/kg BW, respectively. Additionally, the total number of newly hatched larvae per tank was recorded as 34,481.2, 83,768.58, and 39,586.68 for fish injected with 4000, 5000, and 5200 IU HCG/kg BW, respectively

2.2. 2nd trials using different doses of CPH

Table (1) shows that the fish weights are 480 ± 20g, 440 ± 25.6g, and 400 ± 20g per tank for those injected with 3.5, 4.0, and 4.5mg CPH/kg BW, respectively. Table (1) also indicates that the number of fertilized eggs per female varies with different doses of CPH. The number of fertilized eggs recorded was 2981.5, 3813.25, and 2321.25 per female for fish injected with 3.5, 4.0, and 4.5mg CPH/kg BW, respectively. Consequently, the total number of fertilized eggs produced per tank was 45,370, 94,122, and 57,372 for fish injected with 3.5, 4.0, and 4.5mg CPH/kg BW, respectively. Additionally, the total number of newly hatched larvae per tank was 13,834.16, 22,879.5, and 10,213.5 for fish injected with 3.5, 4.0, and 4.5mg CPH/kg BW, respectively

2.3. 3rd trial control

From Table (1), it was observed that the fish weight was approximately 404 ± 14 g per tank. Fish in this trial were injected with 0.9% saline water. The total number of fertilized eggs per tank was recorded as 9157.36. The number of fertilized eggs per female was 11,144.67. Additionally, the total number of newly hatched larvae per tank was 4120.82.

All three HCG treatments improved the spawning performance compared to the CPH and control groups (Table 1). The total number of fertilized eggs per tank was significantly higher in the HCG-treated groups compared to the control. Specifically, the total fertilized eggs were 45,370 per tank, with an average of 5671.25 per female for the

4000 IU HCG injection; 94,122 per tank, with an average of 11,765 per female for the 5000 IU HCG injection; and 57,372 per tank, with an average of 7171.5 per female for the 5200 IU HCG injection.

The highest number of fertilized eggs per tank injected with different doses of CPH was 30,506 per tank, with an average of 3813.25 per female for the 4.0mg CPH/kg BW dose, compared to the control group, which recorded 9,157 per tank, with an average of 1,144.67 per female.

T-test analysis of the data in Table (2) indicates significant differences in the reproductive performance ($P< 0.05$) of *Solea aegyptiaca* treated with different doses of HCG and CPH compared to the non-injected fish.

Table 1. Spawning characteristics of *Solea aegyptiaca* treated with different doses of HCG injection (4000, 5000, and 5200 IU/ kg BW), different doses of CPH (3.5, 4.0, and 4.5mg/kg BW) and control (0.9% of saline water injection), Spawning was recorded daily from the day of treatment (25th December) until (5th March). Total body weight (g), /tank, total number of fertilized egg/ tank, ($P<0.05$), number of fertilized egg/g of female, number of fertilized egg/ female, number of newly hatched larvae, ($P<0.05$) and percentage of hatching, ($P<0.05$).

Item	Exp. of treatment with different hormones							sig
	HCG of IU/Kg B. Wt.			CPH of mg/ kg. B. Wt			CONT.	
	4000	5000	5200	3.5 mg	4.0 mg	4.5 mg		
Wt of female ,g. / tank	**520±24**	**440±20**	**469±12**	**480±20**	**400±25.6**	**400±20**	**404±14**	
r F of ♀ /g before injected	**442.26**	**460**	**438**	**366**	**440**	**440**	**440.0**	
No. of F. eggs spawned/ g of ♀	**87.25**	**213.91**	**115.67**	**49.69**	**43.27**	**46.43**	**22.76**	
Total No. of F. eggs spawned/ ♀	**5671.25**	**11765**	**7171.5**	**2981.5**	**3813.25**	**2321.25**	**1144.67**	0.054
T. No. of F. egg spawned / tank	**45370 [a]**	**94122 [a]**	**57372 [a]**	**23852 [a]**	**30506 [a]**	**18570 [a]**	**9157[a]**	0.008
No of newly hatching larvae	**34481.2 [b]**	**83768.5 [b]**	**39586.68 [b]**	**13834.16 [b]**	**22879.5 [b]**	**10213.5 [b]**	**4120.8 [b]**	0.024

rF: relative Fecundity of female / g. before injection, F. egg: Fertilized egg.

From Fig. (2), it is clear that the highest total number of fertilized eggs per tank was produced in January across all trials, including the control. The highest numbers were 75,780 and 16,120 eggs per tank for fish injected with 5000 IU HCG and 4.0 mg CPH/kg BW, respectively. The lowest number was recorded in the first week of March across all groups.

A T-test analysis of variance was conducted to evaluate the significance of the differences in the total number of fertilized eggs across the four months of the spawning period in the experimental tanks. The results showed significant differences at the 1% significance level, leading to the rejection of the null hypothesis. This supports the alternative hypothesis that there are substantial differences due to the varying periods and the effects of hormones.

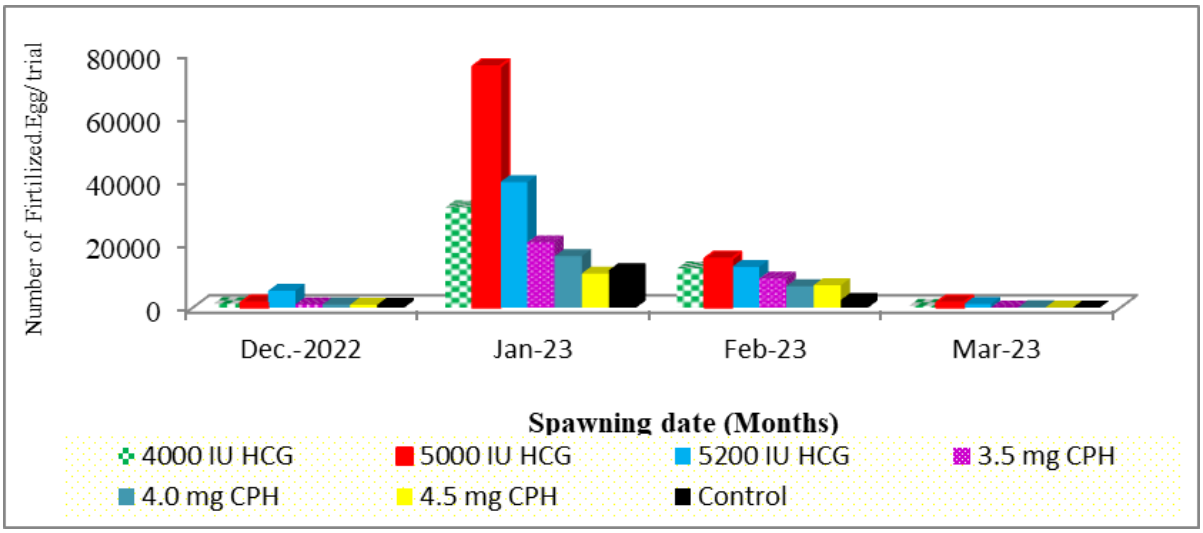


Fig. 2. The relationship between the number of fertilized eggs of *Solea aegyptiaca* resulting from treatments and different hormones at different doses (4000, 5000 and 5200 IU HCG and 3.5, 4.0, and 4.5mg CPH/ kg BW) compared with non-injected fish through different months of the spawning season, from late December 2022 to March 2023

As shown in Table (2), the highest total fecundity was recorded in fish injected with 5000 IU HCG/kg BW, at 460,000 eggs per kg, while the highest value for fish injected with 4.0 and 4.5 mg CPH/kg BW, and the control group, was 440,000 eggs per kg. Moreover, Table (2) indicates that the number of ovulation events varied with different hormone doses. Fish injected with 4000, 5000, and 5200 IU HCG/kg BW had ovulated 18, 28, and 24 times, respectively. Fish injected with 3.5, 4.0, and 4.5mg CPH/kg BW ovulated 12, 15, and 11 times, respectively, while the control group (non-injected) ovulated 8 times.

It was also observed that fish injected with HCG produced different numbers of spawning batches per female over the spawning period: 325.07, 420.18, and 298.81 for tanks injected with 4000, 5000, and 5200 IU HCG/kg BW, respectively. Fish injected with CPH had different numbers of spawning batches: 248.46, 254.22, and 165.80

batches per female for tanks injected with 3.5, 4.0, and 4.5 mg CPH/kg BW, respectively. In comparison, the non-injected fish (control) produced 8 batches, with an average of 143.08 eggs per batch.

Table 2. Spawning characteristics of *Solea aegyptiaca* treated with, different doses of HCG injection (4000, 5000, and 5200 IU/ kg BW), different doses of CPH (3.5, 4.0, and 4.5 mg /kg BW) and control (0.9% of saline water injection), Total body weight of fish (g), /tank, total, Fecundity of / kg, daily number of Fertilized egg / tank, spawning duration, egg diameter, and percentage of hatching, (P<0.05)

Item	Exp. of treatment with different hormones							Sig. (p)
	HCG of IU/Kg B. Wt.			CPH of mg/ kg. B. Wt			CONT.	
	4000	5000	5200	3.5 mg	4.0 mg	4.5 mg		
Wt of female ,g / tank	520±24	440±20	469±12	480±20	400±25.6	400±20	404±14	
Total Fecundity of / kg	442260	460000	438000	366000	440000	440000	440000	
Daily no. of F. egg spawned/ tank	2520.56*	3137.4*	2390.5*	1987.67*	2033.73*	1326.43*	1144.67*	<0.05
No. of times of spawning / tank	18	28	24	12	15	11	8	
No. of F. egg in each batch/ female/tank	315.07*	420.187*	298.81*	248.458*	254.216*	165.803*	143.083*	000
Spawning duration*	55	70	60	40	50	45	42	
Egg diameter (μm)	920±6.07	933±12.2	932±9.59	927±5.7	927±7.56	928±6.27	916±6.16	
% of hatchability	76	89	69	58	75	55	45	0.01

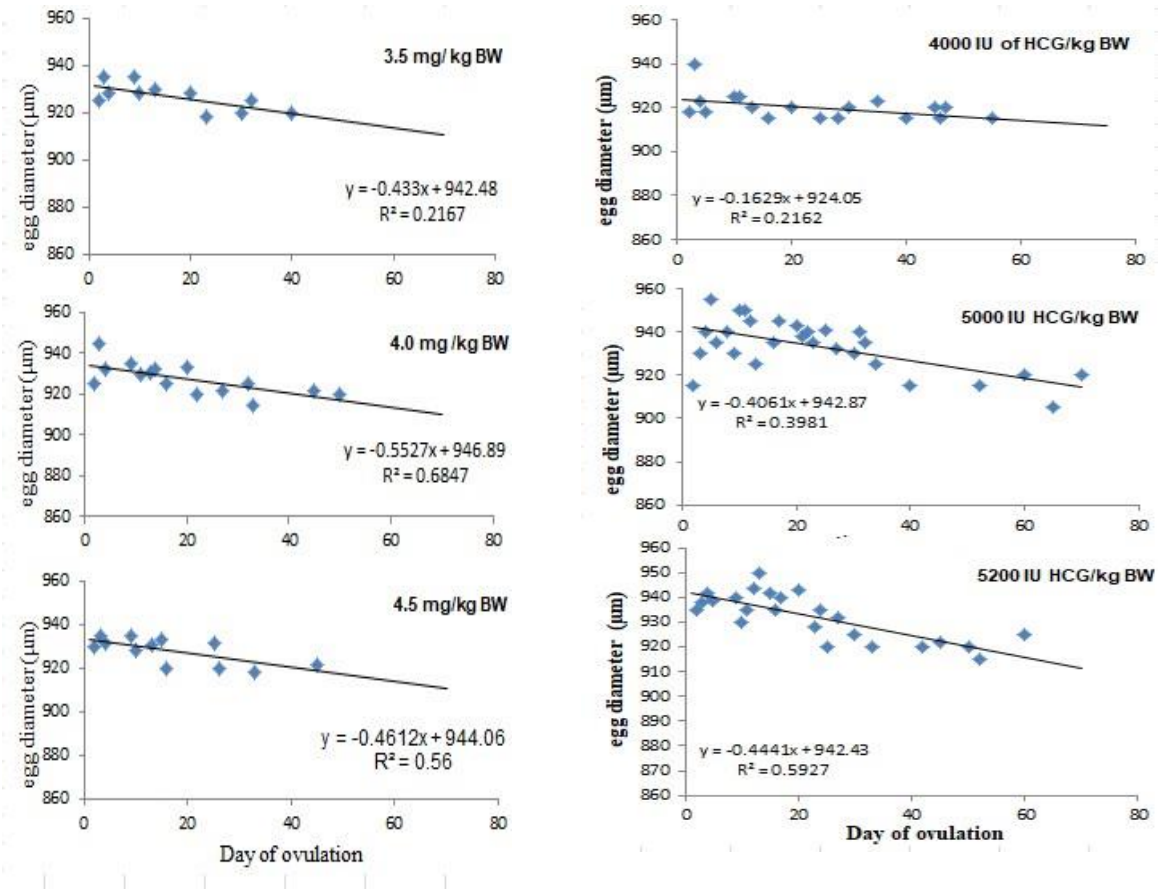
*Spawning duration: the period from the 1st. spawning till the final spawning.

Table (2) shows that the highest hatchability percentages were observed in fish injected with 5000 IU HCG and 4.0 mg CPH/kg, which resulted in 89 and 75% hatchability, respectively, at a water temperature of 15±2°C and a salinity of 34±1 ppt. The lowest hatchability percentage, 45%, was recorded in the control group. These results indicate that the highest daily number of fertilized eggs was recorded as 3137.4 and 2033.73 per female per tank for fish injected with 5000 IU HCG and 4.0 mg CPH/kg BW, respectively. According to the T-test analysis, there were no significant differences (P> 0.05) between all batches throughout the spawning period. However, significant differences (P< 0.05) were found between the fish injected with HCG, CPH, and the control group.

3. Egg diameter and spawning duration

Table (2) and Fig. (3) indicate that the spawning duration varied with different doses of hormones. The spawning period was 55, 70, and 60 days for fish injected with 4000, 5000, and 5200 IU HCG/kg BW, respectively. For fish injected with 3.5, 4.0, and

4.5 mg CPH/kg BW, the spawning durations were 40, 50, and 45 days, respectively. The lowest spawning duration (42 days) was recorded in the control group (no injection). As shown in Table (2) and Fig. (3), no clear variations in egg diameter were observed among females injected with HCG, CPH, or in the control group throughout the experimental period. The egg diameter was 916.5 ± 6.16 µm in the control group, while it increased to 933.0 ± 12.2 µm for fish injected with 5000 IU HCG and 928.0 ± 6.27µm for fish injected with 4.5 mg CPH/kg BW.



CPH **HCG**

Fig. 3. Correlation between the mean diameter of fertilized eggs and the spawning duration of *Solea aegyptiaca*, treated with saline (Control) and different doses of 4000, 5000, and 5200 IU HCG, and 4.0, 4.5, and 5.0 mg CPH/kg BW (represented as groups 1, 2, and 3, respectively).

Table 3. The juvenile reproductive development of *Solea aegyptiaca* throughout the 2022/2023 spawning season, showing the rearing of larvae from newly hatched stages to the juvenile stage (60 days post-hatch, dph) for each trial. The differences were analyzed using a T-test ($P < 0.05$) across rows.

Items/tank	Exp. of treatment with different hormone							Sig.
	HCG of IU/Kg B. Wt.			CPH of mg/ kg. B. Wt			CONT.	
	4000	5000	5200	3.5	4.0	4.5	control	
No of newly hatching larvae	**34481.2**	**83768.58**	**39586.68**	**13834.16**	**22879.5**	**10213.5**	**6870.6**	0.024
No. of larvae at 10 dph	**31757.19**	**80836.68**	**35430.07**	**9725.414**	**19813.65**	**7476.282**	**5503.35**	0.024
% of larvae at 10 dph	**92.1**	**96.5**	**89.5**	**70.3**	**86.6**	**73.2**	**80.1**	
No. of survival larvae at 30 dph	**17240.6**	**59475.69**	**23752.01**	**6917.08**	**13727.7**	**4596.075**	**2748.24**	0.048
% of survival larvae at 30 dph	**50**	**71**	**60**	**50**	**60**	**45**	**40**	
No. of survival Juvenile at 60 dph	**6896.24**	**20942.15**	**8313.20**	**2490.15**	**5033.49**	**1940.57**	**1374.12**	0.04
% of survival juvenile at 60 dph	**20**	**25**	**21**	**18**	**22**	**19**	**20**	

4. Larval production

Table (3) illustrates that Solea aegyptiaca injected with different doses of HCG and CPH were compared with non-injected fish during the spawning period. It was observed that the number of newly hatched larvae collected from the spawner tanks varied. The highest percentage of newly hatched larvae, 89%, was recorded in trials with 5000 IU HCG and 4.0mg CPH/kg BW, respectively. This result was compared with non-injected fish (control group), which had the lowest hatchability percentage.

Table (3) also shows that the highest survival rate percentage at 10 days post-hatch (dph) was 98.5% and 86.6% for fish injected with HCG and 4.0mg CPH/kg BW, respectively, compared to 80.1% in the control group. The survival rate decreased over time, reaching 50, 71, and 60% at the complete metamorphosis stage (28±2 days) for fish injected with HCG. The highest survival rates at this stage were recorded from trials with 3.5, 4.0, and 4.5mg CPH/kg, with averages of 50, 60, and 45%, respectively. The lowest value recorded was around 40%.

After 60 dph, mortality increased due to environmental factors. The survival rate decreased to 25% for HCG-injected fish and 22% for CPH (4.0 mg/kg), while the control group showed a decrease to 20%.

Table (3) further shows that a T-test analysis of variance was conducted to evaluate the significance of the differences between the data across the rows. The total numbers of newly hatched larvae, total number of survival larvae at 10 dph, total number of survival larvae at complete metamorphosis (30 dph), and total number of survival juveniles at 60 dph were recorded for each group throughout the spawning period. The results showed significant differences at the 1% significance level, leading to the rejection of the null hypothesis. This supports the alternative hypothesis that there are substantial differences due to the varying doses of hormones and their effects over time.

DISCUSSION

To meet the increasing demand for fry, which is currently scarce in the wild, hatcheries use hormones to stimulate spawning. This approach is effective in ensuring a steady supply of fry and fingerlings. The experimental applications of induced spawning on *Solea aegyptiaca* were carried out during the spawning season, using different doses of two types of hormones. The first group received human chorionic gonadotropin (HCG), and the second group was injected with carp pituitary homogenate (CPH). The aim was to assess their effects on ripening, ovulation, spawning duration, reproductive performance, and the survival rate of fry at the metamorphosis and juvenile stages (60 dph).

In the present study, it was recorded that the ovaries of fish showed ripening and hydration of oocytes after injection. This is similar to results obtained in previous research **(Bahiker & Ibrahim, 1979)**. Hydration of oocytes after injection has been reported in various species, including the striped mullet **(Shehadeh & Ellis, 1970; Ishida *et al.*, 1972)**. **Rowland (1983)** and **Harmin and Crim (1992)** suggested that HCG treatment promotes water absorption and hydration of the ovary during ovulation.

The initial egg diameter, before hormone injection with different doses of HCG and CPH, was >550 ± 50µm. After 3 ± 1 days of injection, while during ovulation and spawning, the diameter increased to 905 ± 30µm. These findings are consistent with those of **Rodriguez (1984)** and **Garcia-López *et al.* (2006a)**, who found that in Senegalese sole, GnRHa induction increased the oocyte diameter from 550 to 950µm and stimulated the maturation of other smaller oocyte batches. Similar results were observed in other flatfish species, such as the summer flounder **(Watanabe *et al.*, 1998)** and the greenback flounder **(Sun & Pankhurst, 2004)**.

Solea aegyptiaca ovulated and shed eggs 3 ± 1 days after injection during the ovulation and spawning period. This is in line with **Salvatori *et al.* (1985)**, who reported that *Solea solea* treated with HCG laid eggs 3 to 5 days after injection. **Abd El-Kawi (1995)** also recorded ovulation in *Solea vulgaris* 3 to 5 days after HCG injection. However, other fish species, such as the goldfish **(Lin *et al.*, 1991)**, ovulated within 11 to

14 hours after GnRH and dopamine injection. While, *M. ambigua* spawned 26 to 38 hours after injection with 5-15mg/ kg carp pituitary gland **(Rowland, 1983)**. This variation in ovulation response time is due to the stage of ripening in the fish and environmental conditions.

Hormone treatments were effective in inducing ovulation during the spawning season. Female fish injected with 4000, 5000, and 5200 IU/kg BW of HCG responded quickly, producing a high percentage of fertilized eggs. The best dose for a higher number of fertilized eggs was 5000IU HCG/kg BW. These results confirm earlier findings by **Crim and Glebe (1984)** and **Mok (1985)**, who successfully induced ovulation in fish during peak spawning by treating them with 5600 IU/kg HCG. In this respect, **Zaki and Hamza (1986)** successfully induced spawning in *Solea* species using HCG. Additionally, **Ramos (1986)** reported successful induction of spawning in the common sole using HCG injections. The lowest doses of HCG (250-500 IU/kg) produced the greatest number of fertilized eggs, while higher doses (750-1000 IU/kg) resulted in poor-quality eggs or no spawning. **Abd El-Kawi (1995)** reported successful spawning induction in *Solea vulgaris* with 5600 IU/kg HCG.

In contrast, some studies have shown that HCG failed to induce spawning in certain fish species, such as major Indian carps. However, combining HCG with lower doses of carp pituitary extract (3-6 mg/kg) enhanced spawning **(Khalil, 1985)**. Additionally, HCG failed to induce spawning in *Clarias lazera* when injected with 2000 IU/kg **(Khalil, 1985)**. The best dose for successful spawning in these species was 4.0 mg CPH/kg BW. Other studies have shown that the best quality and quantity of fertilized eggs were produced in *Cyprinus carpio* when injected with 2.5mg CPH/kg BW **(Abd El-Hakim, 2000)**. **Benini *et al.* (2022)** also reported that the European eel females treated with CPE produced fewer fertilized eggs.

Spawning duration varied from 45 to 70 days, with the highest value recorded in fish injected with 5000 IU HCG/kg BW, compared to 42 days for the control group. **Guzmán *et al.* (2009)** reported that Senegalese sole treated with GnRHa implants had a spawning duration of 3-4 weeks during the mid-spawning period, possibly due to inhibition of spawning in the absence of exogenous GnRHa. In this study, the peak spawning activity and ovulation of *Solea aegyptiaca* occurred in January, with the lowest ovulation was recorded in the first week of March across all treatments. These results contrast with those of **Ashour (1971)**, who found that *Solea vulgaris* spawned from late November to early February, with a maximum spawning in early February. **Boulos and Ashour (1973)** showed that *Solea* species spawn multiple times during the long spawning period. It is possible that climate change has affected the spawning period of *Solea aegyptiaca*.

In this study, the highest number of fertilized eggs released was 94,122 per tank, with an average of 11,765.25 per female, from *Solea aegyptiaca* injected with 5000 IU HCG/kg BW. In comparison, fish injected with CPH (3.5, 4.0, and 4.5mg/kg BW) released the highest number of fertilized eggs (30,506 per tank, with an average of 3,813.25 per female), with the 4.0 mg CPH/kg group showing the highest values. The control group (non-injected) released the lowest number of fertilized eggs (15,268 per tank, with an average of 1,908.5 per female).

The rearing period for larvae is critical, especially during the first phase, as it depends on feeding (algae, rotifers, and Artemia) and environmental parameters. This is consistent with previous studies **(Abd El-Kawi, 1995; Assem, 1995)**.

The reproductive performance and hatching rates were significantly higher in the HCG-treated group compared to the CPH group. The highest hatchability rate, 89%, was recorded in the HCG-treated fish, compared to 75% for the 4.0 mg CPH/kg group and 45% for the control group. The highest survival rate at the metamorphosis stage was 71% in the HCG group, while the control group had the lowest survival rate (40%). These findings are consistent with the outcomes of **Palazzi *et al.* (2006)**, who achieved an average survival rate of 43% at metamorphosis in *Solea solea* after using commercial feeds. Additionally, juvenile survival rates of 25% were recorded after 60 dph, confirming similar results by **Zaki and Hamza (1986)**, **Dinis (1992)** and **Abd El-Kawi (1995)**.

CONCLUSION

The present results indicate that cultured *Solea aegyptiaca* female breeders can be induced to undergo ovarian maturation (OM) and can spawn spontaneously in a daily rhythm after 3 ± 1 days of treatment with HCG and CPH. Based on our findings, the optimal dose for *Solea aegyptiaca* is 5000 IU HCG/kg of body weight (BW), compared to other treatments. The highest dose of carp pituitary extract is 4 mg/kg BW. Although this extract was the least expensive, its quantity was low. Overall, the highest hatchability and survival rates were recorded with 5000 IU HCG/kg BW. However, there is a critical period from the newly hatched larvae to the metamorphosis stage. To enhance the population of *Solea aegyptiaca* fingerlings, improvements in the larval rearing system are needed to achieve the best survival rate for larvae.

REFERENCES

Abdel Hakim, E. E. (2000). Induced spawning of cultivated common carp, Cyprins carpio in fish farm. Bull. Nat. Inst. of Oceanogr. & Fish., A.R.E., 2000. Vol. (26): 211-221

Abd El-Kawi, S, S (1995). induced spawning and Larval rearing of *Solea vulgaris* in Lake Qarun. M.Sc. Thesis, Fac. Sci. Cairo Univ., Egypt

Abd El-Kawi, S, S (2002). Breeding and feeding behavior of mullet species in Lake Qarun. Ph.D thesis, Faculty of Science Benha University pp. 210

Agulteiros, M.J.; Anguis, V.;Cañavate, J.P.; Martinez-Rodríguez, G.; Mylonas, C.C. and Cerdá, J., (2006). Induction of spawning of captive reared Senegalese sole (*Solea Senegalensis*) using different delivery systems for gonadotropin-releasing hormone agonist. Aquaculture 257, 511-524.

Ahmed, A. I.; Sharaf, M. M. and Laban, H.A. (2010). Reproduction of the Egyptain sole, *Solea aegyptiaca* (Actinopterygil: Pleuronectiformes: Soleidae), from Port Said, Egypt, Mediterranean Sea. Acta Ichthyologica et Piscatoria. 40 (2): 161–166

Ashour, M. B. M. (1971). Studies on the female reproductive cycle of the fishes, *Mugil capito* and *Solea vulgaris* at lake Quarun (Fayoum, Egypt) M.sc. Thesis, Cairo University

Assem, S. S. (1995). Reprodctive physiology and induced spawning of Solea species. Ph.D thesis, Faculty of Science Alexandria University pp. 270

Badran, M.; El-Danasoury, M.; Sharaf, S. and Hassan, A. (2019). Biological measurements and hormonal profile in female Gilthead sea bream (*Sparus aurata*) in response to Human Chorionic Gonadotropin (HCG) injection. Suez Canal Veterinary Medical Journal. SCVMJ, 24(1): 103-112.

Benini, E.; Politis, S. N.; Nielsen, A.; Sørensen, S. R.; Tomkiewicz, J. and Engrola, S. (2022). Type of hormonal treatment administered to induce vitellogenesis in European eel influences biochemical composition of eggs and yolk-sac larvae. Fish Physiology and Biochemistry, 48(1): 185-200.

Babiker, M. M .and Ibrahim, H. (1991). Studies on the biology of reproduction in the cichlid *Tilapia nilotica* (L.): effect of steroid and trophic hormones on ovulation and ovarian hydration J.Fish Biol., 15, 21-30

Boulos, R. and Ashour, M. B. M., (1973). Studies on the female Reproductive cycle of some fishes at lake Quarun . (Fayoum, Egypt). Bull. Fac. Sci, Cairo University, No. 46;

Bukovala, E.; Cariani, A; Maes, G. E.; Sevilla, R. G.; -Bagnis, V.; Jerome, M.; Guarniero, I.; Monios, G.; Tinti, R.; Filip A. M.; Volckaert, A.; Bautista, J. and Krey, G. (2012). Restriction Fragment Length Analysis of the Cytochrome b Gene and Muscle Fatty Acid Composition Differentiate the Cryptic Flatfish Species *Solea solea* and *Solea aegyptiaca*

Cejko, B.I.; Kowalski, R. K.; Kucharczyk, D.; Targoñska, K.; Krejszeff, S.; arski, D. and Glogowski, J. (2009). Influence of the length of time after hormonal stimulation on selected parameters of milt of ide Leuciscus idus L. Aquacult. Res. 10.1111/j.1365-2109.

Crim, L. W. and Glebe, B. D. (1984). Advancement and synchrony of ovulation in Atlantic salmon with pelleted LH RH analog. Aquaculture, 43: 47-56

Dinis, M. T. (1992). Aspects of potential of Solea senegalenisis kup for aquaculyure, larval rearing and weaning. Aquaculture Fish -Manage. Vol. 23,(4) , Pp: 515-520

El-Husseiny, M. M. (2001). Reproductive Biology of Solea sp. in Lake Quarun. MSc. Thesis, Faculty of Science, Ain Shams University, Egypt.

Fahmy, A. F. and El-Greisey, Z. A. (2014). Induced spawning of *Liza ramada* using three different protocols of hormones with respect to their effects on egg quality. African Journal of Biotechnology. Vol. 13 (40), pp. 4028-1039

Fish Official Statistics Year Book (2021). Report of Fish Resources Protection and Development Agency . 115 Pp..

Fuchs, J. (1979) Technque d'elevage larvaire et production intensive de jvueniles chez la sole (*Solea solea*) These de specilite Optio Oceanologie Biologique, Universite de Morseille II.

Gabr, M. H. (2015). Capture production and stock assessment of *Solea aegyptiaca* Chabanaud, 1927 (Soleidae: Pleuronectiformes) in Bardawil Lagoon, Egypt. Egyptian Journal of Aquatic Research, 41, 101-110

Fuchs, J. (1979). Technque d'elevage larvaire et production intensive de jvueniles chez la sole (*Solea solea*). These de specilite Optio Oceanologie Biologique, Universite de Morseille 11

García-López, Á.; Anguis, V.; Couto, E.; Canario, A. V. M.; Cañavate, J. P.; Sarasquete, C.; Rodríguez, M. and Invasive, G. (2006a). Non Assessment of Reproductive Status and Cycle of Sex Steroid Levels in a Captive Wild Broodstock of Senegalese Sole *Solea senegalensis* (Kaup). Aquaculture 254(1–4): 583–93.

Garcia-Lopez, Á.; Couto, E.; Canario, A. V. M.; Sarasquete, C.; Rodríguez, M. and Invasive, G., (2007). Ovarian Development and Plasma Sex Steroid Levels in Cultured Female Senegalese Sole, *Solea senegalensis*. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology 146 (3): 342–54.

Guzman, J. M.; Ramos, J.; Mylonas, C. C. and Mananos, E. L. (2009). Spawning performance and plasma levels of GnRHa and sex steroids in cultured female Senegalese sole (*Solea senegalensis*) treated with different GnRHa-delivery systems. Vol.: 291, Pages 200-209

Harmine, S. A. and Crim, L.W.,(1992). Gonadotropić hormone- releasing hormone analog (GnRH- A) induced ovulation and spawning in female winter flounder pseudopleuronectes and americanus (Walbaum). Aquaculture, 104, 375-390

Ishida, R.; Hirose, K.; and Donaldson, E. M. (1972). Induction of ovulation in ayu, *Plecoglossus altivelis*, with SP gonadotropin. Bull. Jpn. Soc. Sci. Fish. 38(9):1007-1017

Khalil, A. A. (1985). Reproductive Biology of *Clarias lazera* in Lake Manzalah. M. Sc. Thesis, Faculty of Science, Alexandria University

Konsowa, A. H. and Abdellah, R. G. (2002a). Physico-chemical characteristic and their effects on phytoplankton community in Wadi el-Rayan lakes, Egypt: A- first lake J. Egypt. Acad. Soc. Environ. Develop. (D) 3 (2): 1-27. [Data on the limnochemistry and phytoplankton structure of the upper Wadi El-Rayan lake]

Konsowa, A. H. and Abdellah, R. G. (2002b). Physico-chemical characteristic and their effects on phytoplankton community in Wadi el-Rayan lakes, Egypt: B- second lake J. Egypt. Acad. Soc. Environ. Develop. (D) 3 (2): 29-51[Data on the limnochemistry and phytoplankton structure of the lower Wadi El-Rayan lake]

Larsson, D. G. J.; Mylonas, C. C.; Zohar, Y. and Crim, L.W., (1997). Gonadotropin releasing hormone-analogue (GnRH-A) advances ovulation and improves the reproductive performance of a cold-water batch-spawning teleost, the yellowtail flounder (*Pleuronectes ferrugineus*). Can. J. Aquat. Fish. Sci. 54, 1957-1964

Lin, H. R.; Zhou, X. J.; Van Der Kraak, G. and Peter, R. E. (1991). Effect of gonadotropin-releasing hormone agonists and dopamine antagonists on gonadotropin secretion and ovulation in Chinese Loach *Paramisgurnus dobryanus*. Aquaculture, 95: 139-147

Mañanos, E.; Duncan, N. and Mylonas, C. C., (2008). Reproduction and control of ovulation, spermitation and spawning in cultured fish. In: Cabrita, E., Robles, V., Herráez, M.P. (Eds.), Methods in Reproductive Aquaculture: Marine and Freshwater Species, CRC Press, Taylor and Francis Group, Boca Raton, pp. 3-80.

M O A., (2021). Statistics of fish production insect, and food manufacturing. Ministry of agriculture, Egypt.WWW.agri.gov.eg

Mok, T. K. (1985). Induced spawning and larvalrearing of the white seabream, Mylio berda. Aquaculture, 44, 41-49

Morgan, M. J. (2008). Integrating Reproductive Biology into Scientific Advice for Fisheries Management. J. Northw. Atl. Fish. Sci., Vol. 41: 37–51.

Mylonas, C. C.; Fostier, A. and Zanuy, S. (2010). Broodstock management and hormonal manipulations of fish reproduction. Aquaculture, 165(3): 516-534.

Natea, G.; Wakjira, M.; Negisho, T. and Endebu, M. (2017). Spawning response of African catfish (*Clarias gariepinus* Burchell, 1822), Claridae: Teleost) exposed to different piscine pituitary and synthetic hormone. International Journal of Fisheries and Aquatic Studies, 5(2): 264-269.

Palazzi, R.; Richard, J. ; Bozzato, G. and Zane, L. (2006). Larval and juvenile rearing of common sole (*Solea solea* L.) in the Northern Adriatic (Italy). Aquaculture, Vol. 255, 1-4, Pp 495-506

Ramos, J. (1986). Induction of spawning in common sole (*Solea solea* L.) with human chorionic gonadotropin (HCG). Aquaculture, 56(3-4): 239-242.

Rodriguez, R. B., (1984). Biologia y cultivo de *Solea senegalensis* Kaup 1858 en el Golfo de Cádiz. PhD Thesis, Universidad de Sevilla, Spain

Rottmann, R. W.; Shireman, J. V. and Chapman, F. A. (1991). Introduction to Hormone-Induced Spawning of Fish. Southern Regional Aquaculture Center(SRAC)NO.421.

Rowland, S. J. (1983). The hormone induced ovulation and spawning of the Australian fresh water fish golden perch, *Macquaria ambigua* (Richardson) (Percichthyidea0. Aquaculture, 35: 221-238

Palazzi, R.; Richard, J. ; Bozzato, G. and Zane, L. (2006). Larval and juvenile rearing of common sole (*Solea solea* L.) in the Northern Adriatic (Italy). Aquaculture, Vol. 255, 1-4, Pp 495-506

Salvatori, R.; Lenzi, M.; Fommei, F. and Angelini, M. (1985). Experiences of artificial reproduction and larval rearing of Sole (*Solea solea* Flower). OEBALIA, Vol. XI-2, N.S. 737-744 (in Italy)

Shehadeh, Z. H. and Ellis, J. N. (1970). Induced spawning of the striped mullet Mugil cephalus L. J. Fish Biol., 2: 355-360

Szedlmayer, A. T., (1987). Hormone induced spawning of weakfish progressive fisj culturist. Aquaculture, 49: 1158-160

Sun and Pankhurst (2004). Sun, B. Pankhurst, N.W., 2004, Patterns of oocyte growth, vitellogenin and gonadal steroids concentrations in greenback flounder. J. Fish Biol. 64, 1399-1412

Taranger, G. L.; Carrillo, M.; Schulz, R. W.; Fontaine, P.; Zanuy, S.; Felip, A. and Hansen, T. (2010). Control of puberty in farmed fish. General and Comparative Endocrinology, 165, 483–515.

Vachon, J.; Chapleau, F. and Desoutter-Meniger, M., (2008). Revision du genre Solea et réhabilitation du genre Barnardichthys (Soleidae; Pleuronectiformes). In: Cybium 32 (1), 9-26 (English Abstract)

Wafeek, M.; Sharaf, S.; Abdelkawy, S. S.; Abd Elazem, A. M. and Attia, E. I. (2020). Morphological and histological studies on hyprid dole fish (*Solea vulgaris* and *Solea aegyptiaca*). Abbassa Int. J. Aqua., Vol.13 No.(1), 75-94

Watanabe, W.O.; Ellis, E.P.; Ellis, S.C. and Feeley, M. W. (1998). Progress in controlled maturation and spawning of summer flounder (*Paralichthys dentatus*) broodstock. J. World Aqua, Soc. 29, 393-404

Zaki, M. I. and Hamza, A. K. (1986). Reproductive Biology And Induced Spawning Of *Solea solea* ((L.) In Egypt. Bull. Inst. Oceanogr. & Fish. ARE, 12: 115 - 125.