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# **REPRODUCTIVE BIOLOGY AND HISTOLOGICAL CHARACTERS OF MALE RHABDOSARGUS HAFFARA (TELEOSTEI; SPARIDAE) FROM SUEZ BAY, RED SEA**

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## ABSTRACT

The study of reproductive biology of male Rhabdosargus haffara revealed that length at first sexual maturity was 10.3 cm. The over all sex ratio of male to female 1: 1.72. The monthly distributions of gonadosomatic index (GSI) and maturity stages of testes showed that male had a definite spawning season from December to March. Hepatosomatic index (HSI) was changed during the maturation of testes and an inverse correlation with GSI was evident in the months of spawning.

The histological study for testes of Rhabdosargus haffara showed that there are five stages of spermatogenesis; spermatogonium, primary spermatocyte, secondary spermatocyte, spermatid and spermatozoon. Also the maturation of testis can be divided into 8 stages.

## **INTRODUCTION**

*Rhabdosargus haffara* is a tropical fish found in the western Indian Ocean, Red Sea and especially common in the North. It inhibits shallow waters, mainly around coral reefs, and over sandy or mud-sandy bottoms. Feeds chiefly on molluscs and to a lesser extent on crustaceans which are crushed with its developed molars. Its common size is 10 -20 cm and the maximum is 35 cm (Fischer and Bianchi, 1984).

Studies on reproduction of male *Rhabdosargus haffara* are very rarely (AL-Oraimi, 1996; Ibrahim, 1999 and Mehanna, 2001) studied age and growth, the reproductive biology and histological characters in captivity were studied by (EL-Boray, 1997) and the effect of different types of food on the development, growth and biochemical composition (EL-Halfawy, 2001). In recent years the rising commercial demand and farming interest of *R. haffara* in

Suez City and the lack of information of their reproduction in the wild, therefor the present study aims to carried out the reproductive biology and histological characters of male *R*. *haffara*. Specific objectives were clarified to determine length at first sexual maturity, sex ratio, distribution of maturity stages, gonadosomatic index (GSI), hepatosomatic index (HIS) and spawning season.

## **MATERIALS AND METHODS**

Male *Rhabdosargus haffara* were sampled between May, 1999 and April, 2000 by line angling from Suez Bay. For each fish, total length to nearest mm, total weight and gutted weight to nearest gm were recorded. The fish was dissected. Each testis and liver were removed and weighed to nearest gm.

The monthly gonadosomatic index (GSI) and hepatsomatic index (HSI) were calculated respectively as:

 $GSI = \frac{Weight of testis}{Gutted weight of body} X100$ 

 $HSI = \frac{Weight of liver}{Gutted weight of body} X100$ 

At the time of dissection, portions of testes were fixed in Bouin's solution and processed for light microscopy. Transverse sections were taken at 6 µm and stained with haematoxylin-eosin The histological . sections were investigated and the development of spermatogenic cells and maturity stages were identified and assessed respectively. Photomicrograph for sections was made to illustrate the structure of spermatogenic cells and maturity stages.

## RESULTS

### A- Length at first sexual maturity

Fig (1) shows the percentage of mature male *R*. *haffara* versus the total length. It is indicated that length at first sexual maturity ( $L_{50}$ ) for male is 10.3 cm. All males of total length 9 cm are immature while all males longer than 14 cm are fully mature.

## **B-** Sex ratio

From 402 specimens examined, 254 were males and 148 were females. Thus the overall ratio of males to females is 1 : 1.72. Figure (2) shows that males are dominant during the period from September to March. While females are dominated from May to August and in April month too.

However, a significant trend is evident when sex ratio is calculated for each length class (Fig. 3). Males dominate the length intervals between 9 and 14 cm. But females are the most abundant in lengths > 14 cm.

#### C- Gonadosomatic index (GSI)

The monthly changes of the (GSI) value of male R. haffara are shown in Figure (4). The average value (GSI) showed no obvious changes during the period from June to September. A slightly increase observed in October followed by a slightly decrease in November. A progressive increase occurred from November through December and recording a maximum value in January. A significant decrease occurred in February (P < 0.05). The value of (GSI) showed a slight stability in March and April.

### **D-** Hepatosomatic index (HSI)

Fig (4) shows the monthly changes on the average value (HSI) of male *R. haffara*. The value of (HSI) decreased with no significant difference during the period from May to October. In November there is a significant decrease in the average value of (HSI) (P < 0.05) but in December a significant increase is recorded. The average value of (HSI) decreased again in January with no significant difference. The value of (HSI) showed an increase in February, March and April with significant different only in February and April.

### E- Histology of the testis

Testis of *R. haffara* corresponds to a double elongate structure, flattened anteriorly and united at the posterior part, forming a sperm duct that ends at the genital papilla. Internally, each testis is formed of a large number of tubules separated from each other by connective tissue. The tubules contain the germ cells at different stages of maturation and they are connected to a main collecting duct that run longitudinally and leading to a sperm duct. The tubules are surrounded by a tunica consisting of connective tissue and containing the blood vessels supplying the testis.

#### **1- Spermatogenesis**

The study of series of the histological sections of testis allowed the establishment of the following five typical stages of spermatogenesis:

## a- Spermatogonia

Spermatogonia are more or less spherical in shape. They can be found either as individual cells or as groups of cells. The cells have an average of  $10.2 \ \mu m$  in diameter and have a pale cytoplasm. The large central nucleous, 7.5  $\mu$ m in diameter, contains single nucleolus and densely staining chromatin material along the nuclear membrane (Fig. 5).

## **b- Primary spermatocytes**

Primary spermatocytes occur in groups. They are smaller than the spermatogonia. Their diameter is about 5.1  $\mu$ m. They have a very thin cytoplasmic area. The large central nucleus, 3.8  $\mu$ m in diameter, contained patches of intensely staining chromatin along the nuclear membrane (Fig. 5).

## c- Secondary spermatocytes

They are also occurred in groups, contained dense nuclei. They have an average diameter of 4  $\mu$ m. The nuclear diameter equal to 3.19  $\mu$ m (Fig. 5).

## d- Spermatids

The development of spermatids is accompanied by a further reduction in size. They have a diameter of 3.19  $\mu$ m and contained a very densely stained nucleus 2.55  $\mu$ m (Fig. 5).

#### e- Spermatozoa

Spermatozoa with a head diameter of 1.91  $\mu$ m occur in groups in the center of tubules. They are distinguished within the tubules in the early stages, by the presence of a clear zone where the sperm tails are situated (Fig. 5).

## 2- Microscopic structure of maturity stages

According to the aforementioned histological characters of spermatogenic cells and their quantity, structure of the testicular tissue, the maturation of testis of R. haffara was divided microscopically into 8 stages:

### I- Immature stage (Fig. 6)

The infantile testis consists of completely nests of spermatogonia in tubules. The wall of the tubules made up of connective tissue. The lumen of tubules not distinguishable. The median spermatic duct is not detected.

### II- Developing stage (Fig. 7)

In this stage the spermatogenesis proceeds rapidly and all phases of development occure. The mass of cells is spermatogonia. There are nests of primary, secondary spermatocytes, spermatids and spermatozoa (very rare). The lumens of the tubules are noticed in this stage.

## **III-** Maturing stage (Fig. 8)

This stage is characterized by spermatogonia are found largely in the peripheral tubules of the testis while in the tubule's center they are lining the wall. Primary spermatocytes are rare while secondary spermatocytes are plentiful. The tubules are filled with spermatids and spermatozoa in the central lumen of tubules. The median spermatic duct is appeared and sperms are transported to it.

## IV- Mature stage (Fig. 9)

This stage is characterized by the predominance of secondary spermatocytes, spermatids and spermatozoa. Spermatogonia are found rarely in the wall of the tubules. The lumens of tubules are filled with spermatids and sperms. The peripheral tubules of the testis have a high number of spermatogonia. The median spermatic duct increased in volume and charged with sperms.

### V- Ripe stage (Fig. 10)

In this stage the tubules and spermatic duct are packed with spermatozoa. The earlier germ cells are very few in number.

## VI- Spawning stage (Fig. 11)

At this stage the tubules discharged a considerable quantity of sperms. The remnant sperms are found in the lumen. The wall becomes thicker than the previous stage. A reduction in the size of the tubule was observed. Spermatogonia are scattered along the wall of the tubule.

#### VII- Spent stage (Fig. 12)

The tubules contained a very rare quantity of sperms. Therefore clear zones are noticed in the lumen of the tubules. More reduction occurred in the size of tubules. Spermatogonia arranged in row lining the wall of the tubule.

### VIII- Quiescent stage (Fig. 13)

In this stage, brown bodies appeared in the tubules. No evidence of increasing in number of spermatogonia than the previous stage.

## **F-** Monthly distribution of maturity stages

Fig (14) shows the monthly distribution of maturity stages of male *R*.

*haffara*. It is cleared that the first appearance of maturing stage occurres in September and lasts to January. The ripe stage appeares in December and January. The spawning stage occurres from December to February. Spent stage is recorded from January to April. The figure shows that the quiescent stage extendes from March to September.

## DISCUSSION

This study showed that length at first sexual maturity (L50) for male R. haffara was 10.3 cm and all lengths of total 9 cm were immature while those larger than 14 cm were fully mature. EL-Boray (1997) found that  $(L_{50})$  for male *R. haffara* in two different water fish farms were 9.83 cm and 11.22 cm. This can be due to that the average size of maturation is directly related to the population density and ecological conditions, particularly temperature which stimulate sexual maturation (Nikolsky, 1963 and Kashiwagi et al., 1987). Also, Berejikian (2000) found that the length at first sexual maturity was significantly affected by feeding level.

In the present study, the overall ratio of male to female *R. haffara* was 1:1.72. The deviation of parity (1:1) may be attributed to the sex reversal of male to female which is usual phenomenon in sparid fishes (EL-Boray, 1997). Wowever, Ibrahim (1999) found that the overall ratio of *R. haffara* was 1: 1.03. In the present study, the male *R. haffara* dominated during the period from September to March. This may be attributed to the migration of female to the spawning ground.

In this study sex ratio were plotted against lengths. Females were dominant in lengths > 14 cm. This strengths the aforementioned finding of sex reverse of male to female.

Manooch (1976) found that the ratio of male to female of sparid red porgies varied from 1 : 1.9 to 1 : 3.3 within a three year period. So, features of population structure such as sex ratio have widely used to detect

hermaphroditism in teleostes fishes (Sadovy and Shapiro, 1987).

Data of the monthly changes values of (GSI) of male *R. haffara* showed that they discharged their sperms during the period from January to February. While the histological examinations of testes showed that it was from December to March. So, gonadosomatic index value by themselves were not sufficient for identifying specific reproductive events.

The values of hepatosomatic index (HSI) of male *R. haffara* were decreased during November where the testes reach to their activity forming all types of spermatogenic cells. While in the period of ripening, December, the value of HSI increased and decreased again in January. This can give a picture on the role of liver in the maturation of testes of *R. haffara*. These results agree with that of Ibrahim (1999) on *R.. haffara* in the South Sinai\_and also with that of Abdalah and Faltas (1998) where liver of *Trigla lucerna and Trigloporus lastoiza* showed larger indices after breeding season.

In the present study, spermatogenesis was divided into 5 stages according to size difference and to the occurrence of new structures easily recognizable during the different maturation of spermatogenic cells. These cells are spermatogonia, primary spermatocyte, secondary spermatocyte, spermatid and spermatozoon. Selman and Wallace (1986) divided spermatogenesis into three main phases: spermatogenesis (formation of primary spermatocytes from spermatogonia). Meiosis (formation of secondary spermatocytes and spermatids). Spermiogenesis (transformation of secondary spermatids into spermatozoa). Spermatogenesis process was proceeded rapidly in R. haffara, therefore all phases of development were observed. Each stage of maturity was characterized by the presence of these cells.

The maturity stage of male testes were divided into 8 stages namely immature, developing, maturing, mature, ripe, spawning, spent and quiscent stages. The monthly distribution of these stages and examinations of histological sections of testes showed that male R. haffara sheds their sperms in December and lastes to March. Males with spawning testis were obtained in this period. AL-Oraimi (1996) in Suez Canal. EL-Boray (1997) in fish farms and Ibrahim (1999) in South Sinai showed that R. haffara is a winter spawner. Therefore, the spawning season of male R. haffara did not affect by regional variation but it varied in the starting month. From the aforementioned results, it could be concluded that male R. haffara in Suez Bay have a definite spawning season started from December and ended in March. some species of the family Sparidae Also, (R. sarba and Diplodus noct) on Southern Sinai Coasts (Ibrahim, 1999) and D. noct in Suez Bay (Mahmoud, 2002) are winter spawners.

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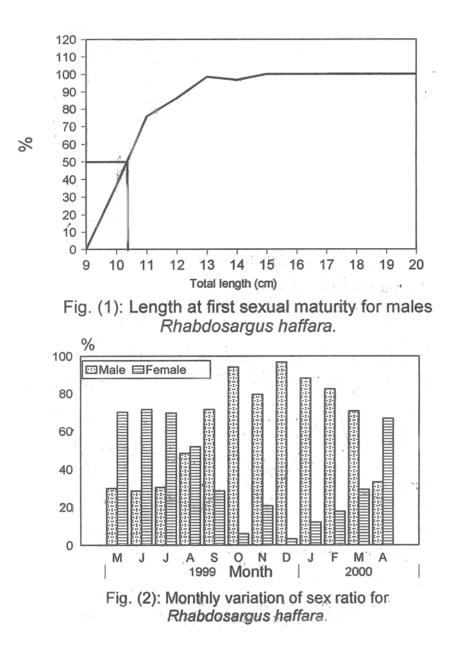
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## List of photomicrographs

- Fig (5): Photomicrograph of a T. S. of testis *R. haffara* shows different types of spermatogenic cells (A) spermatogonia, (B) primary spermatocytes, (C) secondary spermatocytes, (D) spermatids and (E) sperms. X 250
- Fig (6): Photomicrograph of a T. S. of testis *R. haffara* shows immature stage where all spermatogenic cells are spermatogonia. X 100
- Fig (7): Photomicrograph of a T. S. of testis *R. haffara* shows developing stage, (A) spermatogonia, (B) primary spermatocytes, (C) secondary spermatocytes (D) spermatids and (E) sperms. X 100
- Fig (8): Photomicrograph of a T. S. of testis *R. haffara* shows maturing stage (A) spermatogonia,(B) secondary spermatocytes ans (C) sperms. X 100
- Fig (9): Photomicrograph of a T. S. of testis *R. haffara* shows mature stage where the quantity of sperms increased increased (A), (B) secondary spermatocytes. X 100
- Fig (10): Photomicrograph of a T. S. of testis *R. haffara* shows ripe stage where the tubules are filled with sperms (A). X 100
- Fig (11): Photomicrograph of a T. S. of testis *R. haffara* shows spawning stage where small quantity of sperms are found in the tubules (A). X 100
- Fig (12): Photomicrograph of a T. S. of testis *R. haffara* shows spent stage where the lumen of tubules (A) and the median spermatic duct (B) are empty. X 100
- Fig (13): Photomicrograph of a T. S. of testis *R. haffara* shows quiescent stage where the outer tubules are linned with one layer of spermatogonia (arrow). X100

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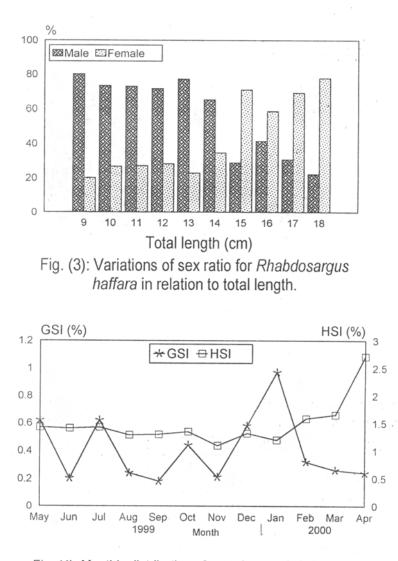


Fig. (4): Monthly distribution of gonadosomatic index and hepatosomatic index for *Rhabdosargus haffara during the reproductive cycle.* 

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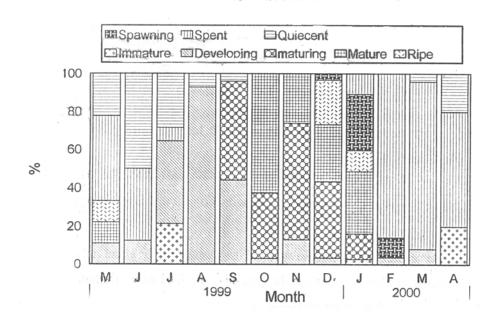


Fig. (14): Monthly distribution of maturity stages for male *Rhabdosargus haffara*.

