Bull. Inst. Oceanogr. & Fish. ARE, 11 (1985): 131 - 147.

SEASONAL DEVELOPMENTAL CAHNGES IN THE OVARIES OF SIGANUS RIVULATUS FROM THE RED SEA

EKRAM MOHAMED AMIN
Institute of Oceanography and Fisheries, Alexandria, Egypt.

ABSTRACT

The annual sexual cycle of the ovary of female S. **rivulatus** throughout its life in the Red Sea can be divided into two prominent oocyte growth periods. The growth period is less active and it includes the mitotic division of reserved oogonia, syanpsis and protoplasmic growth of oocytes. The comprehensive changes occur in the secondary growth period. This highly active period is characterized by a dense deposition of yolk giving rise to a large size of oocyte. The different layers forming the egg membrane are well developed. The nucleus with its contents (Karyoplasm) are subjected to many consecutive changes so as to receive the spermatozoa through the micropyle which is well developed in the transition stage of gonad maturation (IV-V) in the animal pole. The active period of gonad development takes about three months (January-March). Female begins to spawn in March (average diameter eggs are 0.48 $\pm$ 0.015 mm) once a season, hence S. **rivulatus** is a monocyclic fish. Spawning season in the Red Sea extends to about seven months (March-September) during which fishes with ripe ovaries appear in the catch. Other fishes with spent ovaries show the process of atresia where the residual eggs and groups of empty follicle layers are detected.

INTRODUCTION

Rabbit fishes (Siganids) are herbivorous marine fishes, widely distributed in the Indo-Pacific region, and some species were also recorded in the Red Sea (Gunther, 1861; Day, 1878).

Although some investigators had succeeded in inducing spawning and rearing siganids in different parts of the world (Manacop, 1937; Fujjta and Ueno, 1954; Popper et al, 1973; Soh and Lam, 1973; Westernhagen and Rosenthal, 1975; Popper et al, 1979), and some ecological and behavioural aspects of siganids population in the Red Sea and Mediterranean Southern-east coast have been discussed (Popper and Gundermann, 1975), the description of ovarian development for most of the species is still unknown.

The histological studies of gonadal development of this species are essential for the reserach of the biological reproduction and contributive for the techniques to make possible the induction of maturation of such species, and for determining the proper time for induced spawning. Thus contribute to the improvement of the techniques for maturation induction.

The present paper describes the annual sexual cycle of the gonads of female **Siganus rivulatus** taken from Red Sea.

MATERIALS AND METHODS

A total of 300 samples of **S. rivulatus** were collected monthly from September 1980 to August 1981 from the commercial catch in Jeddah region (Red Sea). Ovaries were separated from fresh females and fixed in Bouin solution. Small parts of the gonads were passed through an ascending series of alcohol and embedded in paraffin. Sections of 4-10 µm were stained with Heidenhain iron Haematoxylin and eosin. Definitions of different stages of maturation were based on the scales given by Hjort (1910). Fish age was determined by Peterson's method (1895).

RESULTS

Ovaries of **S. rivulatus** are covered with a thin layer of ovarian wall with under this, a thin connective tissue, from the latter ova bearing lamellae composed of connective tissue fibres and germinal epithelium, project into the ovacoel. Oogenesis of **S. rivulatus** passes through successive degrees of development, multiplication, growth and maturation then ripenning. These different changes are classified into five stages of maturation, followed by the sixth which represents fishes with spent ovaries.

1- Multiplication

In the sexually matured fishes, the oogonia which mainly arise from the definitive germ cells during juvenile stage greatly multiply by their successive mitotic divisions. The newly divided oogonia are usually small in size with 7 µm in diameter (Fig.1), usually found in the ovary of fishes caught in September and October, or in those immature fishes. After the mitotic division, immature ovaries contain large numbers of oogonia.

2- Growth and maturation

Growth commences with the period of synapsis. In this period the chromosomes in the small oocytes are compact together forming a darkly stained mass. Then after the period, the nucleus enlarges in size and has a net-work of chromatin material, the nucleoli lie at one side of the nucleus while the chromosomes are massed at the other (Fig.2).

After the termination of synapsis, growth of oocytes continues through two distinct phases of growth, protoplasmic and trophoplasmic growths.

Fig. (1)
Oogonia in mitotic division (X 800).

Fig. (2)
Oogonia in chromatin nucleolus (X 800).

A- Primary growth

This phase of growth denotes the immature ovaries, it grows slowly within a comparatively long period. Fish having ovaries passing through the protoplasmic growth are obtained in all months of the year and body length vary from 13.0-17.0 cm (2 to 3 years old). Two stages are easily detected during this growths.

Stage I: Ovaries of the first stage of maturation contain large numbers of oocytes, each oocyte contains a small mass of protoplasm, nucleus is centrally positioned, with spherical nucleoli close to the nuclear membrane and some are distributed in the centre. The follicular epithelial cells are arranged in a single thin layer. Average diameter of oocytes measures 0.05 mm, while the nucleus is 0.02 mm in diameter (Fig.3).

Stage II: Two distint features characterise stage II. The appearance of the small vacuoles in the whole cytoplasmic mass, and the deposition of yolk nucleus at one side of the nucleus (Fig.4).

Fig. (3)
Part of ovary in stage I of maturation (X 400).

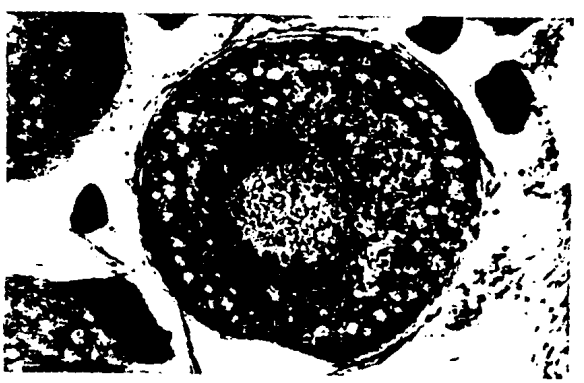

Fig. (4)
Vacuolization of cytoplasm, yolk nucleus at one side of nucleus of oocyte in stage II of maturation.
(X 400).

On further growth, more yolk nuclei appear in the cytoplasm and form a network through the whole cytoplasm (Fig.5). The outer covering layer of follicular epithelial cells is thickened, but still isolated and not differentiated. Average diameter of oocytes measures 0.15 mm. The nucleus is similar to that in previous stages with average of 0.05 mm. in diamtere.

Fig. (5)
Distribution of yolk nucleus in the cytoplasm of oocyte in stage II (X 400).

B- Secondary growth

Trophoplasmic growth takes place within a comparatively short period. Many consecative changes towrads ripening occur, and virtually comparising two stages of maturation (III and IV).

Stage III: The most characteristic feature of this stage is the comprehensive deposition of trophic elements, as a result of which, the oocyte increases in size by increasing the number and size of yolk granules, which are more concentrated around the nucleus. Vacuoles are dispersed in the ooplasm forming two distinct rings, one around and near the nucleus, the other is near the periphery. Yolk vesicles still moving towards the periphery of ooplasm forming there a thick layer just beneath the outer cover and after the vacuole layer (Fig. 6).

The follicular wall of oocyte in stage III begins to be differentiated into three conspicuous layers. The most inner layer is radial noncellular (Zona-radiata). Zona-radiata is surrounded externally with a thinner layer of epithelial cells, and then followed by a connective tissue layer. Average diameter of oocyte in this stage is 0.23 mm. Nucleus occupies a central position with average diamtere of 0.06 mm., nucleoli are spherical, lie adherent to the wall of nucleus.

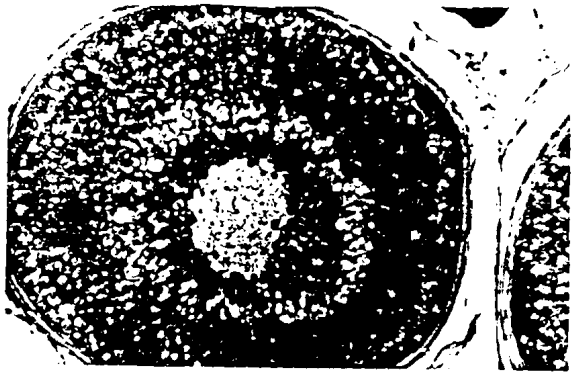

Fig. (6)
Advanced maturation of oocyte in stage II (X 400).

Fishes with maturing ovaries appear in the catch of **S. rivulatus** in the Red Sea from December to the next April. The body lengths are from 17.0 to 20.0 cm and age are from 3 t0 4 years old.

Stage IV: Oocytes in this stage considerably increase in size with dense deposition of yolk granules. The oocytes attain an average diameter of 0.30 mm. Nucleus still in the centre with diameter measuring 0.06 mm and is surrounded by a dark stained layer of cytoplasm. Nucleoli with different sizes, total number about 25, some are close to the nuclear membrane, whereas the other lie in the centre of the nucleus (Fig.7). Egg membrane is highly developed now and is very thick and more prominent, the most inner is a thin layer of minute dark granules, this in turn is surrounded by one layer of Zona-radiata which measures about 0.015 mm in thickness. Zona-radiata is followed externally by single jelly layer surrounded by another layer of epithelial follicle, the most outer layer is connective tissue (Fig. 8).

At this period of vitellogenesis, the nucleus membrane becomes winding, whereas the karyoplasm appears to be more homogenous. The nucleoli which directly adjoin the winding nuclear membrane extremely increase in number, some of them become vacuolated (Fig.9). Another nucleoli become larger in size and move away from the winding nuclear membrane to the cytoplasm around the nucleus (Fig. 10).

The nearly ripe fishes (stage IV) appear in the catch within three months from Jnauary to March, with varying total lengths 19.0 to 25.0 cm and corresponding ages 4-5 years.

Fig. (7)
Oocyte in stage IV, nucleus is surrounded by a dark stained layer of cytoplasm (X 400).

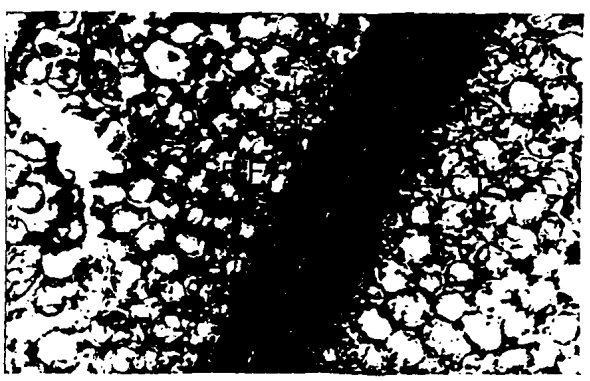

Fig. (8)
The covering layer of oocyte in stage Iv: Z, zona-rediata; J, jelly layer; EF, epithelial follicle; CT, connective tissue (X 800).

3- Ripening

The first sign of oocytes ripening is the migration of nucleus from the centre towards the periphery in a direction. indicating the location of animal pole, at the same time, the nucleus loses its circularity whereas the nucleoli start to migrate to the centre (Fig. 11).

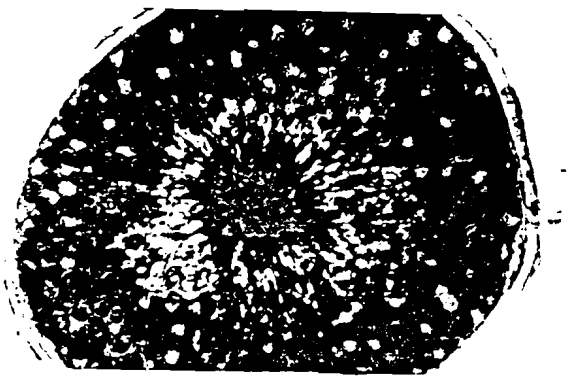

Fig. (9)
The nucleoli adjoin the winding membrane of the nucleus (X 400).

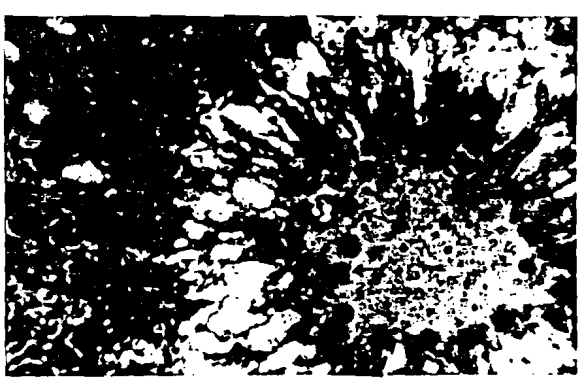

Fig. (10)
The nucleoli become larger, some move away from the winding nuclear membrane (X 1000).

Fig. (11)
Migration of nucleus towards the animal pole (X 400).

Fig. 12 shows the end of migration of the nucleus (germinal vesicle) towards the egg membrane, and the first polar bodies after the miotic division (Telophase period) positioned under the zona-radiata in the perivitelline space.

Fig. (12)
Embryonic sac is close to the egg membrane, the first polar corpucle under the zona-radiata in the perivitelline space (X 600).

On further development of the oocyte towords ripening, the homogenity of yolk granules begins, it is more prominent in the periphery of oocyte (Fig. 13).

In the animal pole, and above the germinal vesicle, which settles now just beneath the egg membrane, there is a funnel shaped or cup-lik depression of micropyle in the egg membrane (Fig. 14).

The peculiar feature which arises in the transition from stage IV to stage V of maturation is the appearance of some nearly ripe oocytes being resorbed, these oocytes are usually of smaller sizes than the others occupying the whole ovary (Fig. 15).

Stage V: The oocytes attain their maximum size with average diameter of about 0.48 mm. The nuclear contents (Karyoplasm) are not visible, the egg membrane becomes relatively tough (Fig. 16).

Ripe eggs of **S. rivulatus** and **S. luridus** noted by Popper et al (1973), are similar in diameter ranging form 0.50 to 0.63 mm. However, these measurements are slightly larger than those of **S. rivulatus** in the Red Sea. Fishes with ripening ovaries are found in the catch from March to September with total lengths at 18.0 to 30.0 cm.

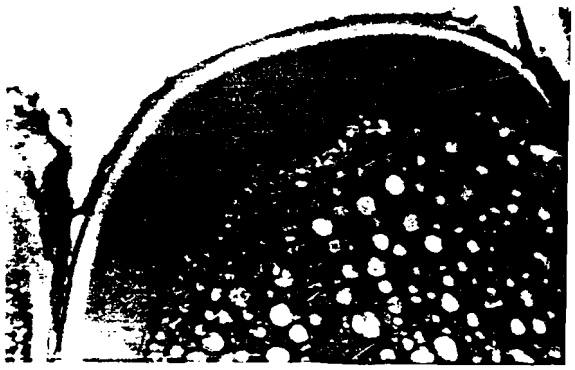

Fig. (13)
Homogenity of yolk granules at the periphery of oocyte (X 600).

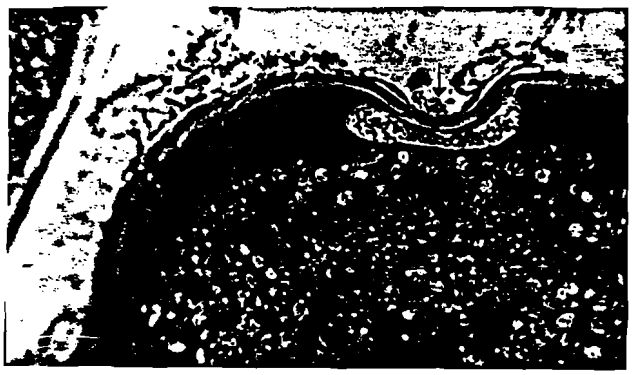

Fig. (14)
Formation of micropyle just above the location of embryonic sac (X 400).

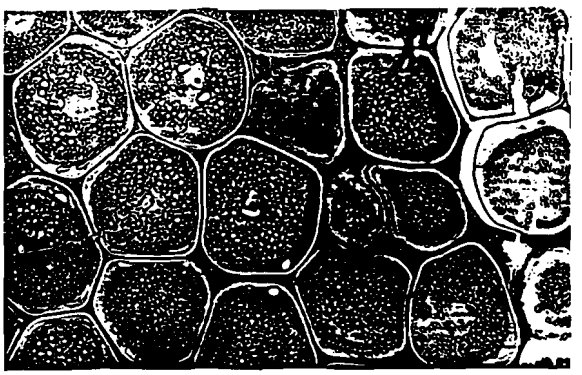

Fig. (15)
Part of the ovary in the transition stage IV - V showing oocyte during the process of resorption (X 150).

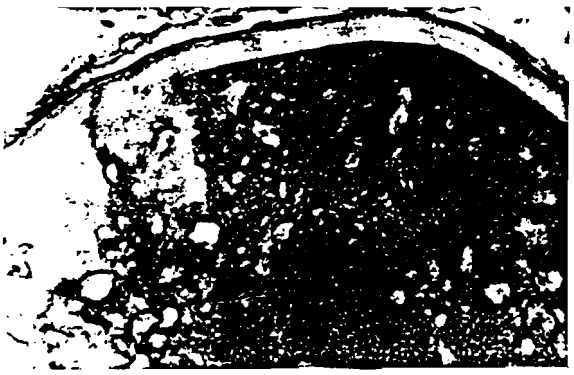

Fig. (16)
Oocyte in stage V of maturation (X 800).

In their ripe condition the ova contain yolk granules in the cytoplasm in the region of the vegetative pole (Fig. 17), these features are characterizing the ova ready for ovulation.

4- Post spawning

Ovaries of spawned fish (post spawning) are extremely reduced in size reaching about one fourth of body cavity, and females are in a spent condition (stage VI). This stage is mainly distinguished by the presence of remnant nonovulated and atretic eggs which are not closely packed together. The follicular epithelial cells, previously associated with the extruded ova begin to hypertrophy and to fill the emptied space (Fig. 18). Spent fishes are obtained in the catch from March to December in various percentages and lengths.

5- Reproductive cycle

The representation of the duration of different stages of maturation of **S. rivulatus** in the Red Sea throughout the year is given in Fig. 19. The frequency of monthly distribution of different maturity stages of female **S. rivulatus** in the Red Sea (Jeddah region) may legitimately determine the spawning season. The first appearance of females with ripe ovaries is noticed in March and their occurrance in the catch extended to September. After September ripe individuals are not present in the collected samples.

Fig. (17)
A ripe ovum in an advanced condition (X 40).

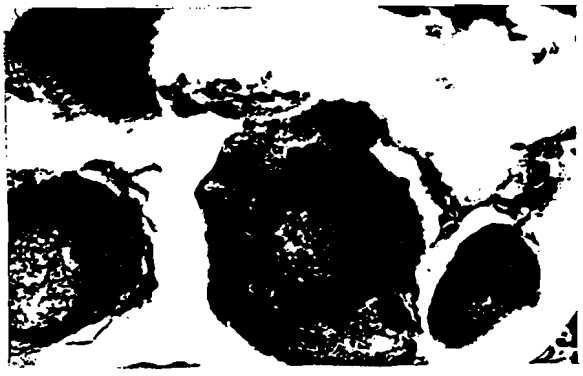

Fig. (18)
Part of the ovary in spent condition (X 40).

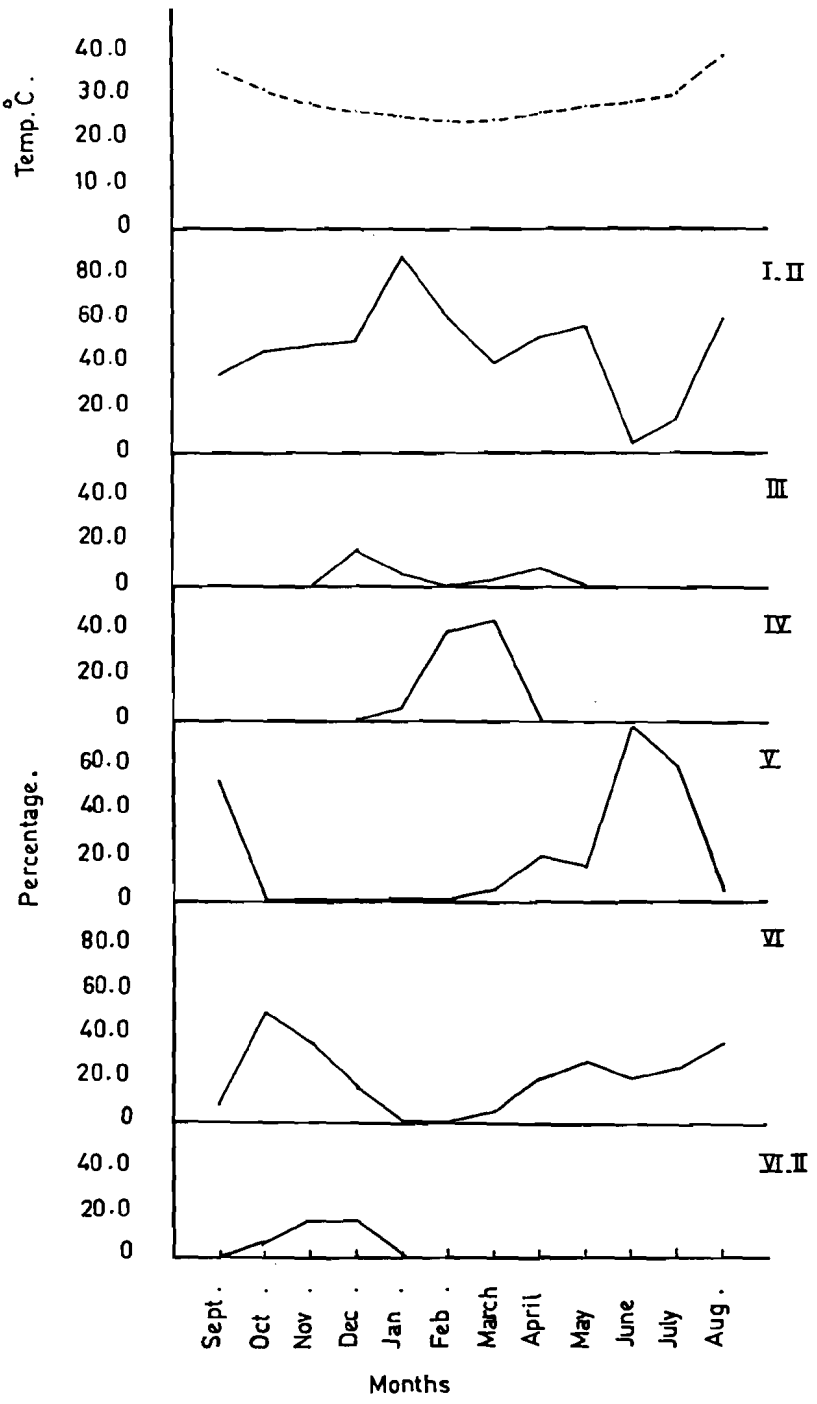

Fig. (19)
Monthly distribution of different stages of maturation of S. **rivulatus.**

DISCUSSION

Oogenesis starts with the mitotic division which begins in the ovaries of **S. rivulatus** in a short time after the preceeding spawning. The active mitotic division is therefore seasonal.

The two characteristic growth periods of the development of the ova begin with a primary growth including the synapsis which may determine the number of ova being spawned by the fish in the current spawning season. Synapsis is directly followed by a comparatively long period of growth which is destined to the increase of protoplasmic mass of the ova, and hence it is called the period of protoplasmic growth. As the mass of the ovum increases, some structures are consequenty added, the most characterizing features are the appearance of yolk nucleus, and vacuoles.

The second growth period is more active and taken place in a comparatively short time. This period is destined for the deposition of trophic elements, and hence is called as the trophic growth. In this period, the granulosa is believed to be responsible for the deposition of yolk in the developing ovum, which greatly increases in size according to this deposition (Hoar, 1970). The formed vacuoles in the periphery of maturing eggs was believed to be of carbohydrate nature (Yamamoto, 1955). The most inner layer (zona-radiata) added to the egg membrane of maturing egg is presumably used for the penetration of nutritive substances from the particular cells of granulosa (Sakun and Butskaya, 1968). Zona-radiata of the maturing ova of **S. rivulatus** is obviously formed of one noncellular layer, in other fish species such as **Gerres oyena**, two layers are forming the zona-radiate (Amin and Bawazeer, 1983). At this period of vitellogenesis the nucleus and its contents (karyoplasm) are more activated, besides, the cytoplasmic mass filling the ovum is further increased.

On further development of the ova (stage IV), the nucleus migrates toward the animal pole, and a funnel shaped depression of micropyle, through which the spermatozoa pass toward the karyoplasm is well developed.

In the transition period, from stage IV to stage V, some nearly ripe eggs being resorbed, this resorption may be related to the specific size of the ovary and consequently to the specific number of eggs being released in the spawning season (Amin et al, 1984).

As the eggs become ripe (stage V), it attains a diameter of 0.48 mm, this in turn is slightly smaller than the measurments of Popper (1979) obtained form **S. rivulatus** and **S. luridus.** This small difference may be due to the difference in average egg diameter (Amin et al, 1984).

Ovaries in the post spawning are characterized by small size, when yolked ova become atretic, the granulosa concerned with yolk phagocytosis in the post spawning (Hoar, 1965).

The timing of reproduction and regulation of the associated morphological changes are very largely dependent on the glands of internal secretions (Hoar, 1969). The monthly distributions of different maturity stages of female **S. rivulatus** determine the frequency of spawning which was shown to extend from March to September. The ripe eggs are laid once a season, as shown from the developing eggs which appear at about the same size, hence **S. rivulatus** does not carry out a multi-spawning, and is a monocyclic fish.

The period of spawning (March-September) is characterized by a comparatively high water temperature ranging from 25.3 to 30.9°C, within this range, the sexually matured individuals, successively take part in the spawning. In the gulf of Aqaba (in the most north of Red Sea) **S. rivulatus** was shown to commence its spawning in June, with a short spawning season, due to the lower temperature in this region than in jeddah region (Popper and Gundermann, 1975). The effect of high water temperature on the duration of spawning season is prominant in many warm water fish species. For instance, fishes inhabiting the tropical waters have the ability to spawn all over the year round (Budnechenko and Dimetreva, 1979).

REFERENCES

Amin, E.M., and F.A. Bawazeer, 1983. Oogenesis and sexual cycle of **Gerres oyena** Forsk. in Red Sea. Egypt., **J.Histol.**, Cairo, 6 (2), 347-357.

Amin, E.M., Kh. A. Hussein and F.A. Bawazeer, 1984. Peculiarities in fecundity of a monocyclic fish (**Gerres oyena** Forsk.) in the Red Sea. **Bull.Fac.Sci.** Alex. 24: 49-60.

Budnechenko, B.A., and O.C. Dimetreva, O.C., 1979. The biology of spawning of Saurida undosquamis (Rich) and Saurida tumbil (Bloch) family Synodontidae in the Arabian Sea.

Day, F., 1878, Bernard Quaritch. London, 165-168.

Fujita, S., and M. Ueno, 1954. On the development of the egg and prelarval stages of **Siganus fuscescens** (Houttuyn) by artificial insemination. **Jap. J. Icthyol.** 3(3-5): 129-132.

Gunther, A., 1861. Catalogus of the Acanthepterygian Fishes in the collection of the British Museum. London, 313-324.

Hjort, J., 1910. Reportion Herring investigation untill January 1910 **Publ. Circons. Cons. Perm. Internat. Explor. Mer.**

Hoar, W.S., 1965. Comparative physiology. **Ann. Rev. Physiol.** London, 27: 51-70.

Hoar, W.S., 1969. Reporduction, Fish physiology, **Acad. Press**, New York, 2: 15-20.

Hoar, W.S., 1970. Fish physiology, **Acad. Press**, London, V, III, 16-18.

Manacop, P.R., 1937. The artificial fertilization of danget, Amphacanthus oramin (Bloch and Schneider)(Probably Siganus canalichlatus).**Philipp. J. Sci.**, 62: 229-237.

Petersen, J., 1895. Einige methoden zuk bestimmung des alters und wachaces der fische. Milteil.D.Deutsch,Seefisherelvereins.

Popper, D.H., H. Gordin, and G.W. Kissil, 1973. Fertilization and hatching of the rabbit fish (**Siganus rivulatus**). **Aquaculture**, 2 (1): 39-44.

Popper, D.H., and N. Gundermann, 1975. Some biological and behavioural aspects siganid populations in the Red Sea and Mediterranean coasts of Israel in relation to their suitability for aquaculture. **Aquaculture**, 6: 127-142.

Popper, D.H., R. Pitt, and Y. Zohar, 1979. Experiments on the propagation of Red Sea Siganids and some notes on their reproduction in nature. **Aquaculture**, 16: 177-181.

Sakun, O.F., and H.A. Butskaya, 1968. Determination of maturity stages and study of sexual cycle in fishes. **Min. Fish., Econ.** USSR. Mormansk, 10-14.

Soh, C.L., and T.J. Lam, 1973. Induced breeding and early development of the rabbit fish, **Siganus oramin** (Schneider). **Proc. Symp. Biol. Res. and Nat. Dev.**, 49-56.

Westernhagen, H.V., and H. Rosenthal, 1975. Rearing and spawning siganids (Prisces: Teleostei) in a closed sea water system. **Helgolunder wiss. Meeresunters**, 27: 1-18.

Yamamoto, K., 1955. Studies on the formation of fish eggs. V: The chemical nature and the origin of the yolk vesicle in the oocytes of the herring, Clupea pallasii. **Annot. Zool.**, Japan, 28 (3): 158-162.