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Research Article

Variation in the Reproductive Biology of Female Rabbitfish *Siganus rivulatus* with Histological and Ultrastructural Evidence

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Abstract

Background and Objective: Rabbitfish *Siganus rivulatus* is one of the ecologically and commercially important marine fish inhabiting Egyptian northwestern Mediterranean Coastal regions. Studying the reproductive biology of fish species is among the most important life-history parameters that researchers must establish. The present study was carried out to test the theory of variation in the reproductive biology of rabbitfish *Siganus* genus and determine the exact spawning season of *Siganus rivulatus* species. **Material and Methods:** Random monthly samples of wild fresh dead fish were collected from Alexandria (December, 2016 to November, 2017). Biometric measures (total length, weight, gutted weight and ovary weight) were carried out. The fish was dissected and ovarian samples were collected and weighed monthly (average n = 20) throughout the year to identify the macroscopic and microscopic features of different maturity stages, determine gonadosomatic index values and fecundity. **Results:** Monthly analysis of the maturity stages distribution and gonadosomatic index recognized one distinct peak in June, identifying the spawning season (late May to mid of July). The morphological appearance and histological examination of the ovaries together with the ultrastructural changes in the oocytes wall showed that this species is an annual synchronous oocyte developer and their release takes place over a short period. **Conclusion:** *Siganus rivulatus* has a short spawning period extending from late May to mid of July (the current study) with an annual seasonal variation depending on different environmental factors. This study recommends the determination of the exact timing of the spawning season for this fish species annually as a basic mariculture practice due to its ecological and economical implication.

Key words: Reproductive biology, histology, ultrastructure, oocyte, gonadosomatic index, *Siganus rivulatus*

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rabbitfish belonging to genus *Siganus* of the family Siganidae include a group of potentially important aquaculture species in the Middle-East and the Indo-Pacific, Indian Ocean, Red sea and Mediterranean region. Rabbitfishes are subtropical euryhaline herbivorous fishes and with rapidly growing market demand in coastal areas[1]. They attracted the interest of Aquatic Biology Scientists due to their pre-dominantly herbivorous feeding habits. It considered among the most successful invaders in the Mediterranean sea[2]. Rabbitfishes have since established large populations in the Mediterranean region and have acquired great economic importance[3]. In nature, they found in reefs among sea grass, mangroves and in shallow lagoons and algae form a major part of their food. The geographical distribution of *Siganus rivulatus* (Forsskal and Niebuhr, 1775) extends between the shores of Libya and Cyprus, in the neritic waters from the Israeli shores to Anatolia[4]. Various biological and ecological traits of these species have been studied or reviewed and new information now exists on their reproduction[5,6]. Investigating fish reproduction can give useful information to support or improve fish stocks and for management purposes[7]. The reproductive biology of the white-spotted rabbitfish *Siganus canaliculatus* (Park, 1797) was studied at east of Saudi Arabia[8]. Ovarian development was determined by macroscopic observations without histology or biological indices[9-11]. Gonadal maturation and oocyte development for *Siganus canaliculatus* and *Siganus spinus* have been explained in the Gulf water off Saudi Arabia[12]. Different authors are working on preliminary reproductive biology studies of *Siganus* rabbitfish[13-16]. Ovarian weights used to determine the gonadosomatic index (GSI) values that reliable indicator of sexual maturity. The spawning season determined from the variations in gonadosomatic index values studied in different months of the year. Fecundity is a key index measures the number of eggs presents in the gravid female fish. Study of fish fecundity is a prime factor in investigating the biology of fish species. Length-weight relationships and fecundity estimates were also included in Lagos lagoon, Nigeria[17]. Maturity stages and fecundity in warm and cold-water fish and squids have been assessed as well[11,18-20]. Moreover, the histological study is very essential to decide the reproductive state of female fish[21]. Histological and ultrastructure characterization of the ovary in mudskipper *Periophthalmus papilio* investigated from the mangrove swamps of Lagos Lagoon, Nigeria has been reported by Lawson[22]. In addition, several authors illustrated the maturation, histological and ultrastructural characteristics of non-related species[23-25]. Histological analysis of gonadal development is the most exact method to decide the seasonal changes in the oocytes associated with sexual ovarian maturation[23,26].

From previous works, there is an annual seasonal variation in the reproductive biology of this genus. The present works studies the most important parameters in the reproductive biology of *Siganus rivulatus* to determine the exact spawning season during the annual environmental differences in region at studied year that could be served for fishery managements and aquaculture.

MATERIALS AND METHODS

Collection of specimens: Random monthly samples of wild fresh dead Siganidae fish *S. rivulatus* were collected from Egyptian northwestern Mediterranean Coastal region in Alexandria, between December, 2016 to November, 2017 and transported to the Hatchery Marine unit in National Institute of Oceanography and Fisheries (NIOF).

Morphometric measurements: Morphometric features measured for each fish where the total length was to the nearest centimeters (ranged from 14-24 cm) and fish total weight and gutted weight was to the nearest gram (ranged from 57-120 g). The fish were dissected to decide sex and gonad maturity stages. Sexes and gonad maturity stages were examined by naked eye examination and under compound microscope. Different maturity stages of fish in different seasons and months to cover a whole annual cycle were measured microscopically (under the light and transmission electron microscope). The ovaries were removed and weighed to the nearest 0.1 g using electronic balance and preserved in 10% neutral formalin for histological examination. Another sample 1 g was removed from each ovary weighed and counted to estimate fecundity. This was measured monthly by research microscope with an ocular micrometer at a power of 40 X. Absolute Fecundity (AF) was determined from a total number of vitellogenic oocytes of a weighed sub-sample of a weighed ovary and then multiplied up to represent the total egg number of that ovary. Absolute fecundity (AF) estimated using the equation[27]:

$$AF = \frac{\text{Ovary weight} \times \text{egg number in the sub-sample}}{\text{Sub-sample weight}}$$

Maturity index and reproductive mode: Maturity stages were evaluated by visual analysis of external features of the gonad and the spawning season was determined from the monthly changes of the GSI. The GSI throughout the year calculated for males and females separately[28] as:

$$GSI = \frac{\text{Gonad weight}}{\text{Gutted weight}} \times 100$$

Histological and ultrastructural preparation methods: A routine histology method was applied[29]. Fixed ovaries were dehydrated through successive grades of 70, 80 and 95% absolute ethyl alcohol before clearing with xylene. Then the sample was embedded in paraffin wax. Sections of 5-7 μm thickness were stained and slides examined for histological analysis with light microscope. While for ultrastructure observation small blocks (2×2 mm) of ovary specimens was fixed at 4°C in 2% glutaraldehyde in 0.2 M sodium cacodylate buffer pH 7.2, then kept in 1% osmium tetroxide for 1 h at room temperature, dehydrated through a graded ethanol series, cleared in propylene oxide and embedded in epoxy resin. By using an ultra-microtome with glass and diamond knifes, semi-thin sections of one micron thick were used and stained with 1% toluidine blue in and 1% sodium borate.

Finally, the ultrathin sections placed on 200 mesh copper grids and stained with uranyl acetate (saturated in 70% ethyl alcohol) followed by lead citrate. Transmission electron microscope was used for sections examination.

Statistical analysis: All data were expressed as mean and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Pearson coefficient was used to correlate between quantitative variables. Simple linear regression was assessed to find the equation of some parameters. Significance of the obtained results was judged at the 1% level.

RESULTS

Biological studies: The general ways of determining the reproductive state in female fish are by staging of ovaries using: macroscopic criteria, calculation of gonadosomatic index (GSI), classifying ovaries histologically and ultrastructurally.

Maturity stages of the ovary: The macroscopic aspects of the ovaries in Siganidae fish *S. rivulatus* during the annual reproductive cycle was examined by dividing the process into 6 stages, according to the size and weight of the ovary and the degree of occupying in the body cavity. These 6 stages of oocyte development includes: Stage I (Immature), Stage II (Maturing 1), Stage III (Maturing 2), Stage IV (Nearly ripe), Stage V (Ripe and Spawning) and Stage VI (Spent) as shown in Table 1 and Fig. 1.

Monthly distribution of maturity stages: The monthly distribution of maturity stages of female *Siganus rivulatus* throughout the different seasons indicated an annual reproductive pattern (Fig. 2). Immature females were found throughout the whole year except at the period of spawning season (from May until July) then began to appear again at the beginning of August, while mature females started to appear from March and ended after two consecutive months and reached the greatest percentage in April (Fig. 2). The period of ripening and spawning were intertwined with each other. The ripening stages appeared for the first time at late May while spawning stages appear in high proportion in June. Spawning had ceased in mid of July and the majority of females were in spent stage (Fig. 2).

Table 1: Macroscopic characteristics of the ovary maturity stages in rabbitfish *S. rivulatus* collected from Egyptian northwestern Mediterranean Coast in Alexandria

	Ovary of female	
Stages of maturity	Shape and its extent in the body cavity	Appearance of the ova
Stag I (Immature)	Small, pale ovary and occupying very small amount up to 1/6 of the body cavity, ova not visible to naked eye (Fig. 1a)	-
Stage II (Maturing 1)	Pale yellow swollen granular ovary visible with naked eye and occupying about 1/3 of the body cavity (Fig. 1b)	Medium size opaque and cannot be easily separated from each other
Stage III (Maturing 2)	Pale yellowish ovaries and riches with blood vessels on the dorsal side, ova clearly visible with naked eye and occupying about ½ of the body cavity (Fig. 1c)	Medium size and opaque and fully yolked
Stage IV (Nearly ripe)	Pinkish yellow ovaries with prominent blood vessels and occupying the whole body cavity (Fig. 1d)	Large mature and semitransparent ova prominently visible
Stage V (Ripe and Spawning)	Orange and reddish ovaries, large hydrated ova clearly visible and occupying full length of the body cavity (Fig. 1e)	Large ripe and transparent ova prominently visible
Stage VI (Spent)	Empty collapsed reddish ovaries with collapsed lopes and occupying about 1/2 of the body cavity (Fig. 1f)	Trapped scattered unshed medium size ova

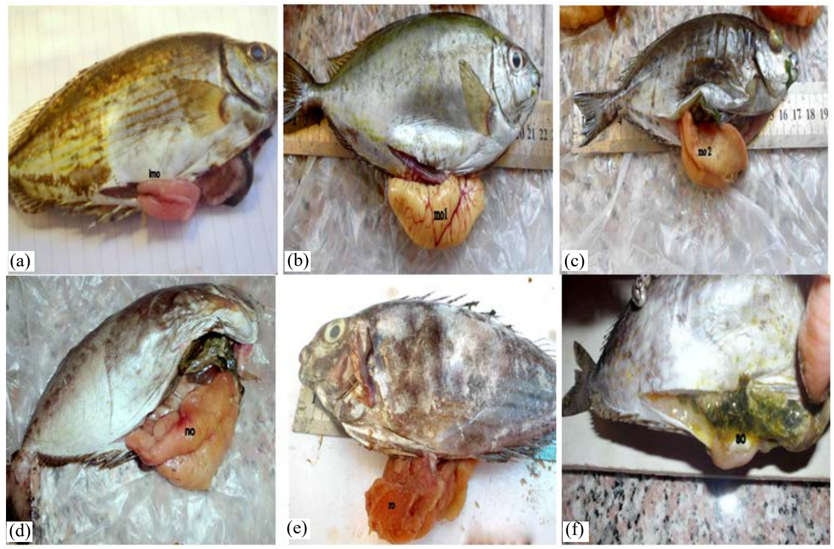

Fig. 1(a-f): Photomicrograph of whole mount of female rabbitfish *S. rivulatus* showing the ovary maturity stages along the year in eastern Mediterranean Coast of Alexandria, (a) Stage I (Immature), (b) Stage II (Maturing 1), (c) Stage III (Maturing 2), (d) Stage IV ((Nearly ripe), (e) Stage V (Ripe and Spawning) and (f) Stage VI (Spent)
Imo: Immature ovary, mo1: Mature ovary1, mo2: Maturing ovary 2, no: Nearly ripe ovary, ro: Ripe and spawning ovary, so: Spent ovary

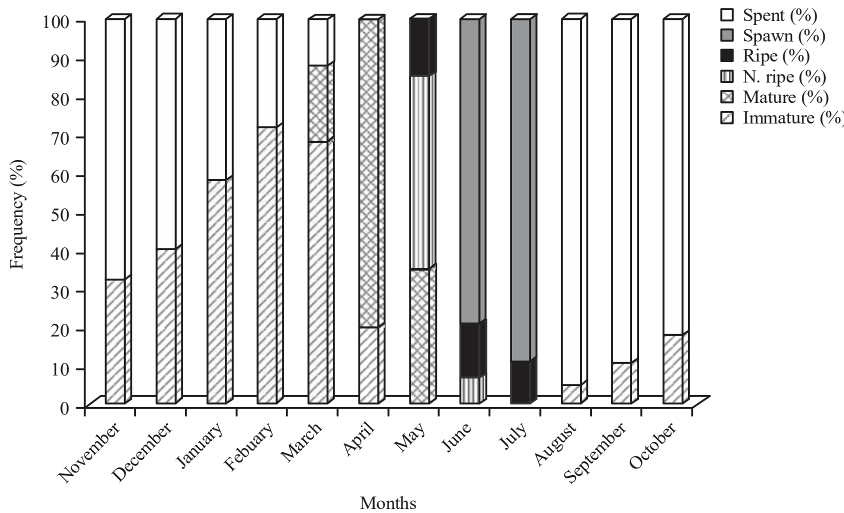

Fig. 2: Histogram of percentages frequency of monthly distribution of maturity stages in females *Siganus rivulatus* throughout different seasons at 2017

Length at first sexual maturity: The length at which 50% of fishes reach their sexual maturity was considered as the length at first sexual maturity. The percentages of maturity distributions for each length group were applied as a marker to detect the onset of the sexual maturity. The length at first sexual maturity was represented in Fig. 3. The result showed that sizes of females smaller than 15 cm were immature fish. The female L_{50} (50% of mean total length) attained first sexual maturity of *S. rivulatus* were found when the females size reaches 16 cm (Fig. 3). Finally, it was found that all females size larger than 18 cm in total length were fully mature.

Gonadosomatic index (GSI): The monthly variations of the mean GSI values of female *S. rivulatus* were directly related to the ovarian maturity stages. At the beginning of the pre-spawning period, ovarian weights started to increase and the peak of the breeding season was in June with highest GSI values corresponding to the ripe and spawning stages (Fig. 4). The GSI values began to decrease towards mid of July which is indicative of the end of spawning season. All specimens during August until December showed small gonad weight and had lowest GSI values which concurrent with spent period Fig. 4.

Fecundity: Females with ripe ovaries used to estimate fecundity. For estimation of absolute fecundity (Fa) only total number of ripe eggs that only spawned by one female in spawning season was considered while on the other hand relative fecundity (Fr) designated the number of eggs spawned per unit length or weight of fish.

Fecundity-length relationship: The relationship of the absolute and relative fecundity with the Total Length (TL) of *Siganus rivulatus* is represented in Table 2 and Fig. 5-6. Absolute fecundity ranged from 200504-881320 eggs for females with lengths from 15-24 cm (Table 2). The relationships between absolute fecundity (observed and calculated) and the length group provided a positive linear relationship where the absolute fecundity (Fa) increased significantly with total length of fish, $R^2 = 0.9713$ and this relation expressed in Fig. 5. Similarly, relative fecundity ranged from 13366.9-36721.6 eggs for females with lengths from 15-24 cm (Table 2). A positive linear relationship between relative fecundity (observed and calculated) and length was shown where the relative fecundity (Fr) increased significantly with total length of fish, $R^2 = 0.958$ and this relation expressed in Fig. 6.

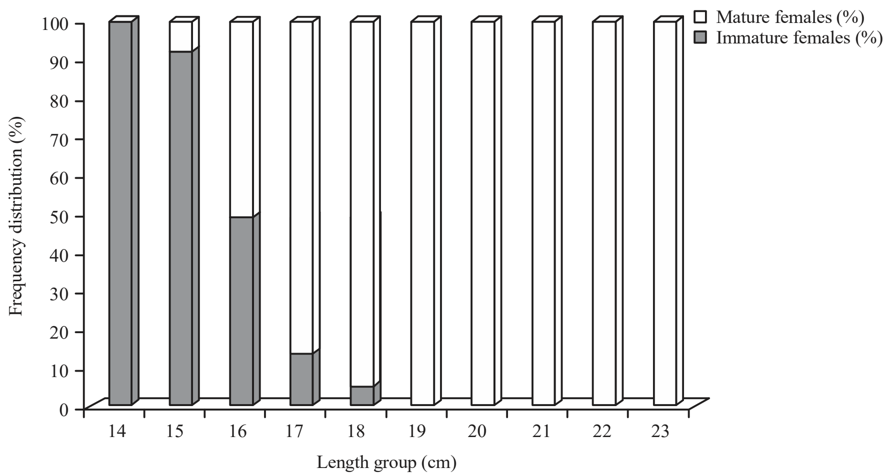

Fig. 3: Histogram of percentages frequency distribution of mature and immature females *Siganus rivulatus* throughout different seasons at 2017

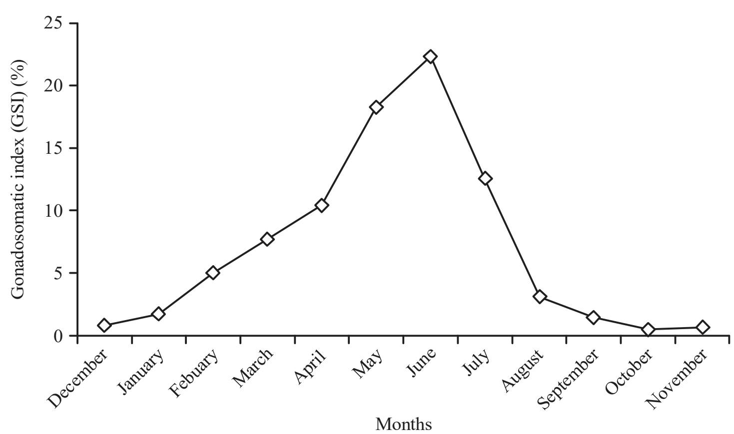

Fig. 4: Monthly variation of gonadosomatic index (%) (GSI) in female *Siganus rivulatus* throughout different seasons at 2017

Table 2: Relationship between fecundity and the Total Length (TL) of the rabbitfish *Siganus rivulatus* collected from Egyptian northwestern Mediterranean Coast in Alexandria

No. of fish	Fish length	Average absolute fecundity	Calculated absolute fecundity	Average relative fecundity	Calculated relative fecundity
5	15	200504	210980	13366.9	12802
4	16	270310	291712	16894.3	15628
6	17	310118	372444	18242.2	18454
5	18	345617	453176	19200.9	21280
3	19	410551	533908	21607.9	24106
6	20	541332	614640	27066.6	26932
4	21	663422	695372	31591.5	29758
3	22	787766	776104	35807.5	32584
2	23	801876	856836	34864.2	35410
2	24	881320	937568	36721.6	38236

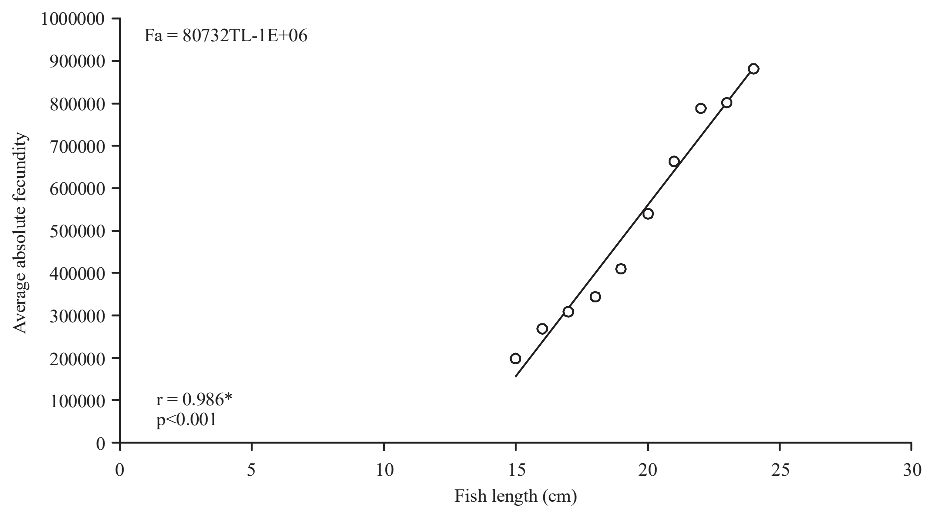

Fig. 5: Relationship between the average absolute fecundity (Fa) and the average fish length of rabbitfish *S. rivulatus* in throughout different seasons at 2017

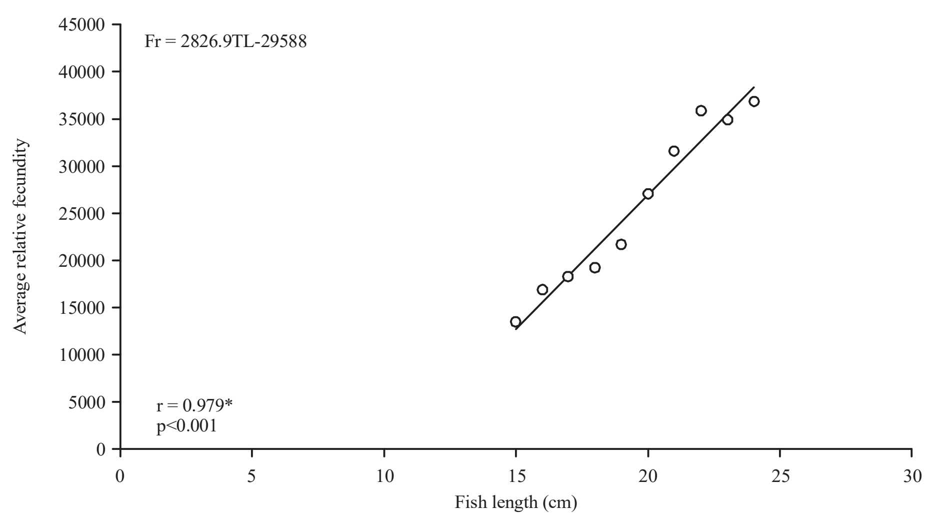

Fig. 6: Relationship between the average relative fecundity (Fr) and the average fish length of rabbitfish *S. rivulatus* in throughout different seasons at 2017

Fecundity-weight relationship: The relationship of the absolute and relative fecundity with the fish gutted weight of *Siganus rivulatus* were represented in Table 3 and Fig. 7-8. Absolute fecundity ranged from 200504-881320 eggs for females with gutted weight (w) from 57-120 g (Table 3). The results revealed that the absolute fecundity (Fa)

Table 3: Relationship between fecundity and the average gutted weight (w) of the rabbitfish *Siganus rivulatus* collected from Egyptian northwestern Mediterranean Coast in Alexandria

No. of fish	Average gutted weight	Average absolute fecundity	Calculated absolute fecundity	Average relative fecundity	Calculated relative fecundity
5	57	200504	513534	3517.6	3585.56
4	68	270310	619871	3975.1	4135.34
6	70	310118	639205	4430.2	4235.3
5	75	345617	687540	4608.2	4485.2
3	110	410551	1025885	3732.3	6234.5
6	103	541332	958216	5255.6	5884.64
4	99	663422	919548	6701.2	5684.72
3	110	787766	1025885	7161.5	6234.5
2	115	801876	1074220	6972.8	6484.4
2	120	881320	1122555	7344.3	6734.3

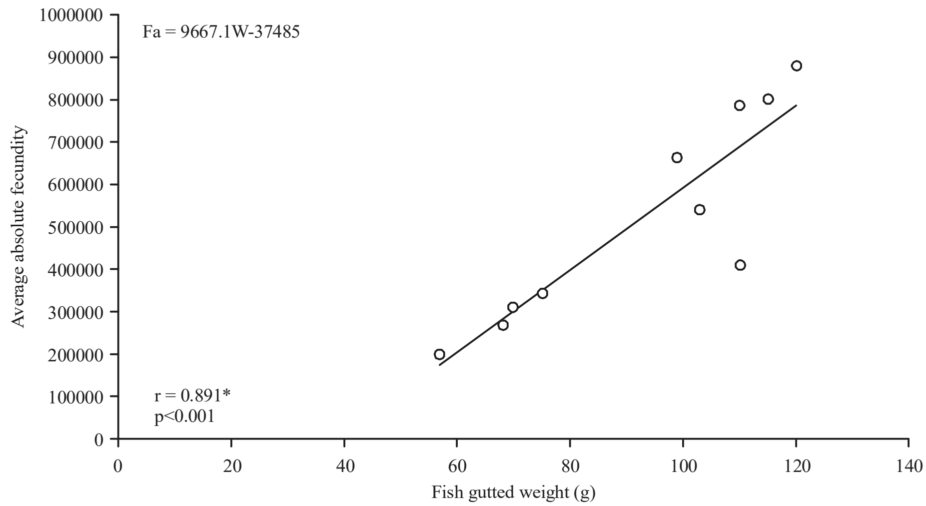

Fig. 7: Relationship between the average absolute fecundity (Fa) and the average gutted weights of rabbitfish *S. rivulatus* in throughout different seasons at 2017

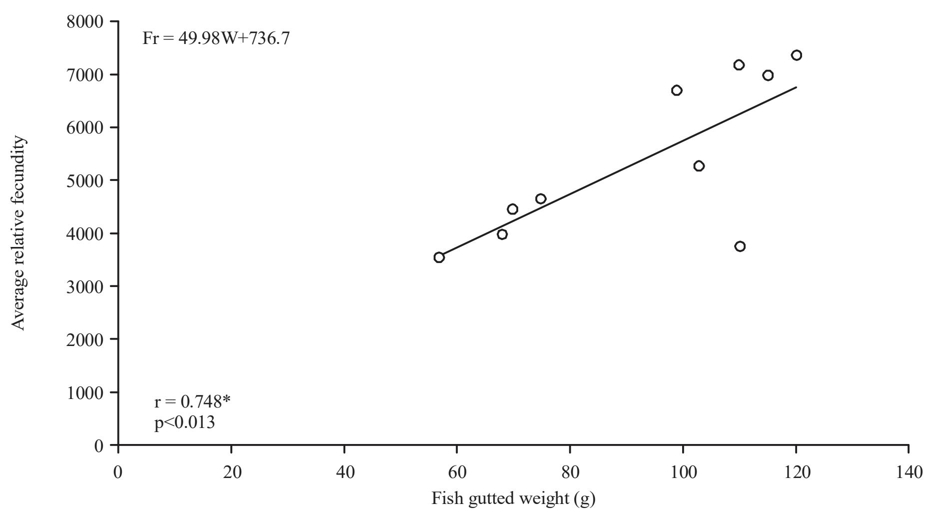

Fig. 8: Relationship between the average relative fecundity (Fr) and the average gutted weights of rabbitfish *S. rivulatus* in throughout different seasons at 2017

increased significantly with gutted weight (w) of the fish, R^2 = 0.7935 with a positive the linear relationship was expressed in Fig. 7. Relative fecundity ranged from 3517.6-7344.3 eggs for females with gutted weight (w) from 57-120 g (Table 3). Likewise, a positive linear relationship between relative fecundity (Fr) and gutted weight (w), R^2 = 0.5595 for female *Siganus rivulatus* was established by the regression equation (Fig. 8). The above results showed that fecundity (absolute or relative) had significantly increased with increasing the lengths and gutted weights of the fish.

Seasonal variation in the ovary during the annual reproductive cycle with histological and ultrastructural evidence: The study showed the ovary of the rabbitfish *Siganus rivulatus* undergoing marked cyclic morphological, histological and ultrastructure changes during the annual reproductive cycle in response to fluctuations in the seasonal environmental parameters. During the spawning season, ripe oocytes were characterized with rapidly increased of oocytes diameters and a large amount of migratory nucleus at final ripe stage (germinal vesicle migration) (Fig. 9). The oocyte ultrastructural differentiation with well-developed vitelline envelope (chorion) acted as a good mark that oocytes were entering the ripening and spawning stages (Table 4, Fig. 9). In comparison to the out of season and during oocyte development, oocyte growth started a series of morphological changes leading to subsequent stages of oocyte developments and a drastic histological and ultrastructural changes (Table 4, Fig. 10, 11). Such changes were observed in the oocytes diameters, the structure of the oocytes wall (zona radiate) and oocyte hydration to accommodate the physiological requirements to begin the spawning time (Table 4, Fig. 10, 11). The microscopic analysis in the ovary of rabbitfish *S. rivulatus* indicated a synchronous oocyte development.

Table 4: Differences among stages in oocyte diameter and oocyte appearance during the annual reproductive cycle of rabbitfish *S. rivulatus*

Stages	Oocyte diameter (µm)	Oocyte characteristics
Immature stage	28-45	• Primary polygonal oocytes with faint cytoplasm
Mature stage	94-115	• Follicular epithelial layer around the oocyte
		• Yolk nucleolus
		• Layer of electron-dense (zona radiate)
Vaculized stage	150-215	Primary vaculized oocyte
		• Peripheral vacuoles (cortical alveoli formation)
		Secondary vaculized oocyte
		• Vitelline envelop formation
Vitellogenic stage	230-440	• Small accumulated of yolk granules
		• Few cytoplasmic vacuoles
		• Zona radiate with numerous interdigitating cytoplasmic processes of microvilli
		• Differentiated of granulosa cells
Pre-ovulatory stage	510-530	• Migratorion of the nucleus in the animal pole (germinal vesicle migration)
		• Hydration of the yolk mass
		• Lipid droplets fusion
		• Well developed vitelline envelop (chorion)
Spent stage		• Few atretic residual empty follicular oocytes
		• Hypertrophy of the oocyte wall
		• Digest of yolk granules
		• Next generation of early stages

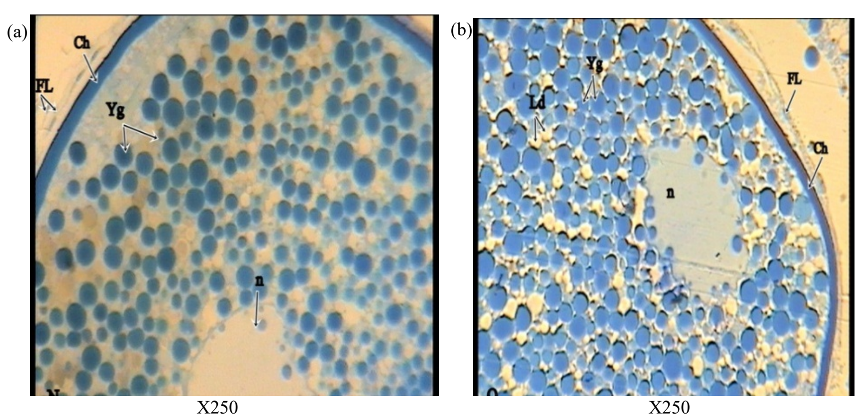

Fig. 9: Continue

(c)

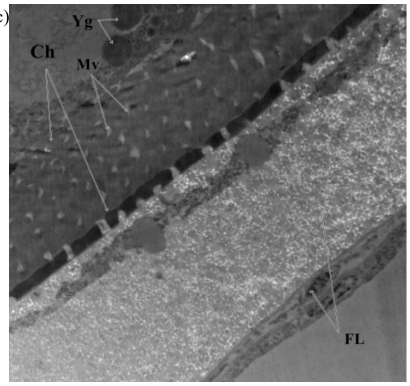

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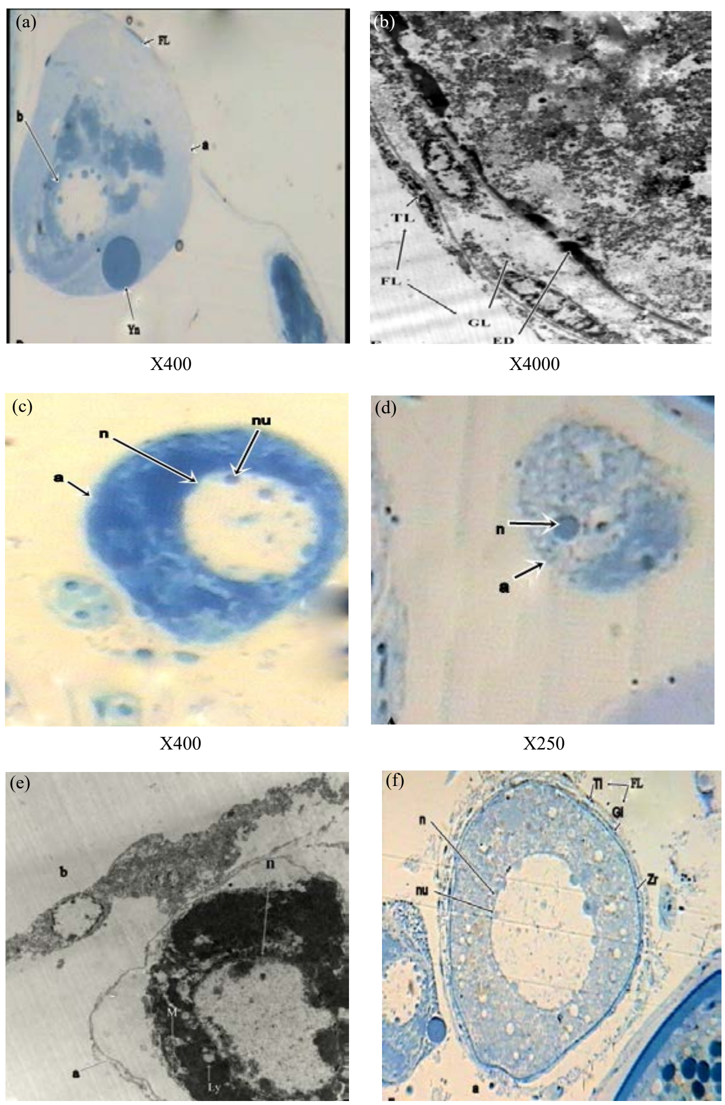
Fig. 9(a-c): Photomicrograph of cross section histological and ultrastructure of the oocyte at ripe stage in *Siganus rivulatus*, (a) Ripe oocytes, (b) Germinal vesicle migration and (c) Wall of ripe oocyte
Yg: Yolk granules ch: Chorion, FL: Follicular epithelial layer Ld: Lipid droplets, Mv: Microvilli



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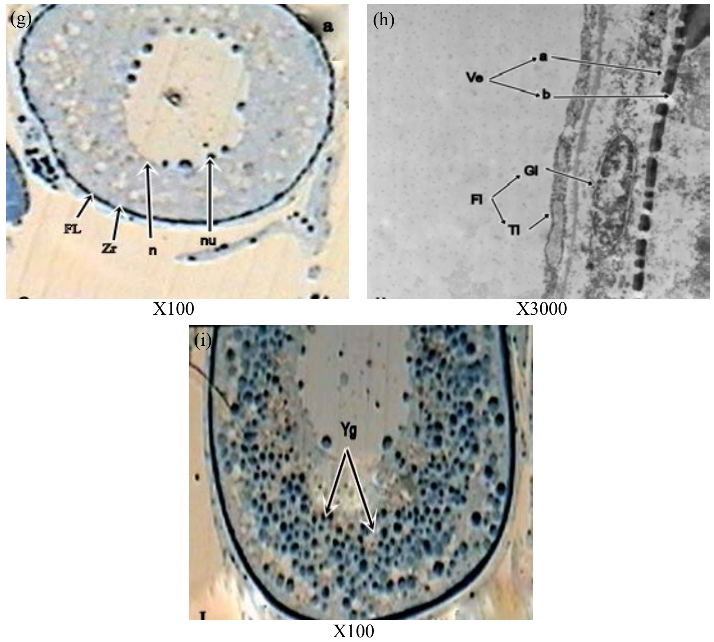

Fig. 10(a-i): Photomicrograph of cross section histological and ultrastructure of the oocyte at different developmental stage in *Siganus rivulatus*, (a) a: Presynaptic oocytes, (b) Oogonia (a: Wall of oogonia, b: Wall of ovary), (c) Early phase of a: Previtellogenic oocytes, (d) Maturing oocyte a: Nucleus b: Yolk nucleus (Yn), (e) Wall of maturing oocyte showing: ED: Electron dense, (f) Vacuolized oocyte showing one row of vacuoles, (g) Vacuolized oocyte showing 2 rows of vacuoles, (h) Wall of vacuolized oocyte showing: 4 different layers: follicular epithelial layer (FL): consists of theca layer (TL) and Granulosa layer (GL), a: Zona radiata externa, b: Zona radiata internal, (i) Early vitellogenic oocyte

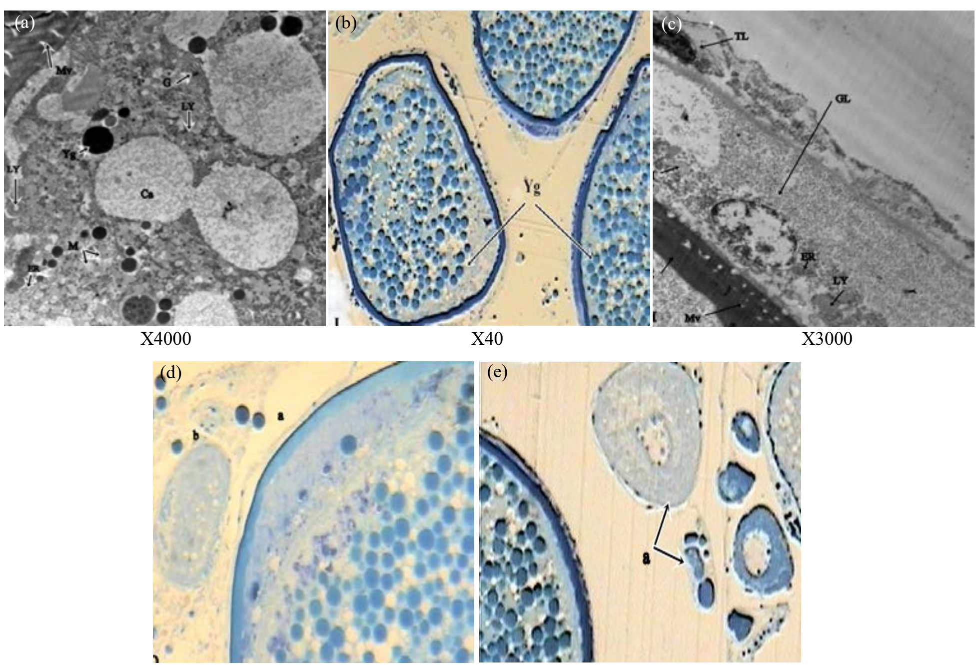

Fig. 11(a-e): Photomicrograph of cross section histological and ultrastructure of the oocyte at different developmental stage in *Siganus rivulatus*, (a) Cytoplasm of early vitellogenic oocyte, (b) Late vitellogenic oocyte, (c) Wall of late vitellogenic oocyte, (d) Hypertrophy of the wall of spent oocytes and (e) New generations of early developing oocytes

nu: Nucleolus, n: Nucleus, Zr: Zona radiata, Yg: Yolk granules, Mv: Microvilli, LY: Lysosome, G: Golgi apparatus, M: Mitochondria, ER: Endoplasmic reticulum, VE: Vitelline envelope, Ld: Lipid droplets, ch: Chorion

DISCUSSION

In the present study, the biometric parameters showed all females whose body length is over 18 cm were fully mature. These results are in concordance with other studies where female rabbitfish *Siganus canaliculatus* attained maturity L_{50} was about 17.2 cm at Qatar coasts or about 18 cm in Arabian Gulf at Saudi Arabia coast and 23.9 cm total length in the Arabian Sea Coast of Oman[12,30,31]. While mean total length at first sexual maturity (L_{50}) for female boarfish (*Capros aper*) was at 9.7 cm total length[32]. Gonadosomatic index (GSI) is an indicator commonly used for depicting annual reproductive cycles in fishes[33]. As a whole, this work's findings obtained from the monthly fluctuations in the gonadosomatic index (GSI) agreed in a great extent with monthly variations of the maturity stages and histological view[31,34]. A spawning peak of *L. elongate* between May and June[35]. However, in the present study, the peak of the spawning season was found in June for *S. rivulatus* when surface water temperature ranged[15,36] between 24 and 29°C. From the present result, the rabbitfish *S. rivulatus* in the northwest Mediterranean Coast of Alexandria, Egypt spawns once a year. Most important economic fish are spawning once a year in short reproductive season[37]. Rabbitfish begin their spawning activities in the early summer months and continue for a short time until mid summer[38]. The oocytes developments have unique characteristic features such as GSI and the oocytes diameter, which are different in different species[39]. The eggs appearances of *Siganus* fish at different maturity stages until spawning conditions were small, spherical, demersal and strongly adhesive[40]. Season influence on the gonad index and oocytes development of *S. rivulatus* are similar to those described for other species[34,35,39,41]. In *Siganus rivulatus* fecundity shows a wide range for a given length but in general, the absolute fecundity has linear regression relationship with length groups and gutted weight. A large fish lays more eggs than a small one, while the correlation of fecundity with weight in most fish is higher than that with length[42]. There is an inverse relationship between egg size and fecundity[43,44]. The work described the seasonal ovarian cycle in *Siganus rivulatus* were described during the annual reproductive cycle and dividing into six stages. All typical stages observed of oogenesis in this species were already described in the literature[32,34,41]. Different developmental stages of the gonads of *Siganus rivulatus* fit with that of most teleosts[19,45]. In the present study, for characterizing the developing oocytes of *Siganus rivulatus* different monitoring criteria were used. These included the size, color of the ovary and the morphological changes of vitelline envelope. The maturation period in *Siganus rivulatus* was characterized by the appearance of follicular layer around the oocyte and yolk nucleus formation. The follicular layer considered as a good proof for synthesis of sexual steroids that meet the requirements of ovulation[46]. Yolk nucleolus is a special structure for the yolk granules development[47]. The first appearance of zona radiata layer was at mature stage as a layer of electron-dense material in the area just under granulose layer. The vacuolization stage of the oocyte was concomitant with cortical alveoli formation[21]. The thecal and granulose cells are the major cellular sites of synthesis and secretion of steroid hormones[48]. The number of organelles was dramatically increased during development as the evidence to produce steroid hormone[49]. This difference may be specific or related to factors such as physiological characteristics to accommodate to the spawning periods. In *Siganus rivulatus*, the yolk deposition period characterized with increase the proportion of oocytes and the yolk granules spread into the centre of the cytoplasm. The nucleus migration initiates to start and act as good evidence of the final maturation process at the end of yolk accumulation stage[50]. In the present work, the oocyte hydration started once vitellogenesis completed and the diameter of the oocyte was rapidly increased. Spawning time confirmed by hydrated oocytes and postovulatory follicle[16]. While in spent stage the ripe unshed oocytes characterized with hypertrophy of the oocyte wall and slightly going to invade inside the cell and digest the yolk granules. The zona radiate lost the reticular network structure and assumed a uniform electron dense structure at the migratory nucleus stage[51].

CONCLUSION

Siganus rivulatus has a short spawning period extending from late May to mid of July (in the current study) with an annual seasonal variation depending on the different environmental factors. The study recommends determining the reproductive biology of this important commercial marine fish species annually to anticipate the accurate spawning time with the appropriate environmental conditions. The work encourages protection of this fish species by prohibiting capturing fish bigger than 18 cm total length especially during the critical spawning season.

SIGNIFICANCE STATEMENT

This study confirms the variation in the time of the spawning season of *Siganus* species which can be useful in planning protection of the important species. Prohibiting capturing it in this critical varied spawning season has huge implications due to its commercial and ecological importance. The study opens the door to researchers to explore seasonal variation in other fish species too, likely due to various environmental factors. Hence a new theory might have been arrived at.

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