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The reproductive biology and sex hormones of the rabbitfish (*Siganus rivulatus*) in the Suez Gulf, Egypt.

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ABSTRACT

Samples of the rabbitfish (*Siganus rivulatus*) were collected monthly during January- December 2016 from the Suez Gulf. The maturity stages of the males and females were morphologically separated according to the changes in shape, size, and color of the gonads in different six successive stages. Stage I (Immature), stage II (Maturing 1), stage III (Maturing 2), stage IV (Mature), stage V (Ripe), and stage VI (Spent). Reproductive characteristics of *Siganus rivulatus* showed that the male reaches first sexual maturity at a smaller body length than the female (15.1 cm and 15.8 cm for male and female, respectively). The gonado-somatic index (GSI) of males was lower than that of females. Where, the maximum GSI values were recorded in July (7.9 % and 6.8 % for females and males, respectively). The absolute fecundity of the present species ranged from 147321 to 384086 eggs. The logarithmic relationship showed that fecundity increased with the increase of length, weight, and gonad weight. K values for combined sex began to increase from January to reach a maximum value (1.65) at April then decreased from May to August. The spawning season of *Siganus rivulatus* in the Suez Gulf may be extending from May to August for females and males. 17β - Estradiol level in females of *S. rivulatus* ranged from 0.076 ± 0.007 ng / ml (recorded in June 2016) to 0.003 ± 0.0005 ng / ml (recorded in December 2016). Estradiol level was low during February but afterward, it increased significantly in June 2016. Testosterone level in males of *S. rivulatus* recorded the highest value of 1.698 ± 0.022 ng/ml in July. Then, it continuously decreased, reaching the minimum value of 0.039 ± 0.007 ng/ml in December 2016 (17.4 ± 1.054).

INTRODUCTION

Rabbitfish belong to the genus *Siganus* of the Family Siganidae **(Woodland, 1990)**. Siganids are herbivorous fishes in marine and brackish waters that are found throughout the indo west Pacific. They are the more common species and represent the objects of traditional subsistence and commercial fisheries throughout this region **(Woodland, 1983)**. Rabbitfish are considered to be excellent food fish in many parts of the world especially in the eastern Mediterranean and Indo-Pacific regions. Egyptian production of rabbitfish amounted by 1363 ton in 2014. Mediterranean Sea fisheries took part in 822 ton production, Red Sea (466 ton) and lakes (75 ton) according to **GAFRD**

(2014). *Siganus rivulatus* are mainly found in shallow water, usually less than 15 meters in depth. This shallow depth should be predictable for species that is almost entirely herbivorous where algae are the more widespread at these depths **(El-Drawany, 2015)**.

In teleost fishes, vitellogenesis and final gonad maturation are regulated by gonadotropins via steroids secreted by the granulosa and theca cells of developing and mature gonad. The occurrence of steroid production in different cells of the ovary may be related to different phases of oocyte development. Of these steroids, 17-β estradiol (E 2) stimulates in turn the hepatic synthesis and secretion of vitellogenin which is accumulated in the gonad. Correlations between changes in plasma levels of gonad steroids and oocyte development have been well documented in a number of freshwater species including Salmon forms **(Whitehead *et al*., 1983** and **Truscott *et al*., 1986)**, Cyprinids **(Kobayashi *et al*., 1987)**, catfish *Heteropneustes fossilis* **(Lamba *et al*., 1983)**, goldeye *Hiodon alosoides* **(Pankhurst *et al*., 1986)**, walleye *Stizostedion vitrum* **(Malison *et al*., 1994)**, and marine species including orange roughly *Hoplostethus atlanticus* **(Pankhurst & Conroy, 1988; Bariche *et al*., 2003)**, Japanese whiting *Sillago japonica* **(Matsuyama *et al*., 1990)**, Japanese sardine *Sardinops melanostictus* **(Matsuyama *et al*., 1991)**, Common snook *Centropomus undecimalis* **(Roberts *et al*., 1999)**, *Caspian kutum* **(Sabet *et al*., 2009)**, catfish *Hemibagrus nemurus* **(Adebiyii *et al*., 2013)** and *Liza carinata* **(Hefny *et al*., 2016)**. Fish have evolved to reproduce under environmental conditions that are favorable to the survival of the young.

Long before spawning, seasonal cues begin the process of maturation. In many fish, this can take up to a year. When the gametes have matured, an environmental stimulus may signal the arrival of optimal conditions for the fry, triggering ovulation and spawning. Examples of environmental stimuli are changes in photoperiod, temperature, rainfall, and food availability. A variety of sensory receptors detect these cues, including the eye, pineal gland (an organ in the dorsal part of the forebrain that is sensitive to light), olfactory organs, taste buds, and thermo receptors **(Sabet *et al*., 2009)**.

The aim of this work was to investigate the reproductive cycle and the physiological role of gonadal steroids, the hormonal profiles of testosterone (T) and 17-β estradiol (E 2) and sexual maturity of rabbitfish (*Siganus rivulatus*) during spawning season.

MATERIALS AND METHODS

Samples of rabbitfish (*Siganus rivulatus*) were collected monthly during the period from January to December 2016 from the Suez Gulf. The fishing ban in the Suez Gulf of was overcome by amateur fishermen through picnic boats. Fish were weighed to the nearest 0.1 g and measured to the nearest 0.1 cm. The gonads were separated, weighed and placed in 10% buffered formalin. The stage of gonad maturity was determined macroscopically. The spawning season was determined by the curvilinear average values of monthly gonadosomatic index (GSI) for both males and females where:

GSI= (gonads weight (g)/gutted weight (g))* 100

The condition factor estimated from the relation below:

$$K = 100\ W / L^3$$

Where, K= Condition factor, W= Weight of fish (g) and L= Length of fish (cm).

To calculate fecundity, all available ovaries (35) recognized as III to V maturity stages were placed in jars with formalin 10%. The egg mass of each ovary was weighed (**w**) and four subsamples (its weight is **x**) were taken containing (**n**) ripe eggs. The total number of ripe eggs (**F**) in the ovary (absolute fecundity) was calculated using the following formula:

$$\mathbf{F = wn / x}$$

Fish were anaesthetized and blood samples were taken from the caudal vessels by using heparinized disposable syringes. Blood sample was centrifuged for 10 min at 3000 rpm and the plasma was stored at -45 °C until steroid analysis. Plasma levels of (E 2) and (T) were measured by radioimmunoassay using the procedure described by **Rinchard *et al*. (1993)**. The test of profile was done in duplicate.

RESULTS AND DISCUSSION

1. Maturity stages

The gonad in *S. rivulatus* during the annual reproductive cycle was examined by dividing the process into six stages, according to the size, color and the degree of occupying in the body cavity. These 6 stages of male and female gonad development includes: Stage I (Immature); Stage II (Maturing 1); Stage III (Maturing 2); Stage IV (Mature); Stage V (Ripe) and Stage VI (Spent). A macroscopic description of each stage is given in **Table (1)**.

Table (1). Maturity stages of male and female *Siganus rivulatus*

Maturity stages	Male	Female
Stage I (Immature)	Small, transparent, pale, occupying a very small portion up to 1/3 of body cavity	Small, transparent, pale, occupying a very small portion up to 1/3 of body cavity, ova not visible to naked eye
Stage II (Maturing 1)	Whitish, translucent, occupying about 1/2 of body cavity	Pale yellow, granular ova visible to naked eye, occupying about 1/2 of body cavity
Stage III (Maturing 2)	Creamy white, occupying about 3/4 of body cavity	Pale yellowish, blood vessels visible on dorsal side, ova clearly visible, occupying about 3/4 of body cavity
Stage IV (Mature)	Creamy white, soft, occupying full length of body cavity	Pinkish-yellow, blood vessels prominent, large ova prominently visible, occupying full length of body cavity
Stage V (Ripe)	Pale whitish, occupying entire body cavity	Orange and reddish ovaries, large hydrated ova clearly visible and occupying full length of the body cavity
Stage VI (Spent)	Empty collapsed reddish ovaries with collapsed lopes and occupying about 1/2 of body cavity	Flaccid, reddish, occupying about 1/2 of body cavity

2. Monthly distribution of maturity stages:

The monthly distribution of maturity stages of female and male *Siganus rivulatus* throughout the different months indicated an annual reproductive pattern **(Figure 1)**. Immature females and males were found throughout the whole year except at the period of spawning season (from May to July) but highest percentage was recorded in February. Maturing stage I started to appear from March and ended after two consecutive months for female but extended to May for males and they reached the greatest percentage in April recording 70 % and 60 % for females and males, respectively. The period of mature stage for males and females were intertwined with each other from May to July. The ripe stages appeared for the first time at May for female and at June for male and appeared in high proportion in July. Spent stage appears with maximum value in August for male and female, and gradually decreased reaching the lowest value in March.

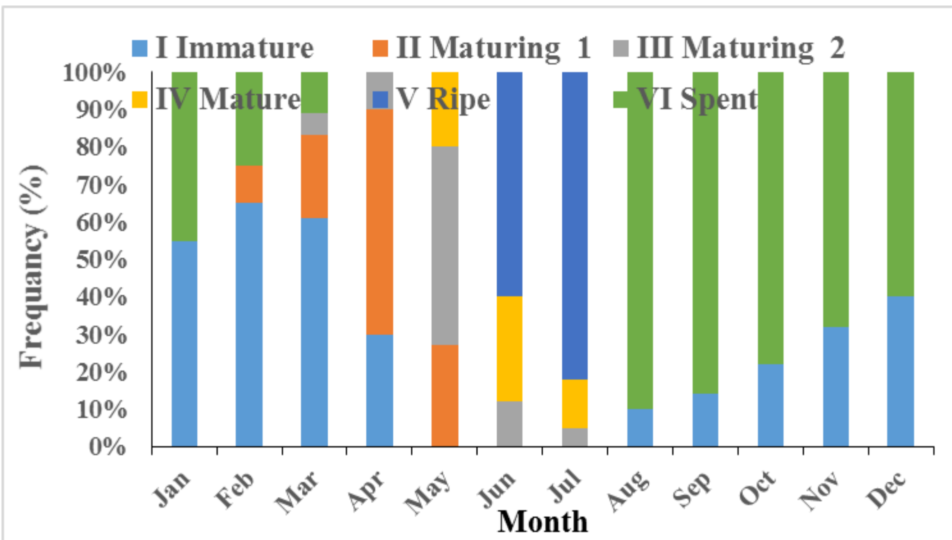

Fig. 1: Monthly distribution of maturity stages of female and male *S. rivulatus* in Suez Gulf.

3. Length at first sexual maturity

The length at first sexual maturity was considered as the length at which 50% of fishes reach their sexual maturity. Males and females of *S. rivulatus* were grouped into 10 mm size groups and the percentage occurrence of fish at the different maturity stages in each size group was calculated. Length at first sexual maturity was represented in **Figure (2)**. Examination of the male and female maturity stages indicated that males and females of *S. rivulatus* matured at about 15.1 and 15.8 cm total length, respectively. All male and females size larger than 18 cm in total length were fully mature.

These results are in conformity with **Shakman *et al.* (2008)** and **Eldrawany (2015)** where their results indicated that males and females of *S. rivulatus* from central Mediterranean and bitter lakes matured at total length about 15.5 cm. Other studies where demonstrated that female rabbitfish, *S. 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 **(Philips & Akel, 2003)**.

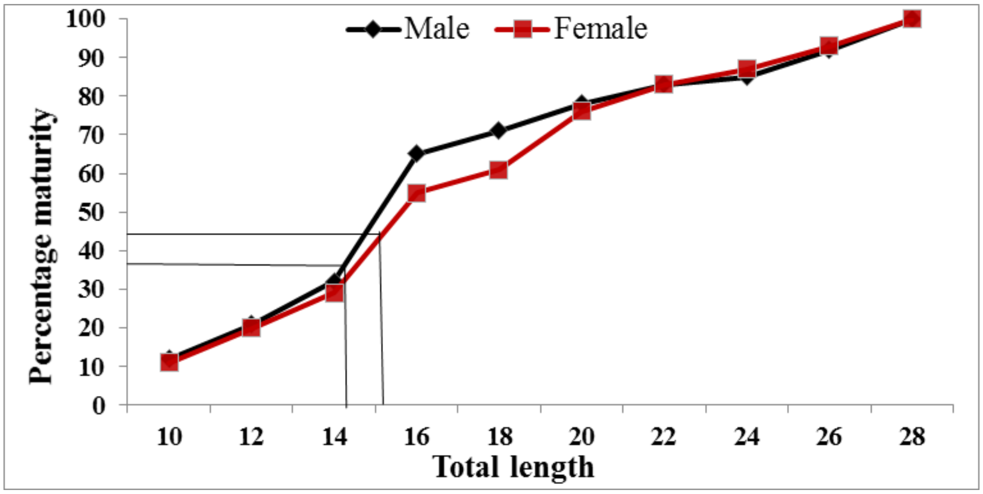

Fig. 2: Size at first maturity of male and female *S. rivulatus* in the Suez Gulf.

4. Gonadosomatic Index (GSI)

The monthly changes in GSI values of males and females **(Figure 3)** revealed that females always had a higher GSI than males. GSI values began to increase after April and reached the maximum values (6.8 in males and 7.9 in females) during July. A drop in GSI values from August to February. This demonstrated that spawning season of *S. rivulatus* in Suez Gulf extend from May to July.

These results are in symmetry with that of **El-Okda (1998)**, who demonstrated that spawning season of *S. rivulatus* in different marine waters of Egypt extends from May to August. **Mohamed (1991)** and **Fahmy (2019)** recorded that the peak of the breeding season (with highest GSI values) of the same species was in June from Mediterranean coastal region of Egypt. These observations are closed to that reported for the same species at Turkey **(Akşiray, 1987)**, at Lebanon **(Bariche *et al.*, 2003).** These results slightly differed from that mentioned in the Mediterranean coast of Turkey (from April to August) by **Torcu (1994)** and **Yeldan & Avsar (2000).** Also, **El-Drawany (2015)** reported that the spawning season of *S. rivulatus* in Bitter Lakes extended from May to July. In the other hand, the spawning season of *Siganus rivulatus* in Red Sea begins earlier than in Mediterranean as reported by **Popper *et al*. (1973)** at the Gulf of Aqaba, by **Hashem (1983)** from March to April and by **Amin (1985)** from February to April at Jeddah region of the Red Sea. This variation of spawning period of siganid species conclude that temperature plays a role in the determination of the timing and duration of spawning season **(Lam & Soh, 1975; Popper *et al.*, 1976** and **Amin, 1985)**.

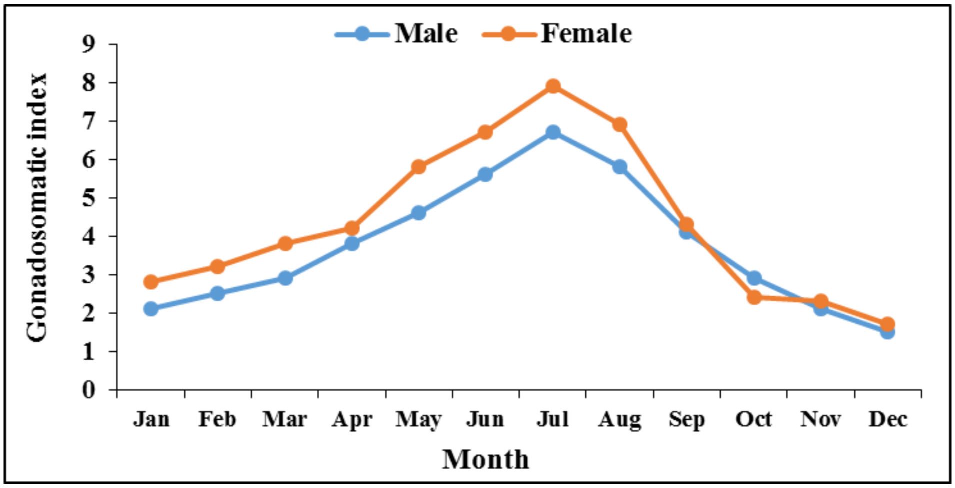

Fig. 3: Monthly variation in gonad-somatic index of male and female *S. rivulatus* in Suez Gulf

5. Condition Factor (K):

Condition factor (K) values for combined sex began to increase from January to reach a maximum value (1.65) at April then decreased from May to June (**Figure, 4)**. Due to some correlation between the condition factor and changes in the food elements deposited in the muscle tissues of fish, it was used to determine the breeding seasons of bony fishes by some authors **(Htun-Han, 1978** and **Avşar & Bingel, 1994)**. There is inverse relationship between the gonadal development and the food elements deposited in the muscle tissue, as the gonadosomatic index increases, the condition factor is expected to decrease.

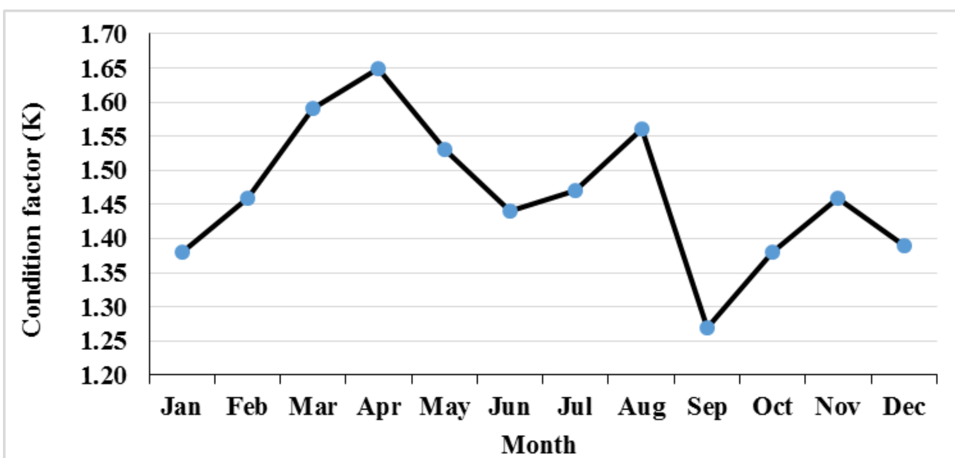

Fig. 4: Condition factor (k) for female and male *S. rivulatus* in Suez Gulf.

It can be inferred that the reason for this decline may be due to the fact that a high portion of the food elements have been used for the development of reproductive cells. By looking at the monthly changes in the condition factor it can be easily shown that the Suez Gulf is similar as the Mediterranean rabbitfish breed between May and August.

6. Fecundity:

The absolute fecundity is the total number of ripe eggs in the ovary. Thus, the ripe egg count in *S. rivulatus* ranged from 147321 to 384086 eggs. Its fecundity increased with the increase in length, weight and gonad weight. The logarithmic relationships between fecundity (F) and length of fish (L), body weight (W) and ovary weight (OW) were expressed by the following equations **(Figures, 5-7)**:

$$\text{Log F} = 2.2833 \log L + 2.2534 \qquad (R^2 = 0.9592)$$

$$\text{Log F} = 0.7558 \log W + 3.5618 \qquad (R^2 = 0.9499)$$

$$\text{Log F} = 2.0975 \log OW + 2.9325 \qquad (R^2 = 0.9078)$$

Estimation of the length of the sexually mature individuals being from 18 to 28 cm and absolute fecundity ranged from 147321 to 384086 eggs makes it possible to compare the rabbitfish from the northeastern and southeastern Mediterranean and those from the Red Sea. Indeed, **Hussein (1986)** carried out a study on the rabbitfish sampled from the shores of the Mediterranean at Alexandria, Egypt and he reported that the sexually mature individuals were 15-28cm in length, and the calculated fecundity values varied between 103200 and 396600. **Hashem (1983)** reported the lengths of sexually mature rabbitfish in the Red Sea to be 15-17cm and that fecundity varied between 40000 and 300000. It can therefore be shown that although the smallest sexually mature rabbitfish individuals of the three regions are about the same length (15 cm); larger individuals are seen more frequently along the Egyptian shores and the highest fecundity values are obtained among the rabbitfish of the northeastern Mediterranean **(Yelden & Avsar, 2000** and **Anand & Reddy. 2017)**.

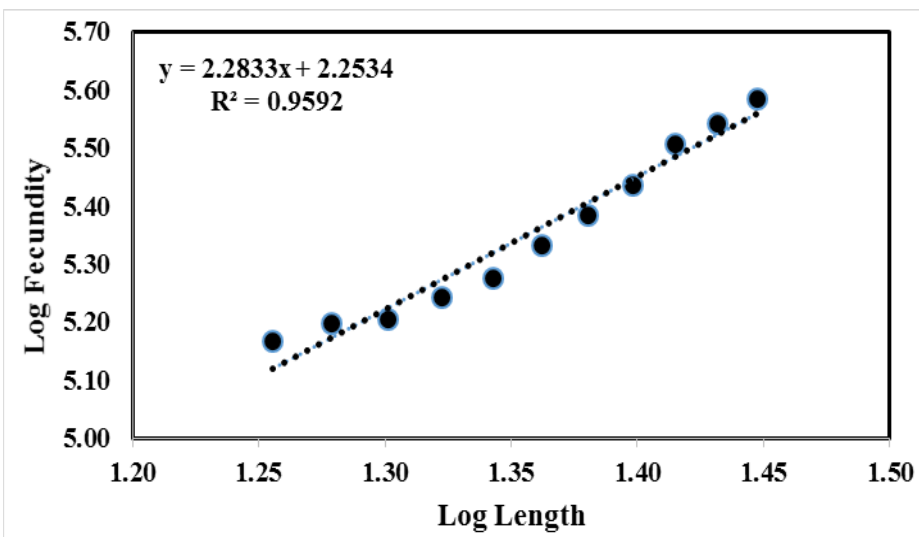

Fig. 5: Absolute fecundity–length relationship of female *S. rivulatus* in the Suez Gulf.

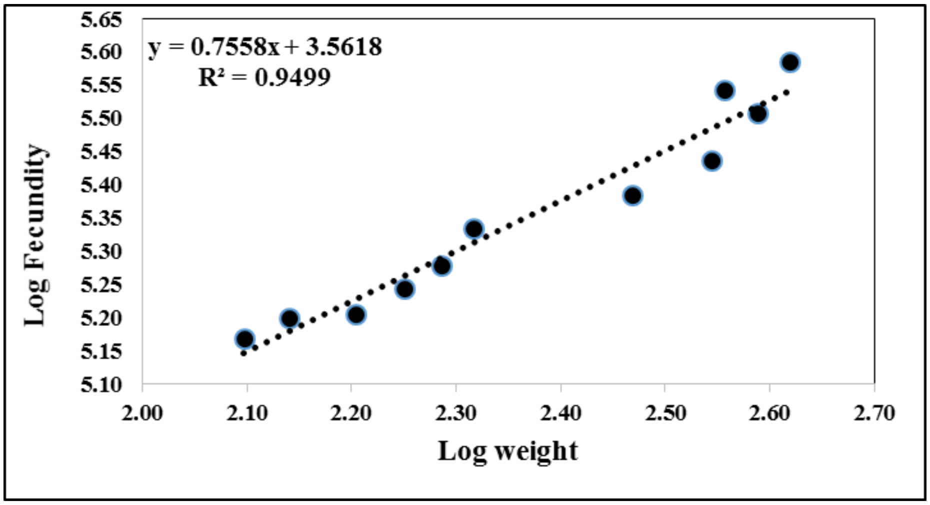

Fig. 6: Absolute fecundity–weight relationship of female *S. rivulatus* in Suez Gulf**.**

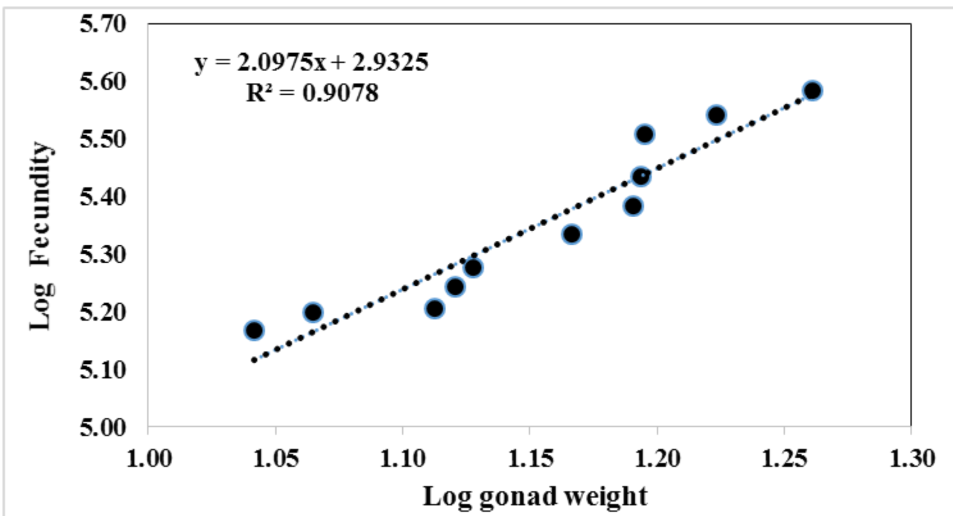

Fig. 7: Absolute fecundity–ovary weight relationship of female *S. rivulatus* in Suez Gulf**.**

7. Sex Steroid Hormones:

17β - Estradiol levels in female *S. rivulatus* ranged from maximum value 0.076 ± 0.007 ng / ml recorded in June 2016 to 0.003 ± 0.0005 ng / ml ml in December 2016. Estradiol levels were low from February but afterward they increased significantly in June 2016 **(Figure 8)**.

Testosterone levels **(Figure 9)** in male *S. rivulatus* recorded highest value (1.698 ± 0.022 ng / ml) in July, and their values are continuously decreased, reaching the minimum value (0.039 ± 0.007 ng / ml of testosterone was observed in December 2016 (17.4 ± 1.054).

Trends of changes in plasma estradiol-17β of female and testosterone of male *S. rivulatus* were somewhat in agreement with development of gonad maturation and GSI changes. In a variety of species, the level of serum E2 begins to increase in accordance with the appearance of active maturing gonad, and reaches the highest levels at ripe stage in the ovary, and sharply declines in fish with spent gonad. **(Smith Haley, 1988; Silvers *et al*., 1993; Kumar *et al*., 2015; Hefny *et al*., 2016** and **Pouresmaeilian *et al*., 2017)** reported an increase in plasma E2 levels once spawning commences and remains high throughout the period of gonad growth.

Fish testes secret steroid hormones in response to stimulation of GTH produced and secreted by the pituitary gland. Of these steroid hormones, it has been reported that testosterone is produced from steroid hormone producing of Leydig cells of interstitial tissue and involved in spermatogenesis as well as secondary sexual characteristics development **(Nagahama *et al.*, 1998)**. The level of testosterone in male *S. rivulatus* was shown to be highest before and after the spent stage which is in agreement with spot lined sardine, *Sardinops melanostictus* **(Matsuyama *et al*., 1991),** and *H. otakii* **(Lee *et al*., 2000)**.

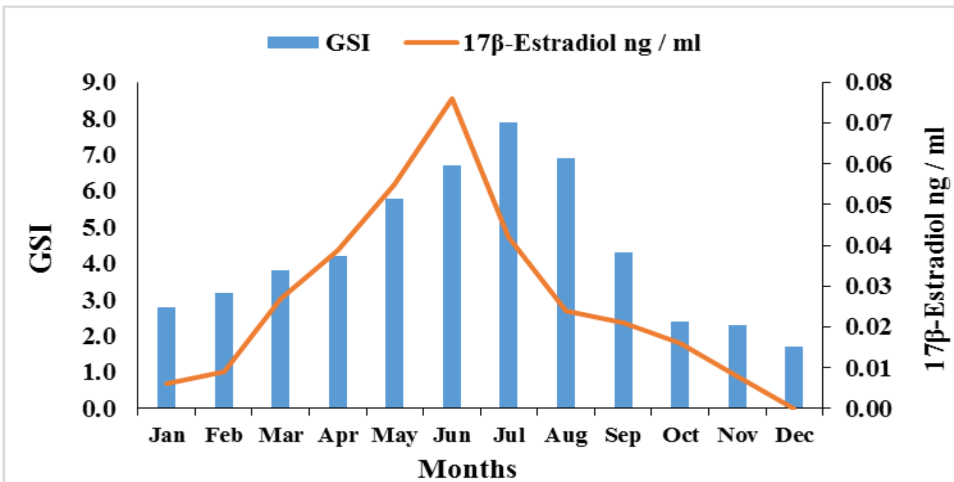

Fig. 8: Monthly changes in concentration of 17β-Estradiol levels and GSI of female *S. rivulatus* in the Suez Gulf.

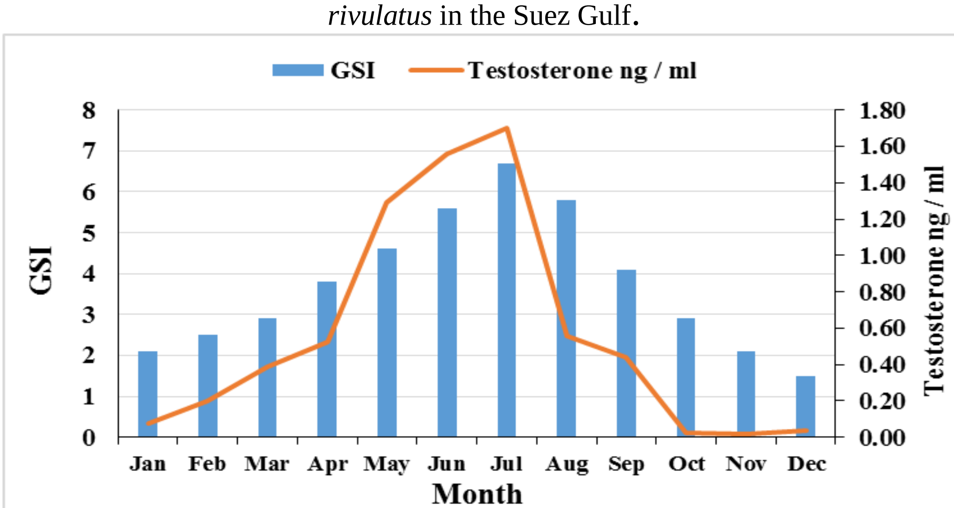

Fig. 9: Monthly changes in concentration of testosterone levels and GSI of male *S. rivulatus* in the Suez Gulf

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