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Reproductive biology and histological features of female thread fin bream *Nemipterus japonicus* in Gulf of Suez, Egypt

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Abstract

In the present study the value of the exponent in the length-weight relationship for females was 2.975 which indicate that this species has isometric growth. The length at which 50% females were mature was 11.1 cm. The GSI increased gradually with ovarian development where recorded maximum value 4.3 in May and minimum value 0.5 in February. Fecundity increased at the rate of 2.8971, 1.0199 and 0.8135 times of the total length, weight of fish and weight of ovary, respectively. Positive correlation was observed between fecundity and total length, weight of fish and weight of ovary. The stages of ovarian development and reproductive season of female thread fin bream *Nemiptrus japonicus* in Suez Gulf was investigated by using the gonado somatic index (GSI) and ovarian histology. The stages of oocyte development were classified into eight developmental stages (Perinuleolus, cortical alveolus, oil droplet, yolk globule, migratory nucleolus, prematuration, and maturation) based on histological characteristics. Oocytes development appears to be apparently spawned more than one time during the prolonged spawning season (summer). Therefore *N. japonicus* belong to synchronus groups. The stage of the most developed oocyte was used to classify the stage of ovarian maturation. Oocytes in the perinucleolus, cortical alveolar and oil droplets stages were present in ovaries throughout the fishing season in the Gulf of Suez. So, the spawning season of *Nemiptrus japonicus* is prolonged one which extends from May to September in Suez Gulf.

Keywords: Nemiptrus japonicus, length-weight relationship, length at first sexual maturity, fecundity, ovarian histology, reproductive biology, spawning season.

1. Introduction

Thread fin bream *Nemipterus japonicus* is one of family nemipteridae which have economic importance in the Gulf of Suez. Its catch represented an important component in the trawl fishery. It is a demersal species, very abundant in costal water, found on mud or sandy bottoms at depth varied from 5 to 80 m, usually in schools of Kerdgari *et al*. (2009). It has a wide distribution from the Red Sea and eastern shores of Africa to the Philippines and Japan (FAO, 1983).

Nemipterus japonicus constitutes an important part of the trawl catch in the South China Sea (Eggleston, 1972; Lee, 1974; Weber and Jothy, 1977, Andaman Sea (Senta and Stan, 1975), Bay of Bengal (Krishnamoorthi, 1971) and also distributed in Persian Gulf and Oman Sea (Valinassab *et al*., 2006). *N. japonicus* considered one of the major species in the southern Red Sea and in the Gulf of Suez (Ben-Tuvia and Grofit, 1973). The presence of *N. japonicus* in the Mediterranean is due to its migration from the Red Sea via the Suez Canal (Golani and Sonin, 2006).

Many authors studied the reproductive biology of *Nemipterus japonicus* such as (Eggleston, 1972; Krishnamoorthi, 1973; Murty, 1984; Bakhsh, 1996; Rajkumar *et al*., 2003; Manojkumar, 2004 and Kerdgari *et al*., 2009). Length-weight relationship (Murty, 1984; Kerdgari *et al*., 2009).

The present study aimed to estimate reproductive cycle of the thread fin sea bream in Suez Gulf by studying the seasonal change in GSI, fecundity and histological observations of ovaries allowing a clear assessment throughout the annual cycle of ovaries development.

2. Materials and methods

Specimens of thread fin bream *Nemipterus japonicus* were caught from Suez Gulf and landed at El-Attaka fish landing center. Collection of samples was done during the period from September, 2006 to May, 2007. Biological measurements including total length (T.L), body weight (BW) gonad weight (GW) and gutted weight (Gut. wt) were done. The gonado somatic index (G.S.I) was calculated according to the formula:

(G.S.I =GW X 100/ Gut.wt)

The length–weight relationship (LWR) was estimated by using the equation:

$$W = aL^{b}$$

where W = weight in grams, L = total length in centimeters, a and b are constants (Pauly 1983).

Three ovaries of fish at different developmental stages were selected to ensure the homogenity of oocyte diameter and number of oocytes. Each ovary was divided into 6 parts (the anterior, middle, and posterior parts of each lobe), 0.05 g taken from each part was placed on glass slides with a grid scale, and all oocytes were measured and counted with Microscope.

For histological studies the ovaries were fixed in Bouin solution and then transferred to 70% ethanol followed by dehydration, and then embedded in paraffin wax. The standard paraffin sections of 5 µm were stained with haematoxylin and eosin as well as milligant trichrome, stain and observed under a NIKON microscope.

3. Results

3.1. Length weight relationship

Length-weigh relationships which represented in Figure (1) were based on samples obtained during various months. A double logarithmic regression analysis was carried out using the formula:

$$Log(W)=Log (a) + b Log (L)$$

Where w= weight (g), L= Total length (cm), a is a constant and b an exponent from the general equation:

$$w=aL^{b}.$$

the results revealed that the regression equation for *N. japonicas* was

$$W=0.0207* L^{2..975}$$

and the correlation coefficient was highly significant (r^2 = 0.921, for females).

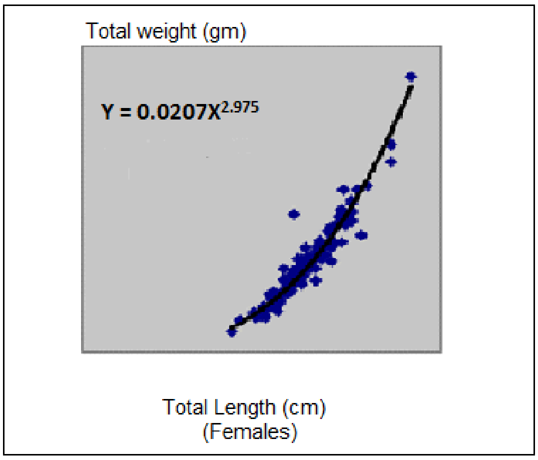

Figure 1. Length–weight relationship of female *Nemipterus japonicus* from Suez Gulf.

3.2. Length at first maturity

The results revealed that the smallest length of mature females was 9.0 cm. The length at which 50% females was 9.0 cm. The length at which 50% females were mature was 11.1 cm. (Figure 2).

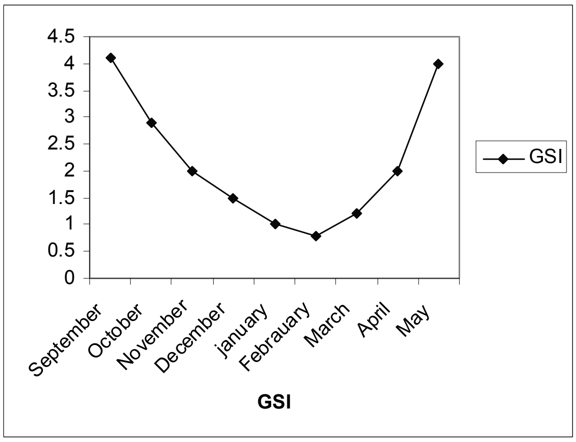

Figure 2. Monthly variation in the gonadosomatic index (GSI) of *Nemipterus japonicus* from Suez Gulf.

3.3. Gonado somatic index (GSI)

The monthly variations of GSI of females *N. japonicus* are given in (Figure 3) and revealed the maximum values were 4.3 and 4.1 in May and September respectively then gradually decreased from October to reach a minimum value 0.5 in February.

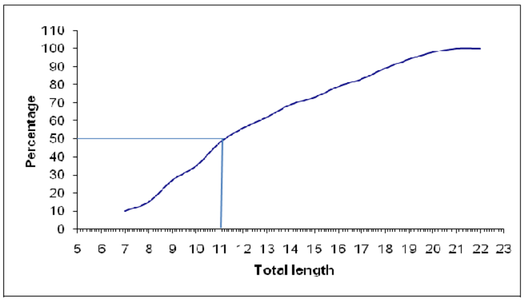

Figure 3. Length at first sexual maturity of female *Nemipterus japonicus* from Suez Gulf.

3.4. Fecundity

Data on observed and calculated number of mature eggs produced by ovaries of different sizes as shown in (Table 1) revealed that the mean total number of mature eggs varied from 14976 ±2100.77 to 74541±1245.922 in individuals of 15 cm to 23 cm. The mean fecundity value of 30 specimens was 42518.2± 5214.24. The relationships between fecundity and total length (cm), total weight (g) and weight of ovary (g) as shown in Table 2 revealed the best fit by a logarithmic equation as:

$$\text{Log } F = -2.0112 + 2.8971 \text{ Log } L \quad (r=0.7268)$$

Where F = Fecundity and L = total length of fish (cm).

The fecundity and weight of fish (g) relationship can be expressed in the form of:

$$\text{Log } F = 2.8536 + 1.0199 \text{ Log } W \quad (r=0.7726)$$

Where F = Fecundity and W = weight of fish.

The relationship between fecundity and weight of ovary (g) can be expressed in form of next regression equation:

$$\text{Log } F = 4.4427 + 0.8135 \text{ Log } w \quad (r=0.7648)$$

Where F = Fecundity and w = weight of ovary in gram (g).

The relationship between fecundity and total length, weight of fish and weight of ovary, it can be seen that there was linear relationship between fecundity and the three variables. Fecundity increased at the rate of 2.8971, 1.0199 and 0.8135 times of the total length, weight of fish and weight of ovary, respectively. Positive correlation was observed between fecundity and total length, weight of fish and weight of ovary. The correlation coefficient values were r = 0.7268, (P<0.05); r = 0.7726, (P<0.05); r = 0.7648, (P<0.05), respectively.

Table 1. Observed and calculated fecundity female *Nemipterus japonicus* from Suez Gulf.

Total length (cm)	No. of fish	Total weight (g)	Ovary weight (g)	Observed Fecundity	Calculated weight		
					Length	Weight	Ovary
15	5	26.80± 2.81188	0.56 ± 0.260576	14976.71± 2100.77	677.14± 159.501	20444.29± 201.213	16899.86± 6471.114
16	5	33.16± 4.206871	1.16± 0.207429	25494.83± 4664.577	25136.17± 1941.78	25965.83± 3478.334	31050.5± 4599.845
17	4	40.66± 2.826753	2.27± 0.281129	41684± 5784.356	28898.33± 1413.931	31251.67± 2216.063	53777.67± 5474.802
18	4	48.06± 2.750576	1.63± 0.443757	40587.2± 5386.982	35749.6± 1786.547	37162± 2303.353	40996.8± 9095.257
19	3	52.86± 7.717677	1.84± 0.241937	54593.33± 7056.223	40001± 1903.524	40844.67± 6077.771	45377± 4836.099
20	3	63.83± 0.572756	2.04± 0.014142	36362.5± 88.38835	44370.5± 1994.748	50211± 1462.297	26803.5± 31483.93
21	2	81.16± 1.378858	3.135± 0.219203	83103± 914.9962	53630± 770.7464	62938± 674.5799	72020± 1091.773
22	2	76.17± 4.815397	1.665± 0.784889	78498.5± 6841.258	64252± 1735.24	59274.5± 3821.912	41375.5± 16136.88
23	2	82.055± 3.500179	1.545± 0.615183	74541± 1245.922	65613.5± 190.2117	77129± 20746.51	31055± 1541.493
Average				42518.2± 5214.24	42519± 1854.52	42122± 5965.82	39564± 8984.527

Table 2. The parameters of the relationship between total fecundity and body weight, standard length and ovary weight in female *Nemipterus japonicus* from Suez Gulf.

Independent Variable	a	b	S.E.	r
Length	- 2.0112	2.8971	0.4218	0.7268
Weight	2.8536	1.0199	0.4102	0.7726
Ovary weight	4.4427	0.8135	0.2951	0.7648

3.5. Histological characteristics of gonads

3.5.1. Oocyte development

The stage of oocyte development in *Nemipterus japonicus* follows the classification of oocyte growth according to Takashi and Robert, (2005).

3.5.1.1. Perinucleolus stage

Oocytes in the perinucleolus stage (Figure 4) have a relatively large nucleus and the cytoplasm is strongly stained with heamtoxylin . Oocytes of this stage occurred throughout fishery season and had a size of 30-60 µm in diameter.

3.5.1.2. Cortical alveolar stage

Cortical alveoli are observed (Figure 5) along the periphery of cytoplasm and these alveoli increase in number and volume with oocyte growth their diameter ranged from 65-100 µm.

Cortical alveoli are located at along approximately half the distance between the nucleus and the periphery of the oocyte (Figure 6). Oil droplets appear and increase in number and volume with oocyte growth; concentric arcles along the periphery of the nucleus. The vitelline membranes enlarge and oil droplets become larger than the cortical alveoli. This stage was observed throughout the fishing season and the oocytes in the stage were 120-200 µm in diameter. Follicular epithelium and zona radiate can be sean differentiated.

3.5.1.3. Yolk globule stage

Yolk globules are strongly stained by eosin and began to appear along the outer layers of the vitelline membrane. These got enlarged progressively. The oocytes in this stage were 250-400 µm in diameter (Figure 7). Follicular epithelium membrane and zona radiate increased in thickness to 2 and 3 µm respectively. Fat vesicles can be seen distributed in the cells and ranged in diameter between 8 and 19 µm.

3.5.1.4. Migratory nucleus stage

The yolk globules began to fuse and eventually form a large yolk mass toward the center of the oocytes and the fat vesicles coalsed and increased in size to 13 µm in average. The nucleus and associated yolk mass migrate toward the animal pole (Figure 8). The oocytes in this stage were 510-600µm in diameter.

3.5.1.5. Pre maturation stage

The nuclear membrane disappears (Figure 9). The oocytes in this stage were 630-900 µm in diameter.

3.5.1.6. Maturation stage

The yolk of this stage liquified and the nucleus is no longer visible and circular shape (Figure 10). The fat vesicles fuse to form a single mass of oil droplet located at the animal pole. The oocytes in this stage were 750-1000 µm in diameter.

3.5.1.7. Atretic oocyte

Atretic oocyte in several transitional stages can be detected (Figures 11 & 12) show the yolk globule stage, vitelline membrane and yolk globules are degraded.

3.6. Maturation stage of ovary

Maturation stages of ovaries were determined based on histological observation of the most advanced oocyte development as follows:

3.6.1. Stage A

The most advanced oocytes are at the perinucleolus stage (Figure 4) .The cytoplasm of the oocyte is strongly stained by heamatoxylin and a few nucleoli are observed in the nucleus. The diameter of the oocytes is smaller than 70 µm.

3.6.2. Stage B

The most advanced oocytes are at the cortical alveoli stage (Figure 5). cortical alveoli ,which are randomly distributed in the peripheral region of the cytoplasm are found. The diameter of the oocytes is 65-100 µm.

3.6.3. Stage C

The most advanced oocytes are between the oil droplet stage and early yolk globule stage. Oil droplet stage increased in size and the cytoplasm. The nucleoli are located in the oocytes at the edge of the nucleus. The diameter of the oocytes is 250-400 µm (Figure 13)

3.6.4. Stage D

The most advanced oocytes are at the migration nucler. Yolk globules increase in size and many fatty droplets are situated around the nucleus which located at the center of the oocytes. The nucleus migrates toward the animal pole. The diameter of oocytes 510-600 µm (Figure 14).

3.6.5. Stage E

The most advanced oocytes reach the migratory nucleus and pre maturation stage. Oil droplets fuse with one another and become a single large oil globule located at the center of the oocyte. The diameter of oocytes is 630-900 µm (Figure 1).

3.6.6. Stage F

Fully ripe ovary not present in the fishing season

3.6.7. Stage G

Atretic oocytes are dominant in the ovary. The cytoplasm of atretic oocytes is a part from the oocyte membrane (Figures 8 &9).

3.7. Changes in the maturation of ovary

Several stages of oocytes were observed in the ovaries, because of this, the stage of oocyte which was most developed in the ovary was used as the stage of ovarian maturation (Takashi and Robert , 2005). All ovaries had oocytes at the oil droplet stage in whole fishing season. Oocyte, in the late yolk globule stage was first observed in March. The percentage of individuals with oocytes that developed from the yolk globule stage gradually increased through April. Oocytes at the maturation stage and post ovulatory follicles were observed in November. The presence of post ovulatory follicle stage increased and all individuals had oocytes of post ovulatory follicle stage in January .peak spawning appears to occur in May fully ripe ovaries were absent from the population.

4. Discussion

The length-weight relationship is an important factor in the biological study and stock assessment of fishes (Abdurahiman *et al*., 2004). This relationship is helpful for estimating the weight of a fish of a given length in which can be used for studying the gonad development, rate of feeding, metamorphosis, maturity stage and condition (Le Cren, 1951). Murty (1984) estimated values of slope for *N. japonicus* males (2.43) and females (2.95) from KaKinda in Indian waters.

Bakhsh (1994) found that this slop to be 2.42 for males and 2.76 for females from the Jizan Region of Red Sea. Vivekanandan and James (1986) estimated this value to be 2.94 for Madras waters in India. In addition, it was 2.66 for Kmataka Region in India. While Kerdgari *et al*. (2009) found slope (3.00) for female of *N. japonicus* in the north of Persia Gulf. This result in agreement with the present study where the slop of females was 2.975 that indicates that this species has symmetrical or isometric growth.

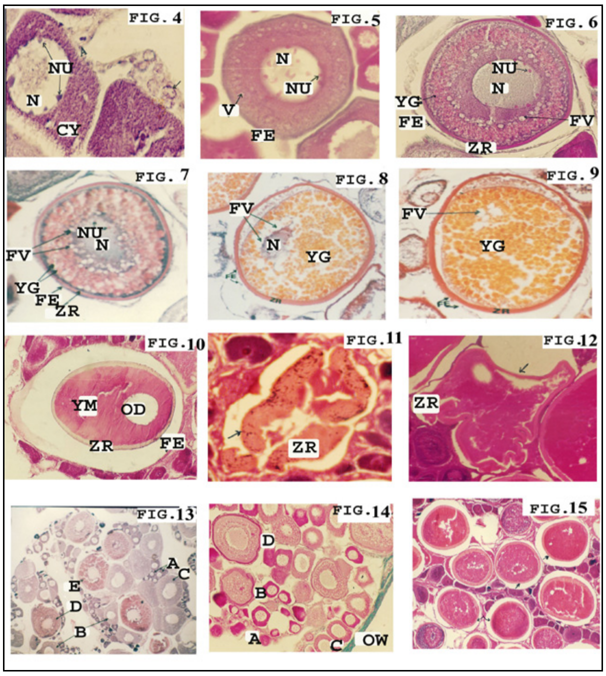

Plate 1. Oocyt maturation of *Nemipterus japonicus*

The results of the present study indicated that females mature earlier than was reported for other areas, this is due to environmental conditions and feeding. Fish showed mature gonads at a length of 9.0 cm and the percentage increased at a length of 11.5 cm, whereas (Murty, 1984; Krishnamoorthri, 1971; Bakhsh, 1996) found the above limit at 12.5 cm, 16.5 cm and 11 cm, respectively.

Fecundity is the most common measure of reproductive potential in fishes. In order to maintain the position of any species in an environment, it required to reproduce to such an extent that would enable it to counteract all physical and biological hazards such as predation, critical stages of its life history, food supply, etc. (Qasim, 1973). In general, fecundity increases with the increase in the size of female, which can be expressed by $F = a L^b$ (Bagenal, 1983). This means that larger fishes produce considerably more eggs than do smaller ones. In the case of *N. japonicus* the linear relationship between fecundity and total length of the fish suggests that the fecundity increases at the rate of 2.987 times the total length. The linear relationships were also noticed between fecundity and weight of fish and ovary weight.

The variation in fecundity estimates for N. *japonicus* in this study appears to coincide with other nemipterid species as reported by other workers (Liu and Su, 1971; Eggleston 1972; Dan, 1977; and Rao and Liu, 1979). The wide variation in fecundity could be associated with the frequency of spawning. Spawned serially, the eggs are shed in batches rather than all at once. The high fecundity estimate at one end of the period probably represents the number of eggs at the onset of the spawning season and the low fecundity estimated at the other end would indicate that the remaining eggs are released in subsequent batches.

The study of reproduction is an important item in fish biology. The reproduction ability of the female can be considered as the key for recruitment of young fish that form the commercial fish stock.

Based on our histological analysis, the ovarian structure of *N. japonicus*, consists of ovigerous lamellae that develop towards the center from the whole area of the interior of ovarian cavity. The ovarian cavity runs throughout the center of the ovary. This type of ovary has also been reported in Seriola quinqueradiata (Takano, 1989).

Histological maturation and G.S.I. values for the ovaries examined, all the fish collected in September to May were spawning with G.S.I about (4.10) having eggs in prematuration and maturation stages. Ovaries maturation were almost all at the oil droplets stages. The presence of oil droplets among oocytes in the maturation stages suggests that, spawned eggs are pelagic this suggestion is confirmed with results obtained from (EL-Halfawy, 1995) and (Takashi-and Robert, 2005). From October to January an decrease in oocyte development occurred, this means complementation of spawning season and the G.S.I. values decreased. The appearance of ovulatory follicles was observed through the period from December to February and suggests the end of spawning season. Peaks in spawning occur in September and May months based on the highest G.S.I values and the highest occurrence of ovaries in the maturation stages. It is considered that spawning activity encompasses the time period from May to September in the summer season, this period is a closed fishing season in the Suez Gulf. Histological observations of ovarian development reported by Bilim *et al.* (1978), El-Halfawy; (1995) and Takashi-and Robert (2005) were similar to our observation, maturity stage are applicable for discrimination between the different condition of the gonads whether in the female grow larger towards the spawning season.

Nagahama (1983) classified the developmental patterns of fish oocytes into 3 types:

(1) Total synchronism, (2) Group synchronism and (3) Synchronism. The developing form of *N. japonicus* oocytes suggests that it may spawn once or more over the prolonged spawning season (in summer)because there are many stages of oocyte developmental stages

in one ovary at least 3 developmental stages. So, it is considered to belong to the group synchronism oocyte development type. These results are confirmed by El-Halfawy (1995) who demonstrated that the *N. japonicus* belonging to group synchronous oocyte development group . Two distinct modes of oocytes diameter distribution for mature fish are present, he reported that the *N. japonicus* thread fin bream in Gulf of Suez waters release one or two batches per season (closed fishing season from May to September). However, we didn't find the full ripe stage oocytes during the collected period. Tormosova, (1983) suggested that stock density; food and water temperature may influence the growth of fish and affect the age at maturity. So the size mode of the female *N. japonicus* reachs sexual maturity at lengths 9.0 cm and bigger than length 11.1 cm at which all females reaching ripping in it suggests that it takes at least one year for females to mature. Manojkumar (2004) determined sexual maturity of females at length 140 mm and 50 % maturity at 183 mm Rajkumar *et al*., (2003) recorded the length at first maturity was 128 mm. Bakhsh (1994) reported the smallest size at first maturity was 9.5 cm for females.

The spawning of *N. japonicas* has been studied by Eggleston (1972), Weber and Jothy (1977), Dan (1980) and Murty (1984). They found that the spawning season varies from one area to another according the environmental factors and the percentage of gravid fishes towards the spawning season (Table 3). The peak of *N. japonicus* reaches to its maximum value in May for female and starts to decrease gradually from October to December.

Table 3: The Spawning time of *N. japonicus.*

Location	Date of spawning	Source
South China	May-October	Krishnamoorthi (1971)
Eastern part of Arabian Sea	Year round (Peak October)	Eggleston (1972)
Off Waltair	September-November	Weber and Jothy (1977)
East Malaysian	November – February	Fursa (1979)
At Waltair	December-February and June-July	Dan (1980)
Kakinada	August-April (peak February-December	Murty (1984)
Madras	June-March (peak December-March)	Vivekanandan and James (1986)
Jizan area	Year round (peak November-May)	Bakhsh (1996)
The northen of Persian Gulf	Spring – Autumn seasons	Kerdgari *et al.* (2009)
Gulf Of Suez	Peak of spawning in May	Present study

Recommendations

- To ensure sustainable utilization of this species we recommend protecting adults during the major spawning season. A seasonal closer from May to September can be provide better breeding opportunities for adults and is believed to a good fishery management measure for this species.
- Further work is needed to estimate the reproductive behavior in the closed fishing season and the time for releasing eggs batches.

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بيولوجيا التكاثر والخصائص الهستولوجية لاناث سمكة الصرع بخليج السويس، مصر

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في هذه الدراسة بلغت قيمة الأس في علاقة الوزن بالطول 2.975 لإناث سمكة الصرع بخليج السويس إلى أنه متساوي القياس. وكان طول الإناث الناضجة عند 50 ٪ هو 11.1 سم. زاد معدل النضج الجنسى تدريجيا مع تطور المبيض حيث سجلت 4.3 القيمة القصوى في مايو وقيمة الحد الأدنى 0.5 في فبراير. اوضحت النتائج زياده الخصوبة بمعدل 2.8971 ، 1.0199 و 0.8135 مرات من إجمالي طول ووزن الأسماك والوزن من المبيض ، على التوالي مما يدل على ارتباط إيجابي بينهم. تم تصنيف مراحل تطور البويضة إلى ثماني مراحل تطور أعتمادا على الخصائص الهستولوجية والتي أوضحت النتائج ان المبيض ينتج بويضات ناضجه اكثر من مرة خلال موسم التكاثر فى فصل الصيف. ولقد اكدت النتائج ان موسم التكاثر لسمكة الصرع يمتد من مايو الى سبتمبر بخليج السويس.