



Effect of Female Weight on Reproductive Performance of European Seabass (*Dicentrarchus Labrax*)

Samah S. El-Saiegh^{1*}, Mohamed F. Abd-Elghany², Gomaa A. Khalifa³ and Ahmed F. Fath El-Bab¹

1- Department of Animal Production, Faculty of Agriculture, Damietta University, Damietta, Egypt.

2- Department of Animal Production, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.

3- Alexandria Fish Hatchery, General Authority of Fish Resources Development, Ministry of Agriculture, Egypt.

*Corresponding Author's E-mail:: samahsadek2018@gmail.com ,

Received: July 29, 2021; **Accepted:** August 10, 2021 **published:** 2021 Vol.11 (3):01-18

ABSTRACT

The present study was carried out at Marine Finfishes Hatchery located at the 21 Km. west of Alexandria, Alexandria governorates. The hatchery belongs to the General Authority for Fish Resources Development (GAFRD), Ministry of Agriculture and Land Reclamation, Egypt. The study aimed to study the effect of female body weights on reproductive traits of European seabass (*Dicentrarchus labrax*). The experimental period extended during the period from December 2020 to April 2021. A total number of 30 females as parent stock of European seabass representing three weight groups up to 1500 gm, >1500-2000 gm and > 2000-2500 gm for groups 1, 2, and 3, respectively (10 each) were stocked in three fiberglass circular tanks (at diameter 2.5m and about size 10m³).

Total egg weight (g) per female (EW/F) and egg diameter (μm) (ED) were significantly affected by female weights groups. Absolute and relative fecundity were 56001.10, 60469.10 and

65368.69; 47.51, 43.35 and 38.40 for groups 1, 2 and 3, respectively. Female body weights had significant effect on both absolute and relative fecundity. Hatchability percentage were found to be 63.31, 64.15 and 64.36 % for the three female weights groups 1, 2 and 3, respectively.

The average larvae number per fish as affected by female body weight it was found to be 29822.72, 32242.95 and 34493.22 larvae for the three female weight groups 1, 2 and 3, respectively and the differences among averages of fry number per fish attributed to female weights were significant ($P < 0.001$).

Therefore, the heavier females produced the higher egg production/fish, egg diameter, absolute fecundity, hatchability percentage, larvae number/fish, larvae number/fish. Also, the heavier females produced the lower means of relative fecundity.

Key words: *reproductive performance, female weight, larvae, European seabass (Dicentrarchus labrax).*

INTRODUCTION

European seabass, *Dicentrarchus labrax* (Linnaeus 1758) belongs to the Actinopterygii class of the ray-finned species of the *Osteichthyes* superclass, and more specifically within the *Moronidae* family of the order of Perciformes, which is the largest order of vertebrates and the most diversified of all fish orders, with 62 families and 2,248 species (Nelson *et al.*, 2016). Its *Dicentrarchus* name comes from ancient Greek, with the etymology of di (two), kentron (sting) and archos (anus), possibly referring to its two anal spines, although today they are normally three (Acarli *et al.*, 2014).

Reproduction takes place at sea and has not been observed in lagoons and estuaries. Juveniles and adults from the lagoons migrate offshore where they mate with individuals from the open sea. There is only one spawning season per year, which occurs in winter in the Mediterranean Sea (December to March), and into June in the Atlantic Ocean. Spawning takes place in groups in mid-water, and the eggs can be found throughout the water column. Egg and larval development accelerates at higher temperatures. There is a difference in growth between sexes. Established “size–age” curves distinguishing male and female individuals show that at the same age, the females are larger than males (Fritsch, 2005).

Adult sea bass reproduces sexually by external fertilization. The sea bass is gonochoristic; sex confirmation is possible only during the spawning season. The proportion of females resulting from individual crossings may vary from 1 to 70%. Sex differentiation starts unusually 200 days post-hatching (dph), with females differentiating first. Temperature clearly influences sex ratio; high temperatures favor the development of males (Piferrer *et al.*, 2005).

The aim of the present study is to investigate the effect of fish body weight of European seabass (*Dicentrarchus labrax*) females on reproductive traits

such as total egg weight per female (g) (EW/F), egg diameter μm (ED), absolute (ABS) and relative fecundity (REL), hatchability% (HAT %) and larvae number per fish.

MATERIALS AND METHODS

Location:

The main objective of this experiment is to study the impact of female weight on reproductive traits of European seabass (*Dicentrarchus labrax*) and larvae number per fish. This experiment was carried out at Marine Finfishes Hatchery located at the 21 Km, west of Alexandria, Alexandria governorate. The hatchery belongs to the General Authority for Fish Resources Development (GAFRD), Ministry of Agriculture and Land Reclamation, Egypt during the period from December 2020 to April 2021.

Experimental females:

A total number of 30 females as parent stock of European seabass representing three weight groups (10 each) were stocked in three fiberglass circular tanks (at diameter 2.5 m and about size 10m^3), one tank for each weight group. All females used in this experiment aged from 3-4 years at start of the experiment. In each weight group, the females were randomly selected, and weighed. The three weights studied were up to 1500 gm; >1500-2000 gm and > 2000-2500 gm for groups 1, 2, and 3, respectively at the start of spawning.

Water supply, Drainage, and air supply systems:

Sea water was drawn directly from an intake point in the shore by subs and well point (150 μm slots, 3 pipes PVC, 3m length). Sea water was pumped by 4 KW polypropylene centrifuge pump to three concrete reservoirs of 60 m^3 capacity, 11 m diameter x 1.5 m depth. The reservoir was used as a setting for the coarser suspended particles before the water was filtered. Water was pumped from the reservoir to two constant head tanks (5 m^3 capacity) to distribute for maturation and spawning tanks, and pumped to larval tanks.

All the tank facilities were provided with two drainage systems, one for cleaning water and other to remove water used in experimental female propagation and larval rearing. The air supply system consists of three air blowers that work alternating at 24-hours interval. The air distribution network was constructed of PVC pipes and control valves.

Physico-chemical characteristics of experimental tanks water:

Data from Table (1) show the Physico-chemical characteristics of experimental tanks water during the propagation period and rearing of larvae

from December 2020 to April 2021. Water quality parameters including water temperature ($^{\circ}\text{C}$), dissolved oxygen (DO), pH, ammonia (NH_3 -N), and salinity (ppt %) were measured throughout the experiment. All water quality parameters were within the permissible levels for sea bass aquaculture.

Table (1): Physical and chemical water parameters in experimental tanks:

Parameters	Means
Water temperature ($^{\circ}\text{C}$)	20.6 ± 1.4
Dissolved oxygen (DO), mg/l	6.2 ± 0.2
pH	7.6 ± 0.4
Ammonia (NH_4 -N) mg/l	0.035 ± 0.016
Salinity (‰)	31.4 ± 1.7

Supplementary Feeding:

The broodstocks were fed on a commercial broodstock diet (45% CP, 15 % EE), (Table 2). The basal diet was composed of fish meal (62 % crude protein) (68 %), soybean meal (46 % crude protein) (20 %), extruded full-fat soya (10 %), yellow corn (15 %), wheat bran (21 %), corn gluten meal (60 % CP) (9 %), vitamin-mineral premix and mono-calcium phosphate (4 %), and fish oil (4 %). The broodstock was fed during the experimental period on a basal diet. The feeding rate was 1 % of the broodstock biomass twice a day at 8:00 a.m. and 3:00 p.m. for 7 days a week. In addition, fresh foodstuff (sardine, *Sardinella* spp.) was given to the broodstock in one meal per day at 2 % of the body weight.

The larvae absorbed the yolk sac during 3 days. Then, the unicellular algae as *Chlorella* spp, *Platymonas* spp were added for larvae feeding at a rate of 300,000 to 400,000 algal cells per ml. from the seventh to the fourteenth day, the crustacean, *Artemia salina* . The two species were routinely used. From the eighteenth day, artemia was enriched by fatty acids (C18 fatty acids) but do not contain C20–22 n-3 highly unsaturated fatty acids (n-3 HUFA), such as EPA (20:5 n-3) and DHA (22:6 n-3). On 24 days the natural feeding was partial replaced by a commercial broodstock diet (45% CP) at a rate of 10% of the biomass (Table 2).

Table (2): Ingredients and proximate composition of the commercial broodstock diet used in feeding sea bass from 12-36 months of age.

Ingredients	%
Fish meal (FM)	68
Rice bran	11
Yellow corn flour	10
Soybean meal	3
Cotton seed meal (decorticated)	4
Cod liver oil	2
Mineral +Vitamins mixture*	2
Total	100
Proximate analysis (%):	
Dry matter (DM)	88.4
Crude Protein (CP)	47.6
Total lipids (TL)	17.2
Ash	19.5
Crude Fiber (CF)	1.9
NFE ¹	13.8
Gross energy (GE) (kcal/100 g D.M) ²	434.3

* Each gram of vitamin premix contains (NRC, 1993), 20.000IUvit. A2000 IU vit. D3, 400 vit. E, 20 mg Niacin, 4.5 mg riboflavin, 3mg pyridoxine, 0.013 mg vit. B12, 100 mg chorine chloride and 2 mg vit K. E each gram of minerals contains 0.83 Ca, 0.63P, 0.78Na, 0.018 Mn, 0.011 Zn and 0.001 Cu. The Mixture was prepared by mixing 35parts of dicalcium phosphate, 3 parts of mineral premix, and 2 parts of common salt.

¹ Calculated by differences

²Growth energy was calculated using values of 5.65, 4.1, and 9.45 Kcal/g protein, carbohydrate, and lipid, respectively (Jobling, 1983).

Hatching Technique:

- Spawning:

On the 19th of December 2020 and 2021, (42 days after starting the feeding protocol) for 2 years, respectively. Samples of eggs were taken via ovary biopsy using a polyethylene cannula (E.D. 1.25 mm) to measure the average of egg diameter of the broodstock tanks. The percentage of matured eggs [egg with a diameter range of 260–400 µm (late developing), egg with a diameter range of 400–600 µm (mid-vitellogenic), 600–800 µm (late-vitellogenic)] and hydrated eggs (≥ 800 µm) were estimated per each female (Mayer *et al.*, 1988). Most fish were in the mid-vitellogenic stage. The male maturity was

checked by striping, which found to be in stage two (two striping for milt appearance) (Rottmann *et al.*, 1991).

- Blood sampling:

On the same time, blood samples were collected from a caudal vesicle of anaesthetized males and female broodstock of different groups, with minimum handling stress. Blood samples (~ 0.5 mL fish⁻¹) were collected using a 1-mL syringe containing 50 μ L of heparin and centrifuged ($750 \times g$, 10 min, 4 °C) to obtain plasma. The plasma samples were stored at -80°C until the assays of sexual hormones and biochemical parameters. Prior to blood sampling, hormonal injection and during all further handling procedures, fish were anaesthetized with 50 mg clove oil L⁻¹.

-Hormonal injection:

After six weeks of feeding, twenty broodstock (ten females and ten males from each tank) were selected and transferred to a fiberglass circular spawning tank (10 m³) at a sex ratio of 1 female: 1 male per tank for hormonal injections. Then, the fish were subjected to hormonal injection to activate the maturation of all females and assure synchronization of ovulation. The broodstock were injected intramuscularly into the base of the lateral fin with the LHRHa hormone (des-Gly10, D-Ala6-LH-RH Ethylamide acetate salt hydrate; Sigma-Aldrich, St Louis, Missouri, USA). The LHRHa dose (15 $\mu\text{g kg}^{-1}$ of the BW) (Gopakumar and Nazar, 2012) for females was divided into two injections. The first dose was 5 $\mu\text{g kg}^{-1}$ of BW, and the second dose was 10 $\mu\text{g kg}^{-1}$ of BW with a 6-hr interval with a hormonal solution volume of 0.5 mL kg⁻¹ BW, while the males were injected with a dose of 5 $\mu\text{g kg}^{-1}$ of BW. The spawning tank was fitted with a surface egg collector (a 400 μm mesh sized nylon net) attached to the discharge water pipe.

-Egg management:

The spawned eggs were released 24 h after hormonal injection. After completion of egg collection, the females were checked to assure the number of spent females. The eggs were weighed and the number of eggs (fecundity) was estimated by dividing the weight of the total eggs by the average individual egg weight, which was assessed by weighing and counting 1 g of the eggs. A sample of ~ 200 eggs from each tank were used to measure the average final egg diameter.

-Egg incubation:

The eggs were transferred to incubation tanks for four hours at a stocking density of 100 eggs per liter. The incubation tanks were filled with filtered and sterilized seawater with the same temperature and salinity as in the spawning tank. The total fertilized and non-viable eggs (fertilized sunk egg) were determined during the incubation period via microscopic investigation of ~5 eggs per sample repeated three times (**Carrillo *et al.*, 1989**). Then, the non-fertile eggs were removed by stopping the artificial aeration (1–2 min) and siphoning the eggs at the bottom of the tank.

-Hatchling management:

The fertilized eggs for each female were incubated in hatching tanks (2 m³ conical fiberglass) for 72 h at 15–16°C and a gentle aeration through a fine air stone with 300 % daily water exchange. Every day, artificial aeration was stopped in the hatching tank for 1–2 min, and then, the dead or non-fertilized eggs that settled on the bottom of the cone were drained. After hatching, newly hatched larvae in each hatching tank were counted before being transferred to the larval-rearing tanks to determine hatching (%). The counting technique was conducted by counting larvae present in a 100 mL subsample (five samples each time), and the average number of larvae in a sample was multiplied by the total volume of water in the hatching tank.

-Measured parameters:

Average of eggs weight (EW/F) spawned per seabass female was determined by gram. Number of eggs in one gram eggs weight (NE/G) was determined by weighting one gram of eggs then all eggs presented in this gram weight were counted. Weight of eggs in gram per kg live body weight was calculated by dividing the weight of eggs spawned per female on its live body weight. The absolute and relative fecundity was determined according to **Bhujel (2000)** as follows:

Absolute fecundity (ABS) = total weight of eggs per female (g) × number of eggs in one gram.

Relative fecundity (REL) = absolute fecundity / body weight (g).

Fertilizability was determined by counting the number of fertile eggs in sample as a percentage of the total number in the same sample.

Hatching (%)

= 100 × (No. of hatched eggs / total No. of fertilized eggs in each tank).

-Steroid hormone evaluation:

Follicle Stimulating Hormone (FSH; mIU ml⁻¹) was determined in plasma by the quantitative sandwich enzyme-linked immunosorbent assay (ELISA) kit (Mybioscience, San Diego, CA, USA) with a sensitivity of 0.1 mIU ml⁻¹ according to (**Jimenez *et al.*, 2005**). Fish Luteinizing hormone (LH; mIU ml⁻¹) was determined by the quantitative competitive ELISA Kit (Mybioscience, San Diego, CA, USA) with a sensitivity of 1.2 mIU ml⁻¹ (**Mananos *et al.*, 1997**). progesterone (prog; pg ml⁻¹) in females were measured by a simple solid-phase enzyme immunoassay (ELISA, DRG Diagnostics GmbH, Germany) based on the principle of competitive binding with the sensitivity of 0.083 ng ml⁻¹ and 9.71 pg ml⁻¹, respectively (**Tietz and Andresen, 1986 & Gore-Langton and Armstrong, 1988**).

Histopathological studies:

Seabass females were dissected directly in the hatchery, and the target organs (ovaries) were quickly removed. Histological techniques were performed according to **Bucke (1994)**. After fixation in Bouin's solution for 24 hr at room temperature, ovaries were dehydrated and routinely processed for paraffin embedding. Then, 5–7 µ thick sections were made in a rotary microtome and stained with hematoxylin and eosin. Ovaries were microscopically examined for a variety of histopathological features then photographed.

Statistical Analysis:

Differences among means were considered significant if P was less than 0.05. Significant differences ($P \leq 0.05$) among means were tested by the method of **Duncan (1955)**.

The statistical analysis of data was carried out by applying the computer program SAS (2012) by adopting the following fixed model:

$$X_{ik} = \mu + F_i + e_i$$

Where:

X_{ik} = The K^{th} observation for the i^{th} female weight.

μ = Overall mean.

F_i = The effect of i^{th} female weights.

e_{ijkl} = Random error.

RESULTS AND DISCUSSION

Total egg weight per female (g) and egg diameter (μm) for European seabass (*Dicentrarchus labrax*):

Total egg weight (g) per female (EW/F) and egg diameter (μm) (ED) for European seabass (*Dicentrarchus labrax*) as affected female weights. Table (3) show that, EW/F were 79.17, 99.77 and 105.27 and averages of ED were 1.00, 1.12 and 1.17 μm for the three female groups (up to 1500 gm), (>1500-2000 gm) and (> 2000-2500 gm), respectively, and the differences among averages due to the effect of the three female weights were significant ($P \leq 0.05$) for each variable.

These results are in accordance with those obtained by **Rana (1988)**. **Soltan et al (2007)** and **Fath El-Bab et al., (2011)** reported about the increase of egg weight produced by large and old fish. **Rana (1988)** found that, the egg number produced by a fish was more related to body weight, while **De Silva, (1986)** claimed that the increase was related to body length. **Cisse (1988)** found no significant correlation between spawner weight and number of spawning.

European seabass is gonochoristic and males mature normally at their second year, while females mature the following year (**Carrillo et al., 1995**). Recommended size for broodstock are males of 700 g and females of 1.5-2 kg (**Oretti et al., 1999**). Vitellogenesis begins in September October, around 4 months before spawning, which takes place from January to March (**Mañanós et al., 1997 and Carrillo et al., 1995**). Values of the diameter of anchovy eggs (longer axis) showed that the biggest eggs were found during sampling that took place in April 2007 and April 2008, while the smallest diameter of the eggs was found in July 2008. The previous findings confirm that the species that have a short lifespan produce largest eggs at the beginning of the spawning season (**Regner, 1989**). That is a kind of adaptation to the conditions of nutrition, which are, as a rule, very weak at the beginning of the spawning season. Larger eggs contain a larger amount of yolk and larvae hatched from those eggs are larger, which gives them a greater chance of survival when environmental conditions are not satisfactory (**Regner, 1989**). The small size of the eggs can be a sign that the spawning season ends (**Regner, 1985 and Gordina et al., 1997**). This reduction in the size of eggs when spawning season ends have been noted in other species – poor cod (*Trisopterus minutus*) (**Hislop, 1975**), and European and American mackerel **Picket and Pawson (1994)**.

Table (3): Least squares means and standard error for some factors affecting on egg weight (g)/fish and egg diameter (μm) for European seabass (*Dicentrarchus labrax*).

Female weight (FW)	No.	Traits	
		EW/F	ED
		LSM \pm SE	LSM \pm SE
FW1 (up to 1500 gm)	10	79.17 \pm 1.33 ^c	1.00 \pm 0.01 ^c
FW2 (>1500-2000 gm)	10	99.77 \pm 1.46 ^b	1.12 \pm 0.01 ^b
FW3 (> 2000-2500 gm)	10	105.27 \pm 1.44 ^a	1.17 \pm 0.01 ^a

Means with the same letter in each column are not significantly differences ($P < 0.05$).

Absolute (ABS) and relative fecundity (REL):

Results presented in Table (4) showed the, averages of absolute fecundity (ABS) as affected by female weights. The absolute fecundity and the relative fecundity were 56001.10, 60469.10 and 65368.69; 47.51, 43.35 and 38.40 \pm 1.07 for groups 1, 2 and 3, respectively. Analysis of variance showed that, females body weights had significant effect ($P \leq 0.05$) on both absolute and relative fecundity.

Fecundity was defined here as the number of eggs in a freshly spawned egg clutch (**Rana, 1988**) Many previous studies of fish have adopted the ‘classical’ definition of fecundity, i.e., the number of maturing oocytes in the ovaries prior to spawning (**Marshall, 1979 & Payne and Collinson, 1983**).

In this respect, **Hashem and El-Agamy (1977), Soltan et al (2003 & 2007) and Fath El-Bab et al. (2011)** revealed that, fecundity is a function related to length, weight and age of different fish species and it increased with increase in these parameters. **Watanabe and Kuo (1985)** reported that, absolute fecundity increased by using large and old fish. **Rana (1988) and Bhujel (2000)** indicated that, absolute fecundity is related to body weight, while **De Silva (1986)** found that, absolute fecundity is related to body length.

Estay et al., (1997) found that, the relative fecundity decreased with increasing of female body weight. On the other hand, **Rana (1986) and Bhujel (2000)** stated that, relative fecundity decreased with the decrease in age, body weight and body length of female Nile tilapia.

Table (4): Least squares means and standard error for some factors affecting on Absolute fecundity (ABS) and Relative fecundity (REL) for European seabass (*Dicentrarchus labrax*).

Female weight (FW)	No.	Traits	
		Absolute fecundity (ABS)	Relative fecundity (REL)
		LSM±SE	LSM±SE
FW1 (up to 1500 gm)	10	56001.10±2112 ^c	47.51±1.08 ^a
FW2 (>1500-2000 gm)	10	60469.10±2489 ^b	43.35±1.24 ^b
FW3 (> 2000-2500 gm)	10	65368.69±2563 ^a	38.40±1.07 ^c

Means with the same letter in each column are not significantly differences ($P < 0.05$).

4. 4. Hatchability % (HAT %) and Larvae number / female LN/F):

Averages of hatchability percentage as affected by female weights, the hatchability percentages were 63.31, 64.16 and 64.36 % for the three female weights groups studied (up to 1500 gm), (>1500-2000 gm) and (> 2000-2500 gm), respectively. these results were indicated that, hatchability percentages were increased with increasing females body weight. and these results are in agreement with those obtained by **Morsy (2001)** found that, averages of hatchability percentages in black carp females were 49.76, 58.44 and 88.69% for the weight groups 5-6, 6.5-7.5 and 8-9 kg respectively. (**Soltan *et al.*, 2003**) who stated that, hatchability increased by the increase in body weight of females.

On the other hand, **Fath El-Bab *et al.* (2011)** who stated that, hatchability decreased with increasing females body weight.

However, **Asturiano *et al.* (2006)** found that, the spawning criteria showed the superiority of big females in the absolute and relative fecundity, fertilization (%) and hatching (%). In the same manner, induced the best quality of eggs and larvae of Sea bass, *D. labrax*, but reduced spawning parameters.

Table (5): Least squares means and standard error for some factors affecting on Hatchability (HAT%) and Larvae number /Female of fish LN/F fish for European seabass (*Dicentrarchus labrax*).

Female weight (FW)	No.	Traits	
		Hat%	LN/F
		LSM±SE	LSM±SE
FW1 (up to 1500 gm)	20	63.31±0.70	29822.72±129 ^c
FW2 (>1500-2000 gm)	20	64.15±0.69	32242.95±112 ^b
FW3 (> 2000-2500 gm)	20	64.36±0.43	34493.22±107 ^a

Means with the same letter in each column are not significantly differences ($P < 0.05$).

The averages of larvae number per fish (LN/F) as affected by female weights are listed in Table (5). As shown in this table, the averages larvae number per fish (LN/F) as affected by female weights 29822.72, 32242.95 and 34493.22 larvae for the three female weights groups (up to 1500 gm), (>1500-2000 gm) and (> 2000-2500 gm), respectively, with significant differences ($P \leq 0.05$) among averages of larvae number per fish attributed to female weights effect (Table, 5).

These results were indicated that, hatchability percentage and larvae number per fish as affected by female weights were increasing in female body weight hatchability % and larvae number/fish increased. **Thorpe (1984)** and **Fath El-Bab et al. (2011)** found that, larger eggs produce significantly larger swim-up larvae. **Springate and Bromage (1985)** indicated that, there is no relationship between egg size and survival rates of the eggs, hatched fry and swim-up.

Reproductive hormones:

Averages of reproductive hormones of European seabass (*Dicentrarchus labrax*) female (LH, FSH and Progesterone) as affected by female weights (Table 6). The FL hormone was 1.11, 1.14 and 1.13 for female weights groups (up to 1500 gm), (>1500-2000 gm) and (> 2000-2500 gm), respectively (Table 6). The FSH hormone was 0.03, 0.04 and 0.04 for the three female weights, respectively. But the prog hormone was 4.12, 4.16 and 4.18 for female weights groups (up to 1500 gm), (>1500-2000 gm) and (> 2000-2500 gm), respectively.

Table (6): Least squares means and standard error for effect study year on reproductive hormones of European seabass (*Dicentrarchus labrax*) female.

Female weight (FW)	No.	Traits		
		LH	FSH	Prog
		LSM±SF	LSM±SF	LSM±SF
FW1 (up to 1500 gm)	20	1.11±0.02	0.03±0.003	4.12±0.02 ^b
FW2 (>1500-2000 gm)	20	1.14±0.02	0.04±0.003	4.16±0.01 ^{ab}
FW3 (> 2000-2500 gm)	10	1.13±0.02	0.04±0.003	4.18±0.01 ^a

Means with the same letter in each column are not significantly differences ($P < 0.05$).

Generally, the reproductive hormones were in the normal range for all female groups. Due to LHRH acts at a higher level of the hypothalamus–pituitary–gonad axis, LHRH can provide a more balanced stimulation of reproductive events and, presumably, a better integration of these events with other physiological functions, by directly or indirectly affecting the release of other hormones necessary for successful FOM, spermiation and spawning, which nearly agree with **Zohar and Mylonas (2001)** and **Ergun (2002)**.

Histopathological observations:

Fig 1 Photomicrograph of cross section in ovary of (*Dicentrarchus labrax*) at maturation stage (A, B): shows a normal structural wall of the ovary (red arrow), numerous mature oocytes with normal zona granulosa (black arrow) with the large nucleus N and yolk globule (yellow arrow) and yolk vesicle (head arrow) and some young oocyte (double arrow) this period is similar to the maturation period of **Al-absawy (2010)** for *Merluccius merluccius*. (C, D) shows oocytes at various stages of development: numerous primary growth oocytes (double arrow), and mature oocytes (black arrow). The yolk deposition in the oocyte of the present specie showed the same picture described by many authors for some other fishes such as **EL-Gharabawy (1996)** for *Lithognathus mormyrus* and **Assem (2000 and 2003)** for *Caranx crysos* and *Pagellus erythrinus*. (E, F) shows a thick wall of the ovary (red arrow) and numerous mature oocytes with normal zona granulosa (black arrow) with the large nucleus N and yolk globule (yellow arrow) and yolk vesicle (head arrow), and this period was agreed with the characterization of **Al-absawy (2010)** for *Merluccius merluccius*.

Fig 1 (A, B): Photomicrograph of cross section in ovary of (*D. labrax*) at maturation stage (X 40).

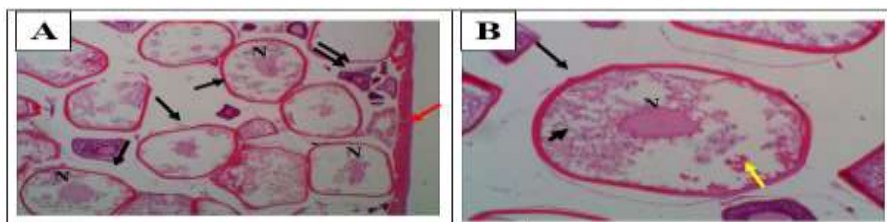


Fig 1 (C, D): Photomicrograph of cross section in oocytes at various stages of development: numerous primary growth oocytes. (X 100).

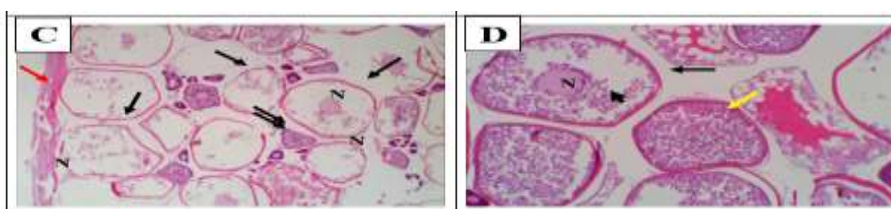
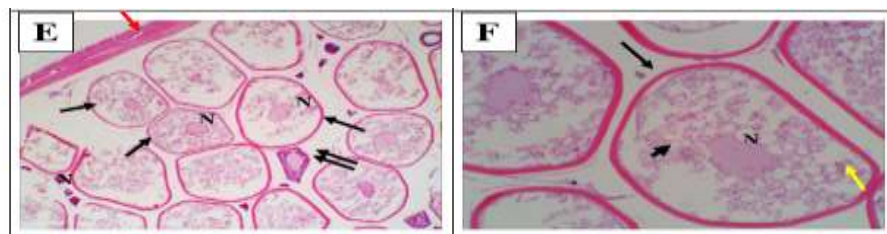


Fig 1 (E, F): Photomicrograph of cross section in a thick wall of the ovary and numerous mature oocytes with normal zona granulosa (X 250).



REFERENCES

- Acarli, D., Kara A. and Bayhan, B.** (2014). Length–weight relations for 29 fish species from homa lagoon, aegean sea, turkey. *Acta ichthyologica et piscatoria* (2014) 44 (3): 249–257.
- Al-Absawy, M. A.** (2010). The reproductive biology and the histological and ultrastructural characteristics in ovaries of the female gadidae fish *Merluccius merluccius* from the Egyptian Mediterranean water. *African Journal of Biotechnology* Vol. 9(17), pp.2544-2559.
- Assem, S. S.** (2000). The reproductive biology and histological characteristics of pelagic Carangid female *Caranx crysos*, from the Egyptian Mediterranean Sea. *J. Egypt Ger. Soc. Zool.*31(C)195 - 215.
- Assem, S. S.** (2003). The reproductive biology and the histological and ultrastructural characteristics of the ovary of the female pelagic fish

- Pagellus erythrinus* from the Egyptian Mediterranean water. J. Egypt. Ger. Soc. Zool. 42: 77-103.
- Asturiano, J. F.** (1999). El Proceso Reproductivo De La Lubina Europea *Dicentrarchus labrax* L. Efectos De Los ácidos Grasos De La Dieta: Estudios in Vivo E in Vitro. Ph. D thesis. Valencia University, Spain, pp. 251.
- Bhujel, R. C.** (2000). A review of strategies for the management of Nile tilapia (*Oreochromis niloticus*) brood fish in seed production systems, especially hapu-based systems. Aquaculture, 181, 37-59.
- Bucke, D.** (1994). Methodologies for demonstrating pathological changes in flounder (*Platichthys flesus*) (L.). In: Diseases and Parasites of Flounder in the Baltic Sea. BMB Publ., 15: 131-143.
- Carrillo, M., Bromage, N., Zanuy, S., Serrano, R. and Prat, F.,** (1989). The effect of modifications in photoperiod on spawning time, ovarian development and egg quality in the sea bass (*Dicentrarchus labrax* L.). Aquaculture 81, 351–365. [https://doi.org/10.1016/0044-8486\(89\)90159-2](https://doi.org/10.1016/0044-8486(89)90159-2).
- Carrillo, M., Zanuy, S., Prat, F., Cerda, J., Ramos, J., Mañanos, E., and Bromage, N.,** (1995). Sea Bass (*Dicentrarchus labrax*), in: Broodstock Management and Egg and Larval Quality, Bromage, N.R. and Roberts R.J., Eds., Blackwell Science, Oxford, UK, 138.
- Carrillo, M., Zanuy, S., Prat, F., Cerda, J., Ramos, J., Mañanós, E. and N. Bromage** (1995). Seabass (*Dicentrarchus labrax*). Broodstock Management and Egg and Larval Quality. Blackwell Science, Oxford.: 138-168.
- Cisse, A.** (1988). Effect of varying protein levels on spawning frequency and growth of *Sarotherodon melanotheran*. In Proc.2nd int. Symp. On tilapia in Aquaculture. No. 15.
- De Silva, S. S.** (1986). Reproductive biology of *Oreochromis mossambicus* populations of man-made lakes in Srilanka: a comparative study. Aquaculture and Fisheries Management, 17: 31-47.
- Duncan, D. B** (1955). Multiple range and multiple F- test. Biometrics, range (1): 1-42. FEAP (2006). Aquaculture statistics. www.feap.org.
- Ehrenbaum, E.,** (1923). Uber die Mackrele. Wiss. Meeresunters. In: Regner, S (1985). Ecology of planktonic stages of the anchovy, *Engraulis encrasicolus* (Linnaeus, 1758), in the central Adriatic. Acta Adriat. 26(1), Series Monographiae, 1: 113
- El-Gharabawy, M. M.** (1996). Histomorphology of ovarian changes during the reproductive cycle of *Lithognathus mormyrus* (Teleostei; Sparidae) J. Egypt. Ger. Soc. Zool. 19(A): 97-115.

- Ergun, B.** (2002). Sea Bass (*Dicentrarchus labrax* L., 1781) Seed production. Turkish journal of fisheries and aquatic sciences, 2:61-70.
- Estay, F., Diaz, N. F., Neira, R. and Garcia, X.** (1997). Reproductive performance of cultured female coho salmon in Chile. PROG. FISH-CULT. 59: 36-40.
- Fath El-Bab, A. F., Farag, M. E., Ramadan, A. A. and Amal. S. Hassan** (2011). Effect of temperature and female weight on reproductive performance of two Nile tilapia (*Oreochromis niloticus*) populations Egypt. J. Aquat. Biol. & Fish., 15 (2):179-193.
- Fritsch, M.,** (2005). Biology and exploitation of the sea bass *Dicentrarchus labrax* (L.) in the French fisheries of the English Channel and the Bay of Biscay. Ph.D. thesis, Université de Bretagne Occidentale.
- Gopakumar, G. and Nazar, A. A.,** (2012). Broodstock Development, Breeding and Larval Rearing of Cobia and Pompano.
- Gordina, A. D., Nikolskiy, V. N., Niermann, U., Binge, F. & Subbotin, A. A.** (1997). New data on the morphological differences of anchovy eggs (*Engraulis encrasicolus* L) in the Black Sea. Fish. Res. 31 (1997): 139-145. harengus). J. Cons. Int. Expl. Mer. 28: 210-240.
- Gore-Langton, R. E., Armstrong, D. T.,** (1988). Follicular stoidogenesis and its control. In: Knobil, E., Neill, J. (Eds.), The Physiology of Reproduction. Raven Press, New York, pp. 331–385.
- Hashem, M. T. and El-Agamy, A.** (1977). Effect of fishing and maturation on the barbusbynni population of the Nozha Hydrome. Bull Inst. Ocean and fish A. R. E., 7:137-151.
- Hislop, J. R. G.** (1975). The breeding and growth of whiting *Merlangius merlangus* in captivity. J. Cons. Int. Explor. Mer., 36(2): 119-127.
- Jimenez, M., Spaliviero, J., Grootenhuis, A., Verhagen, J., Allan, C., Handelsman, D.,** (2005). Validation of an ultrasensitive and specific immunofluorometric assay for mouse follicle-stimulating hormone. Biol. Reprod. 72, 78–85. <https://doi.org/10.1095/biolreprod.104.033654>.
- Jobling, M.,** (1983). A short review and critique of methodology used in fish growth and nutrition studies. J. Fish Biology, 23: 685 - 703.
- Mañanós, E.L., Zanuy, S., and Carrillo, M. P.** (1997). manipulations of the reproductive cycle of sea bass (*Dicentrarchus labrax*) and their effects on gonadal development, and plasma 17 α -estradiol and vitellogenin levels, Fish Physiol Biochem, 16, 211.
- Marshall, B. E.** (1979). Observations on the breeding biology of *Sarotherodon macrochir* (Boulenger) in Lake McIlwaine, Rhodesia. J. Fish. Bioi., 14, 419 -24.

- Mayer, I., Shackley, S. and Ryland, J.,** (1988). Aspects of the reproductive biology of the bass, *Dicentrarchus labrax* L, a histological and histochemical study of oocyte development. J. Fish Biol. 33, 609–622. <https://doi.org/10.1111/j.1095-8649.1988.tb05504.x>.
- Morsy, K. A. I.** (2001): Studies on carp propagation in Egypt. M.Sc. Thesis, Faculty of Agriculture, Zagazig University-Banha Branch.
- Nelson, J. S., Grande, T. C. and Wilson, M. V. H.** (2016). Fishes of the World (5th edn), pp. John Wiley & Sons, Hoboken, New Jersey.
- NRC,** (1993), Nutrient Requirement of Fish. Committee on Animal Nutrition. Board on Agriculture, National Research Council. National Academy Press. Washington, D.C.1993
- Oretti, A., Pedini Fernandez-Criado, M., Cittolin, G., and Guidastri, R.,** (1999). Manual on hatchery production of seabass and gilthead seabream, Vol. 1, FAO, Rome, 194.
- Payne, A. I. and Collinson, R. I.** (1983). A comparison of the biological characteristics of *Sarotherodon aureus* (Steindachner) with those of *S. niloticus* (L.) and other tilapia of the delta and lower Nile. Aquac., (30): 335-51.
- Picket G.D., Pawson M.G.** (1994) Sea Bass; Biology, exploitation and conservation. St. Edmundsbury Press, Suffolk (Great Britain). ISBN 0412 40090 1.
- Piferrer, F., Blázquez, M., Naivarro, L., González, A.,** (2005). Genetic, endocrine, and environmental components of sex determination and differentiation in the European sea bass (*Dicentrarchus labrax* L.). Gen Comp Endocrinol 142:102–110.
- Rana, K. J. and Macintosh, D. J.** (1988). A comparison of the quality of hatchery-reared *Oreochromis niloticus* and *O. mossambicus* fry. In the second international symposium on Tilapia in Aquaculture, ICLARM conference proceedings, 15, (R.S.V. pullin. T. Bhukaswan, K. Tonguthai & J.L. Maclean eds), 497-502.
- Rana, K.** (1988). Reproductive biology and the hatchery rearing of tilapia eggs and fry, In: Muir, J.F., Roberts, R.J. Eds., Recent advances in aquaculture, Croom Helm, London and Sydney and Timber press, Portland, OR, USA. 3, 343 – 406.
- Rana, K. J.** (1986). Parental influences on egg quality, fry production and fry performance in *Oreochromis niloticus* Linnaeus and *O. mossambicus* Peters. Ph D Thesis, Institute of Aquaculture, University of Stirling, UK, 295 pp.

- Regner, S.** (1985). Ecology of planktonic stages of the anchovy, *Engraulis encrasicolus* (Linnaeus, 1758), in the central Adriatic. Acta Adriat. 26(1), Series Monographiae, 1: 1-113.
- Regner, S.** (1989). Reproduction of fishes and ecology of their early stages. Manual. Institute of oceanography and fisheries - Centre for the training of fishing personnel from developing countries, Split, 107.
- Rottmann, R.W., Shireman, J.V., Chapman, F.A.,** (1991). Determining Sexual Maturity of Broodstock for Induced Spawning of Fish. SRAC Publication No. 423.
- SAS** (2012). Institute Inc. SAS/STAT Statistics user's guide. Statistical analytical system, 5th rev ed. Cary, NC, USA: SAS Institute Inc; 2012.
- Soltan, M. A., Hegazi, A. Z., El-Laithy, S. M. M. and Fath El-Bab, A. F.** (2007). Effect of Spawning Season, Male and Female Body Weight on Reproductive Performance of Nile Tilapia, *Oreochromis niloticus*. J. Egypt. Vet. Med. Asso. 67 No. 1:97-108.
- Soltan, Radwan, A. A., Sadek, S. M., Ammar, A. A. and Garrah, K. A.** (2003). Some Factors Affecting on Propegation of Black Carp, *Mylopharyngodon Piceus* Under the Egyptian Conditions. Annals Of Agric. Sc., Moshtohor, Vol. 41(4):1479-1491.
- Springate, W. and Bromage, N. R.** (1985). Effect of egg size on early growth and survival in rainbow trout (*Salmo gairdneri* R.) Aquaculture, 47: 163-173.
- Thorpe, J. E.** (1984). Development rate, fecundity and egg size in Atlantic Salmon (*Salmo salar* L.). Aquaculture, 43: 289-306.
- Tietz, N. W., Andresen, B. D.,** (1986). Textbook of Clinical Chemistry. Saunders, Philadelphia, USA.
- Watanabe, W. O. and Kuo, C. M.** (1985). Observations on the Reproductive Performance of Nile Tilapia (*Oreochromis niloticus*) in Laboratory Aquaria at Various Salinities. Aquaculture, 49, 315-323. [http://dx.doi.org/10.1016/0044-8486\(85\)90088-2](http://dx.doi.org/10.1016/0044-8486(85)90088-2)
- Zohar Y. and Mylonas, C. C.** (2001). Endocrine manipulations of spawning in cultured fish: From hormones to genes. Aquaculture 197(1):99-13. DOI:10.1016/S0044-8486(01)00584-1