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THE REPRODUCTIVE BIOLOGY OF THE GROUPER *Epinephelus chlorostigma* (PISCES, SERRANIDAE) FROM THE RED SEA.

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ABSTRACT

Maturity, spawning season, length and age at first sexual maturity, sex ratio, fecundity and hermaphroditism in E. chlorostigma (C. and V., 1828) from the Red Sea were studied. The spawning season extends from May to end of July with a peak in June. The total length at which 50% of the females reach sexual maturity was found to be 28.0 cm. at an age of 3 years. Females are more numerous than males, the ratio being 2.4 : 1 respectively. The mean observed absolute fecundity increases progressively from age group III to age group VI, then decreases in seven year old fish. The relative fecundity expressed as number of eggs produced per gram of body weight fluctuates between 121 and 776. The mean size of sex reversal in this species is close to a total body length of 48.0 cm.

INTRODUCTION

Family Serranidae is one of the largest and the most important groups of fishes, containing more than 400 species widespread in tropical and subtropical areas allover the world. In the Red Sea, twenty two serranid species belonging to five genera were described (Randall and Ben Tuvia, 1983), of which E. chlorostigma is one of the most economically important and widely distributed groupers in the Red Sea. However, little is known about its biology. The work to be described was undertaken to shed some light on the reproductive biology of this species, as a part of an investigation designed to assess its potential as a fishery resource. The investigations on E. chlorostigma comprised one aspect of a wider research programe, the aim of which is to evaluate and quantify the fish resources in the Gulf of Suez and the Egyptian Red Sea waters.

MATERIALS AND METHODS

Monthly random samples of E. chlorostigma were collected from professional fishermen of Suez and Hurghada commercial fish markets. This collection extended from May 1979 to June 1981.

For histological purposes, samples of **E. chlorostigma** were collected by fishermen at the Hurghada Marine Biological Station. These samples were fished by hook and line and then kept alive by pulling them in a special perforated-woody boat kept under water behind the main boat. They were transferred for sometime to special aquaria. The alive fishes were dissected and the gonads were cut into small pieces and fixed using aqueous Bouin's fluid or Zenker formol. These pieces were then dehydrated in ascending grades of ethyl alcohol, cleared in terpineol, and then embedded in paraffin wax. Sections of 6-8 microns thick were cut. They were then stained with Haematoxylin-Eosin, Mallory's triple-stain and mounted with D.B.X.

For the study of the fecundity, the ripe or nearly ripe ovaries only were removed intact from the fishes collected during the spawning season which extends from May to July. In the present study, analysis of 38 ripe female ovaries was carried out. For each female from which the ovaries were taken for fecundity determination, the following data were recorded: total length to the nearest mm., total weight to the nearest gm., gutted weight to the nearest gm., and date of capture. The ovaries were preserved in 5% neutral formalin. The two ovaries were then weighed. Two subsamples of about 0.1 to 0.2 gm. from each ovary were weighed to the nearest 0.001 gm. and then crumbled in large Petri-dishes containing water. The number of ova ready to be spawned in each subsample was counted. The absolute and relative fecundity were then calculated. The diameters of the ova ready to be spawned in the four subsamples were also measured using a calibrated ocular micrometer.

Age was determined by scalemetry

Gonad Structure:

Accroding to Smith (1965), the gonads of all hermaphroditic serranids are similar in gross appearance. In case of **E. chlorostigma** each gonad consists of a hollow sac, lying below and behind the posterior part of the air bladder and connected with it by mesenteries. The right ovary is usually larger than the left one. The two ovaries join posteriorly forming a common oviduct which open to the exterior by the female genital opening, immediately behind the anus. The urinary bladder and common urinary duct are closely bound to the posterior face of the common oviduct, and open to the exterior by the urinary opening, just behind the female genital opening. The gonads are covered externally with a thick peritoneal layer.

Maturation and Spawning Seasonality:

1. Description of maturity stages:

In the present analysis, the maturity stages system given by Munro (1979) was followed. This system was used by Thompson and Munro (1978) in the study of maturity stages of serranids from the Caribbean Sea. The system is as follows:

Stage 0 (immature):
The gonads are very small, frequently transparent and with no evidence of past spawning. The sex is difficult to be determined (except microscopically).

Stage I (inactive):
The gonad are larger in diameter than the previous stage with a translucent appearance. Testes are smaller than ovaries and usually less translucent.

Stage II (maturing or recovering spent)
The gonads are slightly enlarged but still translucent. No sperm is present. Few small eggs are visible in the ovary.

Stage III (active or developing)
The gonads are larger, testes are opaque and no sperm is expelled by cutting the testis. The ovaries are translucent with small eggs visible microscopically.

Stage IV (active - ripe or developed)
Testes are usually white, and some sperms are expelled from the core when the testis is cut. The ovary is opaque and solid, and eggs are fully formed and numerous, but eggs are not translucent.

Stage V (ripe or gravid)
The gonads are enlarged, occupying a large portion of the visceral cavity. Milt flowed freely when testis is cut. Some of the eggs are translucent.

Stage VI (spawning or ripe - running)
The gonads are enlarged to occupy a major portion of the body cavity. Milt and eggs are expelled from the genetal appertures on application of a slight pressure on both sides of the genital area.

Stage VII (spent):
Testes and ovaries are flaccid. A few degenerating eggs are present in the ovary and the testes are almost empty.

For the general descriptive purposes, stage 0 is referred to as "immature", stages I and II as "inactive", stages III and IV as "active", stages V and VI as " ripe" and stage VII as " spent".

2. Monthly occurence of maturity stages

The maturity stages of the gonads of **E. chlorostigma** from the Red Sea during the different months of the year were followed by applying the foremetioned scale of Munro (1976). The present analysis was carried out on 469 females and 196 males.

Table 1. shows that the first month in which ripe females of E.

Table (1)

Monthly distribution of maturity stages of E. chlorostigma from the Red Sea during 1979 - 1981 (years combined).

Month	FEMALES										MALES				
	No. of Fish	Immature		Inactive		Active		Ripe		Spent		No. of Fish	Active		Ripe	
		No.	%	No.	%	No.	%	No.	%	No.	%		No.	%	No.	%
January	09	03	33.33	01	11.11	05	55.56	-	-	-	-	12	12	100.00	-	-
February	25	09	36.00	08	32.00	08	32.00	-	-	-	-	11	11	100.00	-	-
March	27	08	29.63	07	25.93	12	44.44	-	-	-	-	10	10	100.00	-	-
April	14	01	07.14	02	14.29	11	78.57	-	-	-	-	10	07	70.00	3	30.00
May	40	-	-	07	14.29	24	48.98	18	36.73	-	-	24	22	91.67	2	8.33
June	56	04	7.14	07	12.50	20	35.71	25	44.64	-	-	24	22	91.67	2	8.33
July	94	22	23.70	18	19.15	20	21.28	21	22.34	13	13.83	16	12	75.00	4	25.00
August	68	07	10.29	12	17.65	46	67.65	-	-	03	4.41	12	12	100.00	-	-
September	33	04	12.12	01	3.03	28	84.85	-	-	-	-	19	19	100.00	-	-
October	34	07	20.59	03	8.82	24	70.59	-	-	-	-	13	13	100.00	-	-
November	35	01	2.86	04	11.43	30	85.71	-	-	-	-	23	23	100.00	-	-
December	25	-	-	-	-	25	100.00	-	-	-	-	22	22	100.00	-	-

chlorostigma were detected was May, where 36. 73% of the females examined were found to be ripe. In June, 44.64% of the examined females were ripe. In July, 22.34% of the examined females were ripe, while 13.83% were spent. No ripe females were observed after July, but 4.41% of the examined females in August were spent.

Table 1. shows that all examined males of E. chlorostigma were only active or ripe. It is obvious that the ripe males were observed during the months of April, May, June and July where they constitute 30.00%, 8.33% and 25.00% respectively of the total males examined.

From these results it is clear that the spawning season of E. chlorostigma extends from May to end of July with a peak in June.

3. Spawning

According to Hickling and Rutenberg (1936), measurements of ova diameter in ovaries approaching spawning may give evidence to the spawning nature in a fish whose spawing habits are unknown. If the spawning period is short and definite, the batch of the transparent yolkless small ova destined to be spawned will be withdrawn from the general egg stock in a single group. This single group, in later stages, will be sharply distinguishable from the stock of small eggs from which it was derived.

On the other hand, if the spawning season is long and indefinite, the withdrawal of eggs from the egg stock to undergo maturation will be a continuous process and the eggs will pass continuously from one stage into the other.

To apply this hypothesis to E. chlorostigma from the Red Sea, the percentage frequency distribution in ripe female was calculated. From Figure (1), it is obvious that in ripe female fish there are two batches of eggs.

The first batch represents the immature oocytes, with ova diameter varying from 0.1 to 0.3 mm. The second batch represents the advanced ova, with a peak at 0.6 mm. It is also clear that maturation of ova is still taking place.

Hence, the ovary of a ripe female of E. chlorostigma contains two stages of ova. If we consider the immature ova as representing the stock of oocytes, then we can say that this fish spawns once in the season.

Size and Age at First Sexual Maturity:

The estimation of the size at first sexual maturity has its practical application in the determination of the minimum legal size needed to protect an adequate spawning stock and to ensure at least one spawning for the mature individuals.

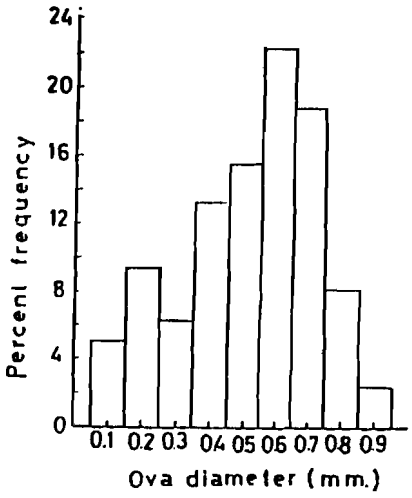

Fig. (1)
Percentage frequency distribution of ova - diameter in ripe female Epinephelus chlorostigma from the Red Sea.

According to Pitt (1970), the length at which 50% of fish reach sexual maturity (L_{50}) is considered to be the length of onset of its sexual-maturity.

From Figure (2), it is clear that for the females of **E. chlorostigma** from the Red Sea L_{50} = 28.0 cm. in total length. This total length corresponds to three years of age. Starting from lengths of 22.0 cm., sexually mature females are frequent. At lengths more than 34.0 cm., all females examined are sexually mature.

On the other hand, the size and age of onset of sexual maturity of males could not be determined in the present investigation, since all collected males were found to be active or ripe. It can be concluded that males of **E. chlorostigma** sexually mature before being vulnerable to the fishery.

Sex Ratio and Sex Reversal:

The study of the length distributions of the collected males and females is very important as being an evidence of protogynous hermaphroditism.

From Table (2) and Figure (3) it is obvious that all young fishes ranging in total length from 14 to 30 cm. were found to be females. Starting from lengths of 30.0 to 56 cm., a broad overlap of the length distributions of the sexes was encountered, while at lengths more than 56 cm., all the examined fishes were found to be males.

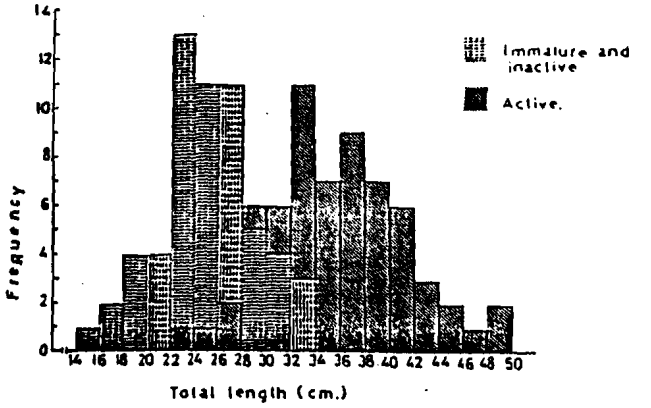

Fig. (2)
Length frequency distribution of immature and inactive and active females per length group during the spawning seasons of **Epinephelus chlorostigma** from the Red Sea.

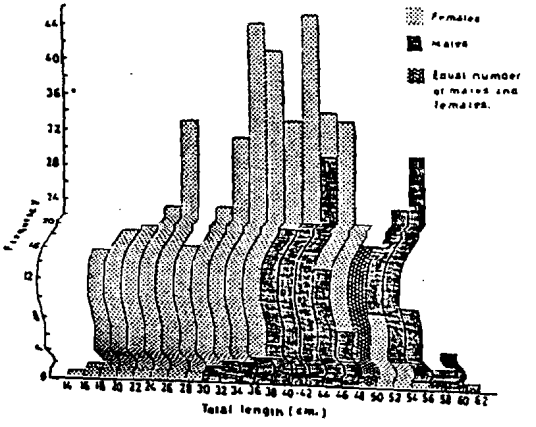

Fig. (3)
Length frequency distribution of male and female **Epinephelus chlorostigma** from the Red Sea.

Table (2)

Length-frequency distribution of male and female E. chlorostigma from the Red Sea, and the percentage of males in successive length groups.

Total Length Group (cm.)	Number of Fish			Percentage of males
	FEMALE	MALE	TOTAL	
14.0 - 15.9	1	0	1	0
16.0 - 17.9	2	0	2	0
18.0 - 19.9	16	0	16	0
20.0 - 21.9	19	0	19	0
22.0 - 23.9	20	0	20	0
24.0 - 25.9	23	0	23	0
26.0 - 27.9	33	0	33	0
28.0 - 29.9	17	0	17	0
30.0 - 31.9	23	2	25	8.00
32.0 - 33.9	31	3	34	8.82
34.0 - 35.9	44	3	47	6.38
36.0 - 37.9	41	4	45	8.89
38.0 - 39.9	33	20	53	37.74
40.0 - 41.9	45	21	66	31.82
42.0 - 43.9	34	29	63	46.03
44.0 - 45.9	33	15	48	31.25
46.0 - 47.9	21	8	29	27.59
48.0 - 49.9	18	18	36	50.00
50.0 - 51.9	10	23	33	69.70
52.0 - 53.9	4	29	33	87.88
54.0 - 55.9	1	11	12	91.67
56.0 - 57.9	0	3	3	100.00
58.0 - 59.9	0	6	6	100.00
60.0 - 61.9	0	1	1	100.00
	469	196	665	

Sex ratio M : F = 1 : 2.39

The percentage of males in successive length groups shows fluctuations in its value from total length 30.0 to 47.9 cm. At total length 48 cm., 50% of the catch were foud to be males. After this point, the percentage of males increases steadily until it reaches 100% of the catch at lengths ranging from 56.0 to 60.0.

From the above mentioned results, it is clear that the mean size at which sex reversal takes place in case of E. chlorostigma to be at a total length of 48.0 cm. Table 2 shows also that the male female ratio is 1 : 2.39.

Fecundity:

The fecundity of a fish is generally defined as the total number of ripe eggs produced by one female in a spawning season or in a year. On studying fish fecundity, two terms are usually applied. These are :

a. Absolute feundity: denoting the total number of ripe eggs in the ovary.

b. Relative fecundity: denoting the number of eggs per unit length or weight of the fish (Nikolsky, 1963).

The fecundity of a fish is not a stable character, but varies according to the species, and to the changes in the environmental conditions. Sometimes there are changes in the fecundity of one and the same species in the different years and different localities (Nikolsky,1963).

From ova diameter analysis, it is obvious that the ripe ovary of a female E. chlorostigma possesses two types of oocytes. The first type represents the immature ova having diameters ranging between 0.1 to 0.3 mm. These ova are very minute in size, transparent and could be considered to represent the oocyte stock. The other type of oocytes are either yolked eggs or transparent ones. These are larger oocytes which are probably destined for spawning in the next season. The yolked eggs are rounded and opaque yellow in colour; with diameters ranging between 0.4 to 0.7 mm. The large transparent eggs are more advanced and have diameters ranging between 0.8 to 0.9 mm. They are more or less oval in shape. Hence, our counts for fecundity estimation of E. chlorostigma were based on the yolked and transparent eggs. Moreover, the ripe ovaries (Fig. 4) taken from females in the spawning season (May to July) were only used.

Fig. (4)
Photograph of ripe ovary of a female
Epinephelus chlorostigma
340 mm. total length.

Table (4)
The mean observed, calculated relative fecundity per total length group of E. chlorostigma from the Red Sea.

Total Length (cm.)		No. of Fishes	Mean Observed Absolute Fecundity	Calculated Absolute Fecundity	Relative Fecundity (F / cm.)
Range	Mean				
30.0 - 31.9	30.50	3	61856.80	86843.77	2028.09
32.0 - 33.9	33.00	2	110408.79	118807.39	3345.72
34.0 - 35.9	35.33	3	252369.50	155851.62	7143.21
36.0 - 37.9	37.00	-	-	187285.01	-
38.0 - 39.9	38.50	2	315406.80	219361.24	8192.38
40.0 - 41.9	40.36	7	307413.24	264649.74	7616.78
42.0 - 43.9	42.36	5	279217.32	320796.19	6591.53
44.0 - 45.9	44.90	5	212786	404422.38	4739.12
46.0 - 47.9	46.33	3	651516.33	458141.77	14062.52
48.0 - 49 9	48.50	4	683731.65	549643.29	14097.56
50.0 - 51.9	51.00	-	-	671297.68	-
52.0 - 53.9	52.50	3	713591.97	573347.05	13592.23
54.0 - 55.9	54.50	1	948620.80	874152.46	17405.89

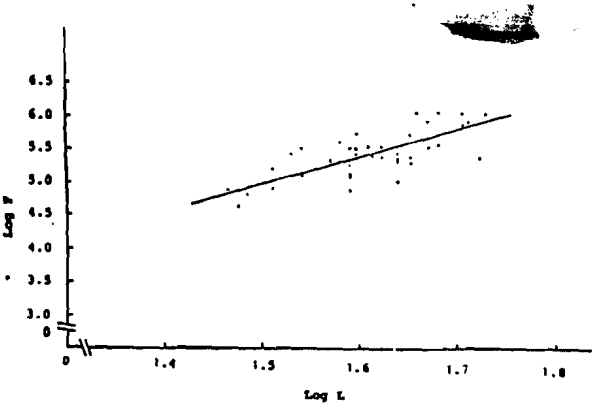

Fig. (5)
Relation between Log absolute fecundity and Log total length of **Epinephelus chlorostigma** from the Red Sea. (The straight line represents the calculated values and the dots represent the observed ones).

Relation Between Fecundity and Total Weight:

From table (3) it is clear that the Log total weight (gm) - Log absolute fecundity relationship gave the highest correlation coefficient. This relationship can be represented by the following equation

$$\text{Log } F = 1.4959587 + 1.3495826 \text{ Log Wt.}$$

Where F is the absolute fecundity and Wt is the total weight in gm. The graphical representation of this linear relationship is shown in Figure (6). Table (5) gives the mean observed, calculated and relative fecundity per total weight group of E. chlorostigma. From this table, it is noted that the values of the relative fecundity ranges from 151.14 to 775.59 per gm.

Relation Between Fecundity and Age:

Fishes of the different lengths and weights were assorted into age groups as previously determined by scale measurements. The relationship between the fecundity and age was based on fishes of age groups III, IV, V, VI and VII. Table (3) shows that for fecundity - age relationship, the highest correlation coefficient was obtained by the log - log relationship. This relationship can be represented by the following formula

$$\text{Log } F = 3.643215 + 2.6949546 \text{ Log } t$$

Where F is the absolute fecundity and t is the age in years.

This linear relationship is graphically represented in figure (7). Table

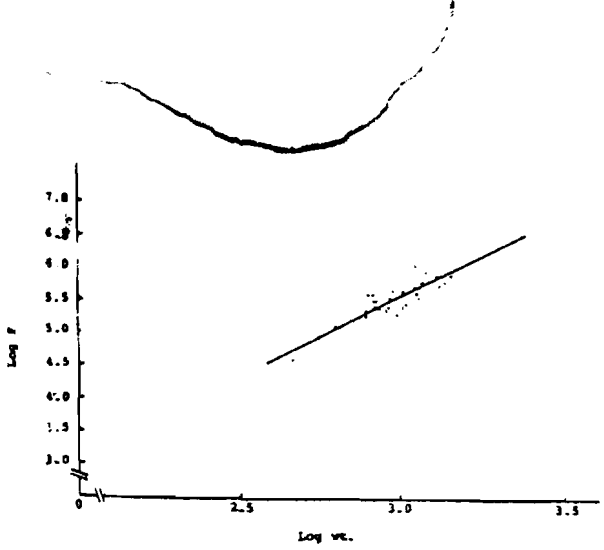

Fig. (6)

Relation between absolute fecundity and Log weight of **Epinephelus chlorostigma** from the Red Sea. (The straight line represents the calculated values and the dots represents the observed ones).

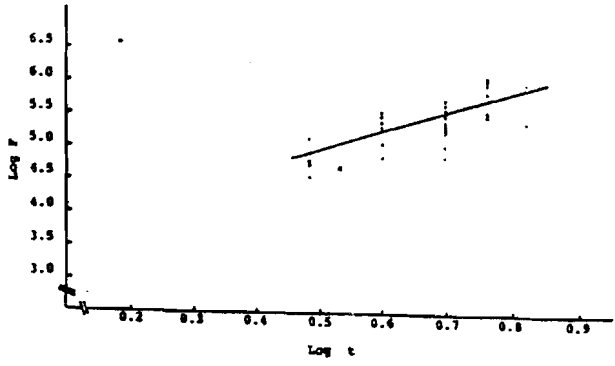

Fig. (7)

The relation between Log absolute fecundity and Log age of **Epinephelus chlorostigma** from the Red Sea.

Table (5)
The mean observed, calculated and relative fecundity per total weight group of E. chlorostigma from the Red Sea.

Total Weight (gm.)		No. of Fish	Mean Observed Absolute Fecundity	Calculated Absolute Fecundity	Relative Fecundity (F / gm.)
Range	Mean				
330 - 429.9	354.25	4	82138.23	86389.91	231.87
430 - 529.9	515.00	1	77835.08	143141.85	151.14
530 - 629.9	574.50	2	195685.86	165900.85	320.62
630 - 729.9	662.50	2	219392.98	201086.16	331.16
730 - 829.9	752.00	4	320359.13	238590.06	426.01
830 - 929.9	871.71	7	281060.73	291228.40	322.42
930 - 1029.9	964.83	6	277868.32	333980.93	288.00
1030 - 1129.9	1120.00	1	253605.30	408441.99	226.43
1130 - 1229.9	1170.00	1	520327.80	433240.50	444.72
1230 - 1329.9	1275.00	2	803641.30	486520.69	630.31
1330 - 1429.9	1363.33	3	478068.39	532551.58	350.66
1430 - 1529.9	1520.00	2	1178899.00	616764.75	775.59
1530 - 1629.9	1600.00	1	683527.60	660972.49	428.20
1630 - 1729.9	1680.00	-	-	705960.00	-
1730 - 1829.9	1780.00	-	-	763253.99	-
1830 - 1929.9	1875.00	1	227110.30	818736.92	121.13
1930 - 2029.9	2020.00	1	948620.80	905322.89	469.61

(6) shows the mean observed and calculated absolute fecundity per age group of E. chlorostigma from the Red Sea

Table (6)
The mean observed and calculated absolute fecundity per age group of E. chlorostigma from the Red Sea.

Age Group (Years)	NO. Of Fish	Mean Observed Absolute Fecundity	Calculated Absolute Fecundity
III	4	82138.23	84924.91
IV	6	244292.87	184390.82
V	19	274933.54	336439.82
VI	7	841616.60	549917.14
VII	2	587865.55	833 35.83

Hermaphroditism:

Serranid Patterns of Heramphroditism:

Smith (1975) had described the different serranid patterns of hermaphroditism. He found that members of the genera Serranus, Diplectrum and Hypoplectrus are synchronous hermaphrodites with well defined ovarian and testicular zones, although both tissues are contained within a common gonadal capsule. In this case, male and female genital cells ripen at the same time. He also described the genera Epinephelus and Mycteroperca as protogynous hermaphrodites in which the male and female elements are completely mixed. In this case, the individual functions at first as a female, then it becomes, by sex inversion, a functioning male. He found that Rypticus, Sacura and Chelidoperca to be protogynous hermaphrodites with a similar intermediate pattern of gonad anatomy, in which the male tissue is confined to the same region of the gonad as in case of Serranus, but the male and female elements lie side by side without an intervening connective tissue wall. Moreover, he found that several serranid species appear to be secondary gonochorists, i.e. when the gonads pass by a stage of intersexuality, one of the sexual categories is becoming functional, while the other is degenerating.

Epinephelus chlorostigma exhibits the "Epinephelus type of hermaphroditism", described by Smith (1965). It is a protogynous hermaphrodite with the entire gonad being formed of a mixture of ovarian and testicular tissues (Fig. 8). The ovarian tissue functions first, then the gonad transforms into a testis (Fig. 9). This phenomenon was also proved by the study of length frequency distribution of male and female E chlorostigma from the Red sea (vide infra). All young fishes examined, ranging in total length from 14.0 to 29.0 cm., were found to be females, while all large fishes, ranging in total length from 56.0 to 60.0 cm., were found to be males.

Fig. (8)
Photograph of a T. S. of the gonads of a specimen of Epinephelus **chlorostigma** 460 mm. total length. Transisional stage, showing developing testicular tissue and young oocytes not yet reabsorbed. (X 92). T. testicular tissue.

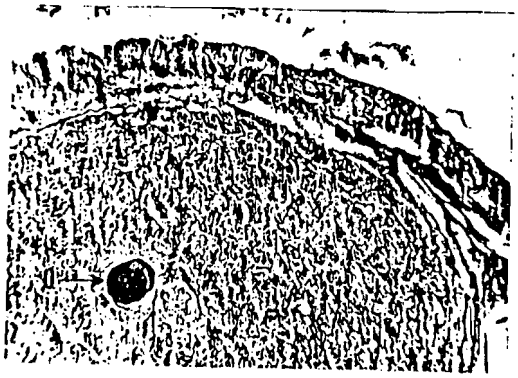

Fig. (9)
Photograph of a T. S. of the gonads of a male
E. chlorostigma, 530 mm. total length.
Mature male phase, showing remnants
of an oocyte. (X 108).
O. OOcyte.

DISCUSSION

Spawning:

In the present study, sexual maturity stages of **E. chlorostigma** from the Red Sea was done based on the maturity scale given by Munro (1976).

Nzioka (1979) has collected one ripe fish of **E. chlorostigma** from the Mafia reef (East Africa) during September. He concluded that the spawning season of this fish is during September. The Spawning season of **E.** chlorostigma from the Red Sea was found to start at May and cnotinued through June and July, with a peak during July. This difference in the spawning period of **E. chlorostigma** between the two areas may be probably due to ecological variations. However, one ripe fish is not enough to precise accurately the spawning season of a studied spcies.

Sex Ratio and Sex Reversal:

According to Thompson and Munro (1978), the sex ratio in groupers is closely related to the size composition of the population. Table (7) summarized their results of sex ratios of five serranid species from Jamaican waters in comparison with our results of sex ratio of **E.**

Table (7)
Comparison of sex ratios of the groupers in Jamaican water (Thompson and Munro, 1978) and the present study (1983).

Species	No. of Specimens	Rate M : F	Area
Epinephelus guttatus	31	1 : 5.60	Port Royal reefs 1970
Epinephelus guttatus	244	1 : 2.81	Oceanic banks
Epinephelus striatus	38	1 : 0.72	Oceanic banks
Mycteroperca venenosa	50	1 : 0.885	Oceanic banks
Cephalopholis fulva	352	1 : 2.14	Oceanic banks
Petrometopon cruentatum	49	1 : 6.0	Port Royal reefs 1970-1971
Epinephelus chlorostigma (Present Study)	665	1 : 2.39	Red Sea.

chlorostigma from the Red Sea. From this table, it is clear that the females are generally more numerous than males. The previous authors explained the increase in number of males than females in case of **Epinephelus striatus** and **Mycteroperca venenosa** by the fact that their collection included mostly large fishes. For **E. chlorostigma** in the Red Sea, it is clear that the females are more numerous than males and the sex ratio lies close to that of **Cephalopholis fulva.**

Thompson and Munro (1978) found the mean size at sex reversal of E. guttatus from the Caribbean Sea was 38 cm. total length. The mean size at sex reversal of **E. chlorostigma** from the Red Sea was found to be in the range of 48 cm. total length.

Fecundity:

Knowledge about the fecundity of the members of the genus Epinephelus is limited in the literature. However, Mikhail (1980), dealing with **Epinephelus aeneus** from Alexandria waters, found that its absolute fecundity varies between 783.988 for a fish of 66.0 cm. total length and 3873271 for a fish of 93.5 cm. total length. Treasurer (1980) has studied the fecundity of the perch, **Perca fluviatilis** L., from the Scotland. He found that its absolute fecundity varies between 6711 and 77978 for females ranging from 14.5 cm. to 34.5 cm. total length. In the present study, the fecundity of **E. chlorostigma** from the Red Sea was found to vary from 42718.2 to 1242724 for females ranging from 30.5 cm. to 47.0 cm. total length. This shows that the absolute fecundity of **E. chlorostigma** is higher than that of **Perca fluviatilis** and lower than that of **E. aeneus.**