Impact of sex ratio and stocking density on the induced spawning of the Egyptian sole (*Solea aegyptiaca* Chabanaud, 1927).

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ABSTRACT

This study was conducted to evaluate the impact of sex ratio and stocking density on the induced spawning and reproductive performance of the Egyptian sole (*Solea aegyptiaca*) broodstock. Broodstock spawners were intermuscularly injected with Human Chorionic Gonadotropin Hormone (HCG) below the dorsal fine base; the dose was 7000 IU/Kg body weight of fish. In this study, three different broodstock sex ratios: 1:1, 1:2 and 2:1 (male ♂: female ♀) were tested. The highest fertilized eggs/ treatment and larvae/ treatment were observed in sex ratio (1♂:2♀). While, the highest fertilized eggs/ female, fertilized eggs/ g female, hatching rate and number of spawning were observed in sex ratio (2♂:1♀). Under the conditions tested in this study, larvae production of *S. aegyptiaca* was affected by different broodstock sex ratios and improved at a sex ratio (1♂:2♀). Also, four different broodstock stocking densities: 0.5, 0.75, 1, 1.25 Kg/m^3 were tested. The highest fertilized eggs/ treatment, fertilized eggs/ g female and larvae/ treatment were observed in stocking density 0.75 Kg/m^3 followed the other stocking densities. The lowest fertilized eggs/ female and/ g female were observed in stocking density 1.25 Kg/m^3. Under the conditions tested in this study, larvae production of *S. aegyptiaca* was affected by different broodstock stocking densities and the best stocking density was 0.75 Kg/m^3. Also, results demonstrated superiority of artificial spawning compared to natural one. From the results of this study, it is recommended to use artificial spawning in *S. aegyptiaca* with sex ratio (1♂:2♀) and stocking density (0.75 Kg/m^3).

Keywords: *Solea aegyptiaca*, induced spawning, reproductive performance, sex ratio, stocking density

INTRODUCTION

The common sole *Solea solea* and the Egyptian sole *S. aegyptiaca* (Family: Soleidae) are the most important sole species that occurs in the Egyptian waters. They tend to occupy shallow, sandy and sandy/muddy habitats as well as the shallow lagoons. The common sole is highly appreciated fish by the Egyptians especially in the coastal communities because of its high quality flesh and is one of the commercially important fish in Egypt providing up to 90 million LE annually. Despite its worldwide importance, little work has been dedicated on its reproductive biology (Mehanna, 2014). The Egyptian sole (*Solea aegyptiaca*) is the most common species of soles that contributed about 6.5% of the total catch of trawl fishery, forming about 13% of the gross revenue of the trawling (Mehanna, 2007). Kariman (2009) recorded that catch composition of sole species during summer and winter seasons in Lake Qarun were more than 50 and 35%, respectively. In Egypt, the information about the reproductive biology of this species is very scarce (Salman, 2014).

El-Husseiny (2001) reported that the Gonado-somatic index (GSI) of female of *S. aegyptiaca,* in Lake Quarun, increased progressively to reach its maximum value in January, while the minimum value was recorded in July, while Ahmed *et al.* (2010) found that the monthly changes in GSI of males and females *S. aegyptiaca* showed a definite breeding season which extends from January to June. Both sexes reached the highest values of GSI in January, while the minimum was in June and August for females and males respectively. Salman (2014) confirmed that the common sole in Bardawil lagoon is a winter spawner and spawned once a year. Mehanna (2014) confirmed that the common sole, *S. solea,* in Bardawil lagoon is a winter spawner and spawn once a year and its spawning period occurs between October and April with a peak activity during December. In the wild, *Solea vulgaris* are reproductively active throughout the period from December to late March (Assem, 1995).

Herrera *et al.* (2008), studied reproduction and breeding of the wedge sole in captivity and indicated that the breeders adapt easily to captivity, and they can spawn in less than one year in captivity. In Egypt, common sole, *S. solea,* was successfully hatched for the first time in 1986 and juveniles were obtained and maintained in healthy conditions (Zaki and Hamza 1986; Assem *et al.,* 2012). Eisawy and El-Bolock (1975) indicated that *Solea aegyptiaca* spawned and propagated successfully in Lake Qarun and it makes up about 36% of the total catch of lake. Artificial spawning of *Solea solea* was recommended by Zaki and Hamza (1988); Assem (1995); Agulleiro *et al.* (2006); Assem *et al.* (2012). Assem (1995) studied the reproductive biology of both *Solea aegyptiaca* and *Solea vulgaris*. Furthermore, he studied induction of spawning by using hormonal treatments, and then studied embryonic and larval developmental stages.

Hormonal induction of spawning is usually carried out with fish which normally do not spawn spontaneously in captivity. For fish species which spawn naturally under confinement, hormonal manipulation is done to synchronize spawnings of several females for mass fry production. Human chorionic gonadotropin (HCG) has been used successfully to induce ovulation and spawning in a number of fish species as reviewed by Donaldson and Hunter (1983). It is used either alone or in combination with other hormones. One advantage in using HCG over other hormones is that its potency can be standardized in international units and results can be compared among users (Lee *et al.,* 1988). Assem (1995) reported that, in artificial spawning of *Solea solea,* the total number of spawned egg varied between 1.3x 10^6 egg per Kg minimally and 2.1x 10^6 egg per Kg maximally at water temperature 17±2.2°C and salinity 34±1‰. Herrera *et al.* (2008), indicated that for Wedge sole (*Dicologoglossa cuneata*) a relative fecundity reached 1.06- 2.33x 10^6 egg/ Kg per spawning season, gametes are released in a wide temperature range; 10-21 °C. Assem *et al.* (2012) reported that, in artificial spawning of *Solea vulgaris*, the total number of fertile ovae varied between 87,000 to 120,000 eggs/ spawn which represent 430±108 eggs/g female. The percent of fertilization varied between 80 and 93% in water temperature of 17±2 and salinity 34±1‰.

The aim of the present study was to evaluate the impact of sex ratio and stocking density on the induced spawning and reproductive performance of the Egyptian sole (*Solea aegyptiaca*) broodstock.

MATERIALS AND METHODS

Broodstock- rearing conditions

Egyptian sole, *Solea aegyptiaca* broodstock (31-83 g/fish, body weight), used in the spawning trials were obtained from Lake Qaroun, El- Fayoum Governorate. They were obtained during the beginning of December 2014 and maintained at National Institute of Oceanography and Fisheries (NIOF), Shakshouk Fish Research Station, El- Fayoum Governorate and acclimated to laboratory conditions for 14 days in rectangular fiberglass tanks (1.5 m^3 capacity) then injected by human chorionic gonadotropin (HCG) hormone and randomly distributed into circular fiberglass tanks (1m^3 capacity). All tanks were provided with continuous aeration. The bottom of each tank was covered by a sand layer about (5-10 cm) for shelter. Fish were held under natural photoperiod condition throughout the spawning trials. Water temperature, dissolved oxygen, pH, total ammonia, un-ionized ammonia, nitrite and nitrate were measured in all trials. The water used in the all trials was obtained from Lake Qaroun. No feed were offered for fish, but zooplankton in water in all trials were count during experimental period (Table 1). The average water quality criteria in all trials are presented in Table (2). About 25% of water tanks were changed twice every day. The trials began 14/12/2014 and ended 14/4/2015, (120 days).

Table 1: Zooplankton species and count during experimental period (No. of zooplankton/ liter) (Mean± S.E).

Species	Natural spawning	Induced spawning	
		Sex ratio	Stocking density
Brchionus plicatilis	2±1	1.5±0.5	1.5±0.5
Nauplis larvae	110±40	87.5±37.5	16±3
Fevelli sp.	19±14	18±16	3±2
Cirripedia larvae	1.5±0.5	1.5±0.5	1.5±0.5
Annelida larvae	1.5±0.5	1.5±0.5	2±1
Mesochra heldti	1.5±0.5	2±1	2±1
Total count zooplankton	135.5±56.5	96.5±37.5	26±2

Table 2: Average values of water quality parameters during experimental periods (Mean±S.E).

Parameters	Natural spawning	Induced spawning	
		Sex ratio	Stocking density
Temperature, °C	16±3	15.5±2.5	16±3
pH	8.16±0.12	8.22±0.11	8.17±0.11
Salinity, ‰	32.5±1.5	32.5±1.5	32.5±1.5
Dissolved oxygen, mg/l	8.3±0.3	8.3±0.3	8.3±0.3
Total ammonia, mg/l	0.38±0.02	0.39±0.01	0.46±0.07
Un-ionized ammonia, mg/l	0.015±0.001	0.015±0.001	0.018±0.003
Nitrite, mg/l	0.125±0.001	0.125±0.001	0.351±0.225
Nitrate, mg/l	0.451±0.029	0.466±0.046	0.857±0.411

Induce spawning

Solea aegyptiaca spawners were intermuscularly injected with HCG, the commercial name Pregnyl® (Pregnyl® N.V. Organon Oss the Netherlands) below the dorsal fine base. The dose was 7000 IU/Kg body weight of fish. The females were ranged from 35 to 83 g/fish in body weight and 15.7 to 20.5 cm/fish in body length, while the males were ranged from 31 to 58 g/fish in body weight and 15.4 to 18.6 cm/fish in body length.

Experimental design

The first experiment was conducted to investigate the effect of different sex ratio (1:1, 1:2 and 2:1, male ♂: female ♀) on induced spawning of *Solea aegyptiaca* with stocking density of approximately 0.5 Kg/m^3. The second experiment was conducted to investigate the effect of different stocking density (0.5, 0.75, 1 and 1.25 Kg/m^3) with sex ratio (1 Female: 1 Male) on induced spawning of *Solea aegyptiaca*. The third experiment to evaluate and compare between induced spawning and natural spawning.

Water quality analysis

Water temperature and pH were measured daily by Combined meter (pH/ EC/ TDS/ temperature, Mi 805). Salinity was measured daily by Refractometer (VITAL Sine SR-6, China). Dissolved oxygen (DO) concentration was determined titrimetrically according to the modified Winkler, full-bottle technique (Method 360.2; EPA, 1983). Water ammonia, nitrite and nitrate were determined by using Spectrophotometer model (LKB Bichrom UV visible spectrophotometer) according to the method described by APHA (1992). To determine un-ionized ammonia concentration, multiply total ammonia concentration by the percentage which is closest to the observed temperature and pH of the water sample (Swann, 1997).

Enumeration and identification of zooplankton in water

Zooplankton samples were collected using zooplankton net. The samples were fixed immediately using formaldehyde solution (4-7%), two ml of Rose Bengal stain (0.5 %) was added after fixation. The samples were examined under a binocular research microscope. The organisms were identified and counted on the counting try with magnification varying from 100X to 400X. Planktonic organisms were identified, classified and described according to description and keys constructed by Ruttner-Kolisko (1974); Campbell (1982); Abdel-Malek *et al.* (1993); Parveen and Mola (2013).

Statistical analysis

The data were analyzed by one-way ANOVA and significant differences were determined by Duncan Waller Multiple Range Test at 5% level using SPSS Statistical Package Program (SPSS, 2008) 17, released version.

RESULTS

During experimental period from 14/12/2014 to 14/4/2015, about 100% spawning success rate for Egyptian sole broodstock injected, using Human Chorionic Gonadotropin Hormone (HCG). After 48 h from the resolving injection the female spawns, the mean total number of fertilized eggs varied between 2200 minimally and 6280 maximally per female and hatching rate varied between 90% minimally and 95% maximally, according to treatments differences (sex ratio and stocking density). The Egyptian sole fish always spawn at night; fish spawn pelagic eggs, which are fertilized externally and float individually near the water surface. The ripe eggs of *Solea aegyptiaca* appeared rounded, colorless and transparent, with a diameter of about 0.85 mm. The surface of the fertilized egg shell is smooth, the fertilized egg appeared rounded and was about 0.92 mm in diameter. The results showed that hatching of *Solea aegyptiaca* eggs occured after 48±3 h from fertilization at temperature 17-19 ºC, while occured after 60-72 h from fertilization at temperature 14-16 ºC. Newly hatched larva was about 1.42 mm in length. In the present study, there were some unfertilized eggs were recorded in all treatments.

The first experiment: Effect of sex ratio on reproductive performance

Reproductive performance parameters of the Egyptian sole broodstock under effect of sex ratio are shown in Tables (3 and 4). The results cleared that the sex ratio had significant effects (P≤0.05) on the reproductive performance parameters such as number of fertilized eggs/ treatment, number of fertilized eggs/ female, number of fertilized eggs/ g female, number of larvae/ treatment and hatching rate (%). While, number of spawning and survival rate (%) showed insignificant differences between treatments.

Table 3: Effect of sex ratio on induced spawning of the Egyptian sole, *Solea aegyptiaca*.

Items	Sex ratio (male ♂: female ♀)			SED*
	1♂ : 1♀	**1♂ : 2♀**	**2♂ : 1♀**	
No. of fertilized eggs/ treatment	30566[b]	43986[a]	30986[b]	341.955
No. of fertilized eggs/ female	5094.33[b]	4398.60[c]	6197.20[a]	52.440
No. of fertilized eggs/g female	94.34[c]	112.21[b]	132.99[a]	1.126
No. of larvae/ treatment	28278[b]	39629[a]	29457[b]	819.174
Hatching rate, %[1]	92.51[ab]	90.09[b]	95.06[a]	1.439
No. of spawning	5	5	7	-
Survival rate, %[2]	100	100	100	-

- (a, b, c) Average in the same row having different superscripts are differ significantly (P≤0.05). * SED is the standard error of difference

1, Hatching rate,% = (Number of larvae/ Number of fertilized eggs per treatment) × 100.

2, Survival rate,% = (Number of fish at end/ Number of fish at start) × 100.

The results also showed that, the highest fertilized eggs/ treatment (43986 egg) and larvae/ treatment (39629 larvae) were observed in sex ratio (1 male ♂: 2 female ♀), thereafter, sex ratios (2♂:1♀) (30986 egg, 29457 larvae) and (1♂:1♀) (30566 egg, 28278 larvae). While, the highest fertilized eggs/ female (6197.20 egg) were observed in sex ratio (2♂:1♀), followed sex ratio (1♂:1♀) (5094.33 egg) then sex ratio (1♂:2♀) (4398.60). The highest fertilized eggs/ g female (132.99 egg) were observed in sex ratio (2♂:1♀), followed sex ratio (1♂:2♀) (112.21 egg) then sex ratio (1♂:1♀) (94.34). The highest hatching rate was observed in sex ratio (2♂:1♀) (95.06%), followed by sex ratio (1♂:1♀) then (1♂:2♀) 92.51 and 90.09%, respectively. Numbers of spawning values were relatively highest with sex ratio (2♂:1♀). These results indicated that the best sex ratio was recorded with (1♂:2♀) at obtained fertilized eggs/ treatment and larvae/ treatment. While, the best sex ratio was recorded with (2♂:1♀) on fertilized eggs/ female, fertilized eggs/ g female, hatching rate and number of spawning under experimental conditions.

Table 4: Effect of sex ratio on induced spawning of *Solea aegyptiaca* (hormone dose, date of injection and date of ovulation).

Items	Sex ratio (male ♂: female ♀)		
	1♂ : 1♀	**1♂ : 2♀**	**2♂ : 1♀**
Hormone dose	7000 IU/kg fish		
Date of injection	14 / 12 / 2014		
Date of ovulation			
The first	16/12/2014	16/12/2014	16/12/2014
The second	25/12/2014	25/12/2014	25/12/2014
The third	3/1/2015	3/1/2015	3/1/2015
The fourth	17/1/2015	18/1/2015	17/1/2015
The fifth	29/1/2015	29/1/2015	28/1/2015
The sixth	- -	- -	8/2/2015
The seventh	- -	- -	12/3/2015

The second experiment: Effect of stocking density on reproductive performance

Reproductive performance parameters of the Egyptian sole broodstock under effect of stocking density are shown in Tables (5 and 6). The results cleared that the stocking density had significant effects ($P \leq 0.05$) on the reproductive performance parameters such as number of fertilized eggs/ treatment, number of fertilized eggs/ female, number of fertilized eggs/ g female and number of larvae/ treatment. While, hatching rate (%), number of spawning and survival rate (%) had insignificant differences among treatments.

Table 5: Effect of stocking density on induced spawning of the Egyptian sole, *Solea aegyptiaca*.

Items	Stocking density (Kg/ m^3)				SED*
	0.5	**0.75**	**1**	**1.25**	
No. of fertilized eggs/ treat.	31386[b]	58840[a]	30636[b]	30986[b]	433.013
No. of fertilized eggs/ female	6277.2[a]	5884[b]	2785.09[c]	2213.29[d]	52.179
No. of fertilized eggs/g female	98.70[b]	129.32[a]	53.00[c]	43.22[d]	0.997
No. of larvae/ treat.	28742[b]	54826[a]	28111[b]	28566[b]	983.589
Hatching rate, %[1]	91.57	93.17	91.75	92.19	1.493
No. of spawning	5	7	7	5	-
Survival rate,%[2]	100	100	100	100	-

- (a, b, c) Average in the same row having different superscripts are differ significantly ($P \leq 0.05$).
* SED is the standard error of difference
1, Hatching rate, % = (Number of larvae/ Number of fertilized eggs per treatment) × 100.
2, Survival rate, % = (Number of fish at end/ Number of fish at start) × 100.

Fertilized eggs/ treatment values were highest with stocking density (0.75 Kg/m^3) followed the other stocking densities. Fertilized eggs/ female values were highest with stocking density (0.5 Kg/m^3), while the lowest value with stocking density (1.25 Kg/m^3). Fertilized eggs/ g female values were highest with stocking density (0.75 Kg/m^3), while the lowest value was with stocking density (1.25 Kg/m^3). Number of larvae/ treatment values were highest with stocking density (0.75 Kg/m^3) followed the other stocking densities. Hatching rates were relatively highest with stocking density (0.75 Kg/m^3) followed the other stocking densities. Number of spawning values were relatively highest under stocking density (0.75 and 1 Kg/m^3) followed stocking density (0.5 and 1.25 Kg/m^3). These results indicated that the best stocking density is (0.75 Kg/m^3) on reproductive performance parameters for the Egyptian sole broodstock under experimental conditions.

Table 6: Effect of stocking density on induced spawning of *Solea aegyptiaca* (hormone dose, date of injection and date of ovulation).

Items	Stocking density (Kg/ m^3)			
	0.5	**0.75**	**1**	**1.25**
Hormone dose	7000 IU/kg fish			
Date of injection	14 / 12 / 2014			
Date of ovulation				
The first	16/12/2014	16/12/2014	16/12/2014	16/12/2014
The second	25/12/2014	14/12/2014	25/12/2014	25/12/2014
The third	3/1/2015	3/1/2015	3/1/2015	3/1/2015
The fourth	17/1/2015	17/1/2015	18/1/2015	17/1/2015
The fifth	28/1/2015	28/1/2015	30/1/2015	30/1/2015
The sixth	- -	8/2/2015	10/2/2015	- -
The seventh	- -	3/3/2015	4/3/2015	- -

The third experiment: Comparative study between induced and natural spawning on reproductive performance

The results (Tables 7 and 8) showed that there are significant differences (P≤0.05) between induced and natural spawning in all reproductive performance parameters, except the number of spawning and survival rate. Reproductive performance parameters values were highest with induced spawning except the number of spawning compared with natural spawning. These results indicated that the induced spawning was better than natural spawning for the Egyptian sole broodstock under experimental conditions.

Table 7: Comparative study between induced and natural spawning of the Egyptian sole, *Solea aegyptiaca*.

Items	Induced spawning	Natural spawning	SED*
No. of fertilized eggs/ treatment	30566^a	21460^b	252.389
No. of fertilized eggs/ female	5094.33^a	3065.71^b	40.311
No. of fertilized eggs/g female	94.34^a	68.35^b	0.785
No. of larvae/ treatment	28278^a	19032^b	665.954
Hatching rate, %	92.51^a	88.68^b	1.662
No. of spawning	5	9	-
Survival rate, %	100	96.43	3.570

- (a, b, c) Average in the same row having different superscripts are differ significantly (P≤0.05).
* SED is the standard error of difference

Table 8: Comparative study between induced and natural spawning of *Solea aegyptiaca* (hormone dose, date of injection and date of ovulation).

Items	Induced spawning	Natural spawning
Hormone dose	7000 IU/kg fish	- -
Date of injection	14 / 12 / 2014	- -
Date of ovulation		
The first	16/12/2014	20/12/2014
The second	25/12/2014	26/12/2014
The third	3/1/2015	17/1/2015
The fourth	17/1/2015	28/1/2015
The fifth	29/1/2015	8/2/2015
The sixth	- -	23/2/2015
The seventh	- -	5/3/2015
The eighth	- -	12/3/2015
The nineth	- -	21/3/2015

DISCUSSION

Broodstock productivity clearly represents the most significant constraint on commercial fish production. Increased knowledge of the factors regulating broodstock productivity is therefore of great importance to the further development of *Solea aegyptiaca* culture. Hormonal induction of ovulation for *Solea aegyptiaca* was successful with human chorionic gonadotropin hormone (HCG). In the present study, artificial spawning of *Solea aegyptiaca* were achieved using HCG (total dose 7000 IU/Kg fish body weight).

In other studies, Assem *et al.* (2012) showed that, artificial spawning of *Solea vulgaris* were achieved using carp pituitary extract (CPE) from 40-70 µg/fish (equal to 200 µg/ Kg fish) or HCG from 2300 to 3000 IU/fish (equal to 10000 IU/ Kg fish) as a priming dose, followed by luteinizing and releasing hormone analogue (LHRHa) from 52- 60 µg/fish (equal to 200 µg/ Kg fish) in the resolving dose. Berlinsky *et al.*

(1997) induced the ovulation of *P. dentatus* with HCG (total dose for ovulation equal to 500 IU/ Kg) and CPE (total dose for ovulation equal to 16 mg/ Kg). Ovulation of *P. tropicus* was obtained with HCG 2000 IU/ Kg (Rosas *et al.,* 1999). Guzman *et al.* (2009) compared the efficiency of two sustained-release delivery systems loaded with GnRHa and a single GnRHa injection on the stimulation of oocyte maturation, sex steroid hormone secretion and spawning of Senegalese sole (*Solea senegalensis*). Agulleiro *et al.* (2006) studied induction of spawning of captive-reared Senegal sole (*Solea senegalensis*) using different administration methods for gonadotropin-releasing hormone agonist and concluded that: female injected with a dose of 5 µg GnRHa/kg three times a week, or treated with a single GnRHa loaded implant (50 µg/kg) showed multiple ovulations and spawns within a period of approximatiple 30 days. Mylonas and Zohar (2001) listed nine flatfish species where ovulation and/or spawning was induced using different GnRHa delivery system ranging from 30 to 1000 µg/kg. From these finding, it is concluded that hormonal induction of ovulation for sole fish was successful with HCG, CPE, LHRHa and GnRHa.

In the present study, *Solea aegyptiaca* fish always spawn at night; fish spawn pelagic eggs, which are fertilized externally and float individually near the water surface. The ripe eggs of *Solea aegyptiaca* appeared rounded, colorless and transparent. The surface of the fertilized egg shell is smooth, the fertilized egg appeared rounded. Many authors were in agreement with this description of the present study Assem *et al.* (2012) for *Solea vulgaris*, Zaki and Hamza (1988); Assem (1995); Baynes and Howell (1996) for *Solea solea.* In this study, the ripe and fertile eggs of *Solea aegyptiaca* was about 0.85 and 0.92 mm in diameter, respectively and newly hatched larvae were about 1.42 mm in length. On the other hand, Assem *et al.* (2012) recorded that, the ripe and fertile eggs of *Solea vulgaris* was about 1.04 and 1.2±0.01 mm in diameter, respectively and newly hatched larvae was about 1.8 mm in length. Also, Yufera *et al.* (1999) recorded that, *S. senegalensis* spawned eggs of about 1 mm diameter. In addition, Herrera *et al.* (2008) mention that, the newly hatched wedge sole larvae were 2.34 mm in length. Moreover, Jimenez *et al.* (2001) reported that, the newly hatched wedge sole larvae total length in wild is 1.57 mm. This value is smaller to that recorded in captivity (2.39 mm). This fact may be related to the large size of the captive breeders, as they were bigger than those found in the wild (Assem *et al.,* 2012).

In the present study, hatching of *Solea aegyptiaca* eggs occured after 48±3 h from fertilization at temperature 17-19 ºC, while occured after 60-72 h from fertilization at temperature 14-16 ºC. These results agreed with the results of Zaki *et al.* (1998) who reported that, the incubation period of eggs of *Solea aegyptiaca* at a temperature ranged from 16-18 ºC is 48±6 h. Also, Herrera *et al.* (2008) reported that, incubation lasted for 36-48 h for wedge sole at 19 ºC. Similarly, Salivatori *et al.* (1985) reported that hatching of *Solea* eggs occured after 45-46 h at temperature 16-18 ºC. Also, Zaki and Hamza (1986) incubated *Solea solea* eggs at temperature ranged between 11-17 ºC and hatched within 72-96 h. On the contrary, Assem *et al.* (2012) recorded that, *Solea vulgaris* eggs hatched after 38±2 h from fertilization at temperature of about 17±2 ºC. Also, Yufera *et al.* (1999) recorded that, *S. senegalensis* eggs hatched after 38 h from fertilization at temperature of about 19.5 ºC. In addition, Bedoui (1995) reported that hatching of *Solea senegalensis* eggs occured after 42 h at temperature 19 ºC. Moreover, Ramos (1986) showed that egg incubated at 20 ±1 ºC and hatching occurred after 40-44 h. Also, Zaki and Hamza (1988) stated that incubation of *Solea solea* eggs varied from 3-4 days at 11-14 ºC. Devauchelle *et al.* (1987) recorded that, *Solea solea* eggs hatched after 120 to 130 h from fertilization at temperature 13 ºC. From these finding, it is concluded that incubation period varied for the same species with variation of water temperature. Also, there is difference in hatching period with other species of fish.

In the present study, the percent of hatching rate varied between 90 and 95% at water temperature of 13-19 °C and salinity 31-34‰. Similar to our results, Salas-Leiton *et al.* (2012) reported that, percent of hatching for *Solea senegalensis* is ranging between 80.7 and 95.7% at water temperature of 20 °C and salinity 18-33‰. Also, Assem *et al.* (2012) noticed that, percent of hatching for *Solea vulgaris* is ranging between 89 and 90% at water temperature of 17±2 °C and salinity 34±1‰. In contrast, Blanco-Vives *et al.* (2010) reported that, percent of hatching for *S. senegalensis* was 78.1% at water temperature of 16-19 °C. Also, Dinis *et al.* (1999) mentioned that, percent of hatching for *S. senegalensis* was 72.1 ± 26.5%. Moreover, a hatching rate of the wedge sole (58.3- 85.2%) were recorded by Herrera *et al.* (2008). As well as described by Anguis and Canavate (2005) for Senegal sole (55.4-70.9%) and other cultured flatfish species as halibut (14-51%) by Mazorra *et al.* (2003). In this study, the high hatching rate is possibly due to the selection broodstock (Fish were healthy, free from parasites) and/or to the quality of the used hormone.

Effect of sex ratio

Reproductive success in many fish species has been shown to be influenced by, among other factors, the broodstock, sex ratio, stocking density, age, size, nutrition and feeding regime (Ridha and Cruz, 1989; Smith *et al.,* 1991; Salama, 1996; Izquierdo *et al.,* 2001; Chong *et al.,* 2004; Tahoun, 2007; Hammouda *et al.,* 2008; Ibrahim *et al.,* 2008).

Most studies on spawning of sole fish were used sex ratio at a rate of 1:1 male: female (Assem *et al.,* 2012 for *Solea vulgaris*), (Blanco-Vives *et al.,* 2010 for *Solea senegalensis*), (Blonk *et al.,* 2009 for *Solea solea*) and sex ratio 1:1-1:1.2 male: female for *Solea senegalensis* by Guzman *et al.* (2009). While Cardinaletti *et al.* (2009) found that, the sex ratio was 1.2:1 male: female during spawning trial for *Solea solea*. Imsland *et al.* (2003) recorded that, the environmental conditions used for *Solea solea* and *S. senegalensis* broodfish we find in published studies: sex ratio of 0.5–3 males to each female. In flatfish species such as flounder (*Paralichthys orbignyanus*) sex ratio used for stocking was 1:0.7 that is ten females: seven males (Bambill *et al.,* 2006). Some authors have recommended a sex ratio of 1-2 males: 1 female for *Paralichthys orbignyanus* reproduction, although the optimal ratio has not been determined yet (Kumagai 1999; Smith *et al.* 1999; Watanabe and Carroll 2001). In a study conducted on *Solea solea* in Bardawil lagoon, Mehanna (2014) found that, the sex ratio was 1:2.11 males to females during spawning season. In the an other study conducted on *Solea aegyptiaca* in Port said, Egypt, Mediterranean sea, Ahmed *et al.* (2010) found that, the sex ratio was 1:1.15 males to females during spawning season.

In the present study, three different broodstock sex ratios: 1:1, 1:2 and 2:1 (male ♂: female ♀) of *Solea aegyptiaca* were tested. The highest fertilized eggs/ treatment and larvae/ treatment were observed in sex ratio (1♂:2♀), thereafter, sex ratios (2♂:1♀) and (1♂:1♀). While, the highest fertilized eggs/ female, fertilized eggs/ g female, hatching rate and number of spawning were observed in sex ratio (2♂:1♀). Under the conditions tested in this study, larvae production of *Solea aegyptiaca* were affected by different broodstock sex ratios and improved at a sex ratio (1♂:2♀). While, fertilized eggs/ female and/ g female, hatching rate and number of spawning improved at a sex ratio (2♂:1♀). These results agreed with the results in other species;

Khalfalla *et al.* (2008) found that, the highest fry production of blue tilapia (*Oreochromis aureus*) obtained with the sex ratio (1♂:2♀) from three different broodstock sex ratios (1:1, 1:2 and 1:3 male: female). Also, Nour *et al.* (2008) evaluated the spawning results of three broad tilapia species, (*Oreochromis niloticus, O. aureus* and *O. galilaeus*) with two sex ratios (1:2 or 1:3 ; male: female) on the criterias of fry production. The results indicated that *O. niloticus* broad with (1♂:2♀) sex ratio produced the highest number of fry. The values of total production fry/ female, number of fry/ female per day, and number of fry/ g female showed the same trends. In addition, Mills and Reynolds (2003), found better performance of females stocked at lower sex ratios 1:2 and 1:3 (male: female) than those stocked at higher 1:4 and 1:5 (male: female) sex ratios. Moreover, Salama (1996) found that the highest fry production of Nile tilapia *O. niloticus* obtained with the lower sex ratio (1 male: 2 females). Similarly, Akar (2012) found that the highest fry production of Nile tilapia *O. niloticus* obtained with the sex ratios 1:2.5 and 1:3 (male: female) from four different broodstock sex ratios (1:1.5, 1:2, 1:2.5 and 1:3 male: female).

On the other hand, Khater (2002) studied the effect of different sex ratio on fry production of both *O. niloticus* and *O. aueus* brood-fish. No significant differences were found between mass spawning of *O. niloticus* and *O. aureus* at the different sex ratio 1:1, 1:2 and 1:3 in number fry/ g female body weight. He found that, the sex ratio of 1 male to 3 females is more economical for fry production. Also, Siddiqui and Al-Harbi (1997) studied four sex ratios of 1:2, 1:3, 1:4 and 1:5 male to female in hybrid tilapia reared in concrete tanks. They stated that there were no significant differences in seed production between all treatments. As well as, Ridha and Cruz (1998) used male to female sex ratio of 1:3, 1:4 and 1:5; their results showed that seed production was not influenced by sex ratio. Nevertheless, M'Hango and Brummett (1998) found that fry production was significantly higher in 1:1 male to female (111 fry/ female) compared to 1:3 sex ratio (66 fry/ female) for *O. shiranus.*

In other species, Targonska and Kucharczyk (2012) recorded that, the effectiveness of reproduction increases when there were more males than females in the spawning shoal, with the sex ratio being not lower than 2:1 male to female for Rosy barb (*Puntius conchonius*) at determination of the number of males in a spawning shoal on the reproduction (1 female and 1, 2, 3, or 4 males). A similar composition of the spawning shoal was recommended in spontaneous reproduction in other species of aquarium fish (Yanong, 1996; Kucharczyk *et al.,* 2008; Spence *et al.,* 2008).

In the present study, larvae production of *Solea aegyptiaca* were affected by different broodstock sex ratios, and this may be attributed to male fertilization efficiency, as it sometimes happens that more than one female is ready to spawn at the same time, while available males are not sufficient to fertilize the eggs, resulting in unfertilized eggs. Also crowding may have a negative effect (Salama, 1996). Some unfertilized eggs were observed in the sex ratio 2:1 male: female, this may be due to competition between males during spawning activity that caused some eggs to remain unfertilized. In addition, Grant *et al.* (1995) stated that higher male density led to increase aggression and male-male competition which could reduce the opportunity for female to spawn. This opinion was supported by Akar (2012). Mills and Reynolds (2003) concluded that there was low competition between males and higher spawning frequency with females occurred when fewer males were encountered during spawning activity.

When the sex ratio is equal to 1:1 and 1:2, male: female a lower hatching rate happens compared to 2:1, male: female. This may happen for a number of reasons, including a low number of spermatozoa produced by one male, which are insufficient to fertilise eggs, which can be attributed to the relatively high fertility of female *Solea aegyptiaca*. The results of studies of other species suggest that the number of spermatozoa per egg should be as high as over 100,000 (Rurangwa *et al.,* 2004). An insufficient number of spermatozoa results in decrease in the percentage of fertilised eggs, which means a reduced in percentage of growing embryos. Furthermore, an excessively high number of spermatozoa compared to the minimum number increases the embryo survival rate, and sometimes even compensates for the effect of other adverse factors (Rurangwa *et al.,* 1998, 2004; Casselman *et al.,* 2006).

The operational sex ratio (ratio of fertilizable females to sexually active males at a given time) is a principal factor influencing the intensity of sexual selection. The overall adult sex ratio is a key factor affecting sexual competition. If the adult sex ratio is biased, potential rates of reproduction are not sufficient to predict the direction of sexual competition. The sex ratio can also be influenced by the distribution of individuals in time and space, temperature and precopulatory guarding of multiple mates (Debuse *et al.,* 1999).

Effect of stocking density

Studies on spawning of the sole fish were used stocking density as followed: stocking density 4 fish/m^3 equal to 1.04-1.24 Kg/m^3 for *Solea vulgaris* (Assem *et al.,* 2012), stocking density range of 0.8-1 Kg/m^2 for *Solea solea* (Cardinaletti *et al.,* 2009). While Guzman *et al.* (2009) reported that, the stocking density range of 3-4 Kg/m^2 for *Solea senegalensis.* Imsland *et al.* (2003) recorded that, the environmental conditions used for *Solea solea* and *S. senegalensis* broodfish we find in published studies: moderate densities (0.6–3.0 Kg/m^3. Dinis *et al.* (1999) recorded that, stocking density in the maturation tanks should be 1–1.5 Kg/m^2 for *S. senegalensis*. Data of Dinis *et al.* (2003) indicated that *S. senegalensis* broodstock density might be up to 5 Kg/m^2.

In the present study, four different broodstock stocking densities: 0.5, 0.75, 1, 1.25 Kg/m^3 of *Solea aegyptiaca* were tested. The highest fertilized eggs/ treatment, fertilized eggs/ g female and larvae/ treatment were observed in stocking density 0.75 Kg/m^3 followed the other stocking densities, the lowest fertilized eggs/ female and/ g female were observed in stocking density 1.25 Kg/m^3. Under the conditions tested in this study, larvae production of *Solea aegyptiaca* were affected by different broodstock stocking densities and the best stocking density 0.75 Kg/m^3. These results agreed with the results in other species; Shubha and Reddy (2011) who indicated that, increase in stocking density significantly reduced fecundity (eggs/ female) for *Oreochromis mossambicus.* Also, Tahoun *et al.* (2008) indicated that increasing broodstock density significantly ($P \leq 0.05$) reduced broodstock fecundity (seed per female) for Nile tilpia (*Oreochromis niloticus*), the lower stocking density had the highest seed/ female, seed/ g female and seed/ day from three different broodstock density (4, 8 and 12 fish/m^2). As well as, Tsadik and Bar (2007) suggested lower stocking density of 3 broodfish/ m^3 to improve seed production of hapa -in- pond tilapia hatcheries. Several researchers studied the effects of different stocking densities on the reproductive performance particularly on Nile tilapia *O. niloticus* have demonstrated that increasing the level of stocking density significantly ($P \leq 0.05$) reduces spawning success and in turn mass production (Little, 1989; Ernst *et al.* 1991; Ridha and Cruz, 1999; Bhujel, 2000). Bhujel (2000) reported that, an inverse relationship between stocking density and the percentage of spawning females has been found in production hybrids of Tilapia, *O. niloticus* and *O. hornorum*, probably due to some chemical or behavioural factors. High stocking density inhibits reproduction possibly due to the presence of a substance in Tilapia mucus, which might cause an auto-allergic response.

These results disagreed with the findings in other species of Obi and Shelton (1988) who found that fry production per unit of area (m^2) in Tilapia, *O. hornorum* (Trewavas) tended to increase with the increase of broodstock stocking density. Also, Maluwa and Costa-Pierce (1993) reported similar observations for Tilapia, *O. shiranus*. Therefore, the conclusion of the above authors that a high number of seed could be produced at a higher stocking density might be true as long as broodstock density did not exceed the optimum density.

Under the conditions tested in this study, the observed higher fertilized eggs and larvae production at the lower stocking density (0.5 and 0.75 Kg/m^3) compared to other stocking densities indicated more synchronous spawning activity. Increasing the density beyond 0.75 Kg/m^3 was not effective to improve larvae production. At high densities, there is a competition for space which increases social interaction and in turn, causes social stress and possibly thereby affecting reproductive efficiency. Present opinion is supported by Shubha and Reddy (2011); Tahoun *et al.* (2008). Under intensive hatchery system, broodstock are often stocked at high densities in small and confined breeding units such as aquaria and tanks, resulting in aggression and fighting among males and thus, affecting seed production (Behrends *et al.,* 1993). Therefore, the manipulation of broodtock density is one of several technique applied to improve mass production of sole fish seed production than higher densities.

In conclusion, from the results of the present study, it is recommended to conduct artificial spawning of *Solea aegyptiaca* with sex ratio (1♂:2♀) and stocking density (0.75 Kg/m^3).

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ARABIC SUMMARY

تأثير نسبه الجنسين وكثافة التخزين على التفريخ الأصطناعي لأسماك موسى المصرية.

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أجريت هذه الدراسة لتقييم تأثير نسبه الجنسين وكثافة التخزين على التفريخ الأصطناعي لأسماك موسى المصرية (*Solea aegyptiaca*). تم حقن الأسماك بهرمون الجونادوتروبين البشري (HCG) بمعدل ٧٠٠٠ وحدة دولية/كجم من وزن الأسماك. استخدم ثلاث نسب جنسية مختلفة (١:١، ١:٢ و١:٢ ذكور: إناث) وأربع كثافات تخزين مختلفة (٠,٥ ، ٠,٧٥، ١ و ١,٢٥ كجم/م[٣]). أظهرت نتائج هذه الدراسة أن لكل من النسبة الجنسية وكثافة التخزين تأثير على مقاييس التناسل (عدد البيض المخصب لكل معاملة وعدد اليرقات الكلي لكل معاملة). كانت أعلى قيم لهذه المقاييس سجلت مع النسبة الجنسية (١:٢ ذكور: إناث) مع كثافة التخزين (٠,٧٥ كجم/م[٣]). نتائج تفوق التفريخ الأصطناعي بالمقارنة بالتفريخ الطبيعي. نتائج هذه الدراسة توصي بالتفريخ الأصطناعي لأسماك موسى المصرية مع استخدام النسبة الجنسية (١:٢ ذكور: إناث) وكثافة التخزين (٠,٧٥ كجم/م[٣]).