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SEASONAL HISTOLOGICAL CHANGES IN THE TESTES OF THE MARINE TELEOST FISH (TRACHURUS MEDITERRANEUS S.)

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ABSTRACT

The survey of the gonadal anatomy and histology has revealed the occurrence of five developmental stages in the testis of the fish **Trachurus mediterraneus** including; 1: Maturing stage (late February to early March); 2: Prespawning stage (late March to April); 3: Spawning stage (late April to early June); and 4: Post spawning stage (June to July) and 5: Resting stage (August to February). Six types of spermatogenic cells could be demonstrated during spermatogenesis- Germ cells and spermatogonia are present in the peripheral zone of the seminiferous lobules the whole year-round being greatly reduced during the breeding period. At the beginning of the spawning season, a complex of seminiferous lobules, which open into the spermatic duct, is formed. Spermatogenesis proceeds within these lobules, which are devided into temporary cysts. The presence of interstitial and lobule boundary cells in the testes of **Trachurus mediterraneus** were observed.

Data on GSI, frequency of the various speramatogenic stages and histomorphology of the testes, analyzed on a monthly basis, indicate seasonal reproductive cycle for this fish.

INTRODUCTION

Although several investigators have studied the gonadal reproductive cycle of female teleosts, similar studies on male fishes are relatively few. However, important contributions on the testicular cycle of teleosts have been made by (Bowers and Holliday, 1961; Khanna and pant 1966; Dadzie 1969; Sanwal and Khanna 1972; Latif and Saady 1973; Bisht 1974; Shrestha and Khanna 1978 & Rijnsdrop 1989).

In the present study, an attempt has been made to describe cyclical changes in the testes of **Trachurus mediterraneus.** The results obtained here will provide ample data for correlating its reproductive physiology with the pituitary gland activity.

MATERIAL AND METHODS

Specimens of Trachurus mediterraneus S. were obtained fresh weekly from the commercial catch at Anfoushy, Alexandria fishing grounds. The sampling period lasted from Dcember 1988 to November 1989.

Fresh testes at different stages of development were histologically prepared. The tissues were fixed in Bouin's fluid, 10 % neutral formalin and Carnoy's fluid. Sections cut at 4 um thickness were stained with Heidenhain's iron alum haematoxylin (HH) (Gatenby and Beams, 1950) and by alcian blue-PAS technique (AB-PAS) (Bancroft and Stevens, 1977).

In the testes, spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa were counted, at x 380 magnification using the eye piece grid of 1 mm squares. The number of each cell type was counted for five fields of view in one TS for each and the mean count for each cell type in each maturity stage was expressed as a percentage of the total. The gonado somatic index (GSI) for every month was estimated, as belows:

GSI = wt. of the gonad/gutted body weight x 100

RESULTS

Morphology of the testes:

The testes are paired, elongated structures, lying on either side of the air bladder, ventral to the kidney and dorsal to the alimentary canal, occupying the posterior region of the abdominal cavity. They are attached to the body wall by means of mesorchia, and are of almost equal size. They are non-pigmented and remain free from one another for almost their entire length except at the hindmost region, where they become united to form a common spermatic duct. The size, shape, vascularity and colour of the testes vary with the season and stage of maturity.

Histology :

The testis is composed of a large number of seminiferous lobules which are held together by means of a thin connective tissue. The lobules vary in size (Figs. 1 - 10) and are highly convulated. They are separated from each other by a connective tissue stroma and communicate with the lumen of sperm duct. Besides the connective tissue, the stroma septum contains blood capillaries and interstitial cells (Fig. 6).

With the approach of the breeding season, the germ cells of the lobules become very active and are seen at variour stages of maturation, viz., germ cells, spermatogonia, spermatocytes, spermatids and sperm. The germ cell (Fig. 10) is a large spherical structure, having an ill-defined nuclear membrane and is lightly stained, the large nucleus

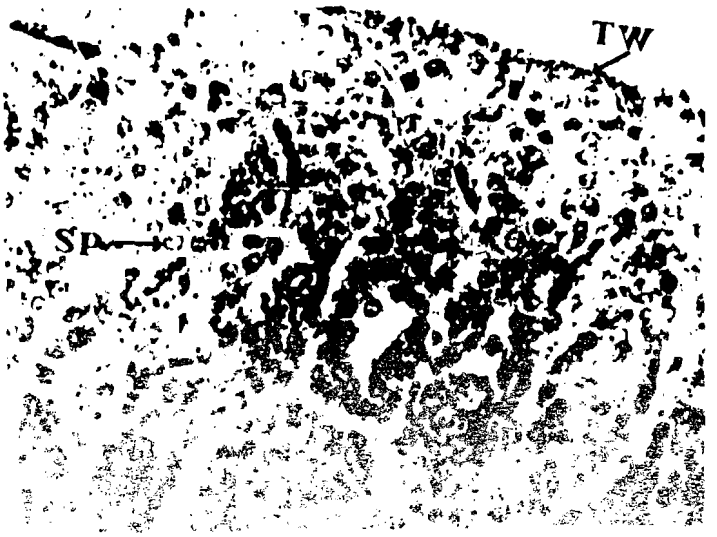


Fig. 1

Maturation stage, February, 17 cm. Note the [illegible] spermatogonia (Sg), cysts of primary spermatocytes (PS), a secondary spermatocyte (Ss), and trabecular wall (tr). HH, X 380

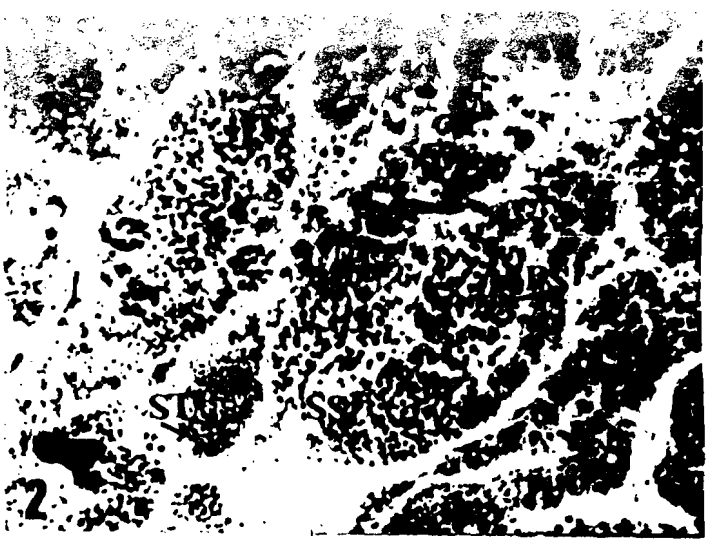


Fig. 2

Maturation stage, February, 18.5 cm, showing cysts of primary spermatocytes (PS) and secondary spermatocytes (SS), and spermatids (St). HH, X 380

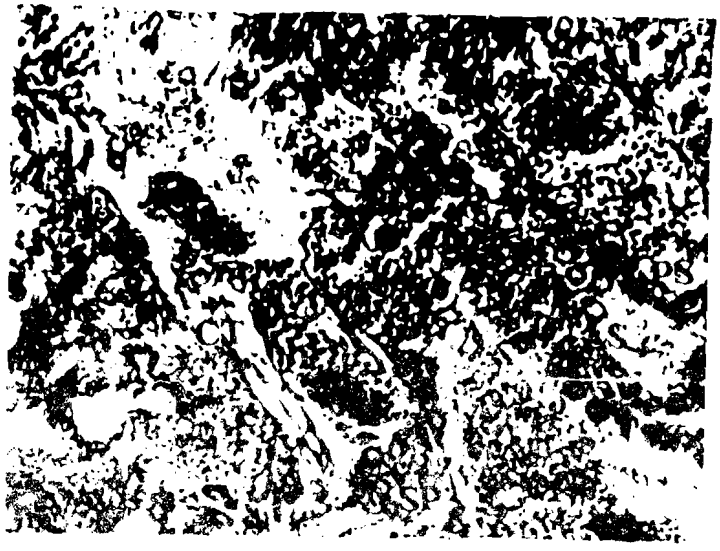


Fig. 3

Maturation stage, early March, 18 cm. Note connective tissue (CT), spermatogonia (SP), cyst of primary and secondary cysts (PS, SS), spermatids (St) and few sperms (S). HH, X 380

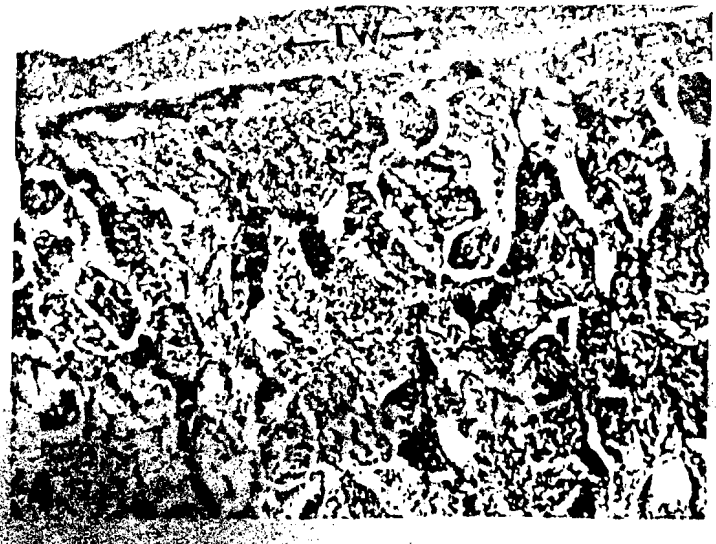
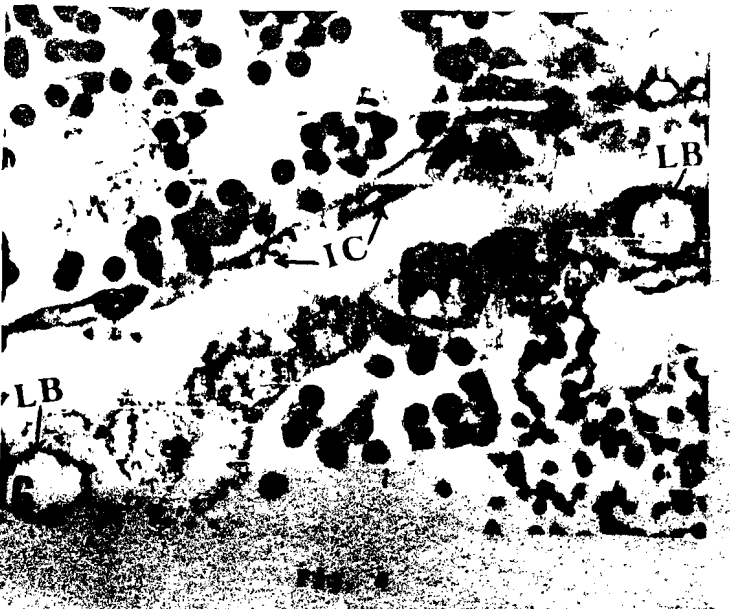


Fig. 4

Pre-spawning stage, March, 20 cm. Testicular wall (TW) composed of a layer of fibrous muscular connective tissue. AB/PAS, X 96

Fig. 5

Pre-spawning period, late March, 21 cm. Showing resting spermatogonia (RS), connective tissue (CT), active spermatogenesis within seminiferous lobule (SL). HH, X 380



Pre-spawning period, April, 22 cm. The lobule boundary cell (LB), with oval granular nuclei and the interstitial cell (IC). AB/PAS. X 1800

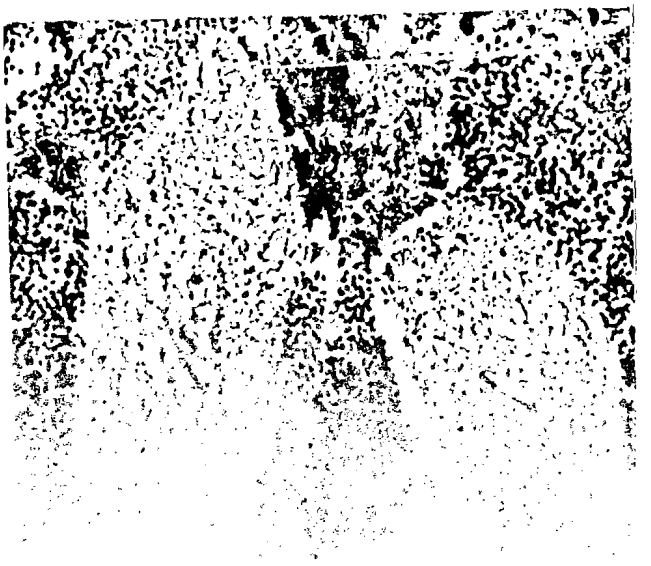


Fig. 7

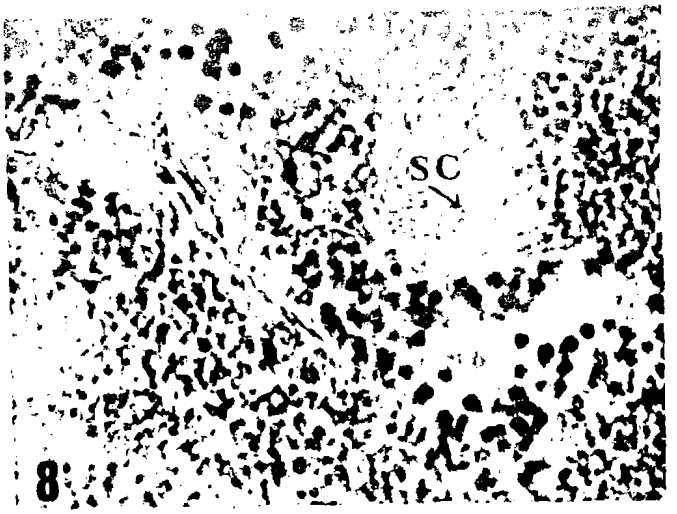


Fig. 8

Magnified part from the above section. Sperm cluster (SC) showing a uniformally chromatic head and faintly stained tail. AB/PAS X 1040

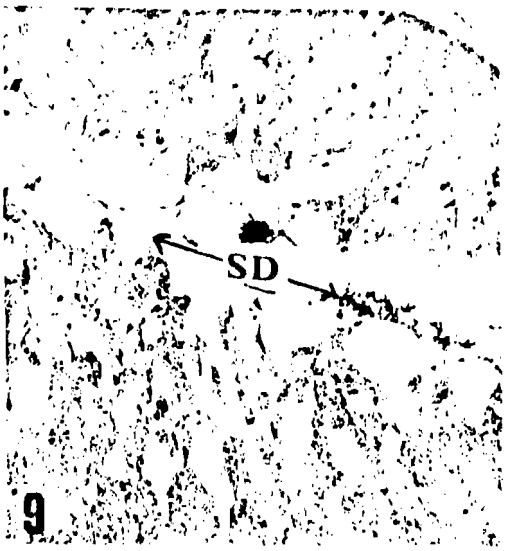


Fig. 9

Spawning stage, early June, 23.0 cm. Sperm duct (SD) full of spermatozoa. Note, lumen of sperm duct is continuous with the lumen of seminiferous lobules. HH, X 96.

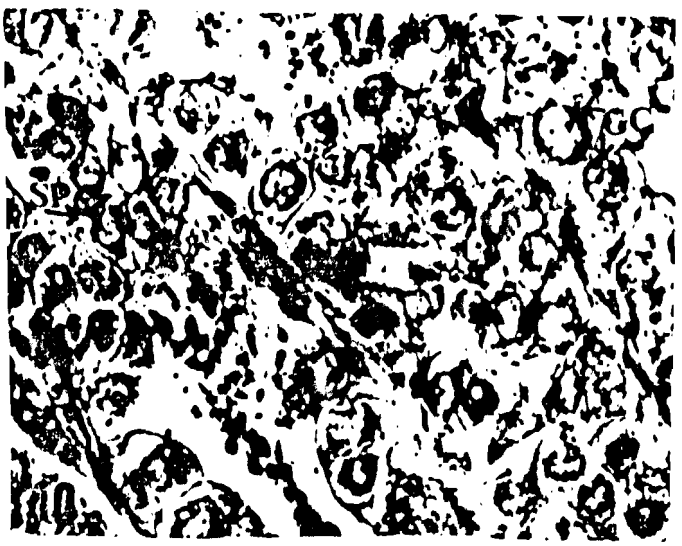


Fig. 10

Post-spawning stage, June, 24 cm. Note germ cells (GC), spermatogonia (SP) and residual spermatozoa (R). HH, X 1040

being central. The nucleus occupies the greater part of the cell. The germ cells multiply to form a large number of spermatogonia (Fig. 10) which exhibit a more or less distinct nuclear membrane.

The spermatogonia increase in size, ultimately giving rise to primary spermatocytes. The primary spermatocytes have darkly stained, smaller nuclei, with indistinct nuclei (Figs. 1 and 2). They are usually manifesting various stages of division.

The primary spermatocytes undergo reduction division and give rise to the secondary spermatocytes, which are smaller in size, having athick clump of chromatin (Fig. 2) and are of short duration. They divide quickly, giving rise to the spermatids. The spermatids can be recognized by their smaller size, spherical structure and highly chromatic nature of the chromatin, which is deeply stained with haematoxylin (Fig. 3). Finally, the transformation of spermatids into sperm occurs. Sperms are similar to spermatids but are further reduced in size and posses a uniformally chromatic head and a transparent tail, (Figs. 7 and 8).

It can be seen from Figures 11 and 12 that spermatogenic activity in Trachurus mediterraneus begins in late February and March, attains its maximum in April, when the lobules are full of spermatids and spermatozoa and GSI reach maximum. The rate of spermatogensis then slows down and by late July it almost stops (GSI minimum). From August to early February (GSI more or less constant), the testes pass through a resting period.

Seasonal Changes in the Testes :

Based on the differences in histomorphology and monthly analysis of GSI and spermatogenic cells. The testes can be divided into the following five stages.

1- Maturation stage:

This stage extends from late February to early March. The testes become slightly enlarged and pink in colour due to increased vascularity. During this period, the spermatogenic activity is in progress. The lobules increase gradually in size and as a result, the connective tissue occupying the interlobular spaces is slightly reduced, at the beginning of this period, the lobules contain germ cells, a large number of spermatogonia and cysts of primary spermatocytes (Fig. 1). With the progress of spermatogenesis, cysts of secondary spermatocytes and spermatids are also observed in the month of March (Fig. 2). The testicular wall (the tunica propria) is clearly defiend as a layer of fibrous connective tissues (Figs. 1 and 4).

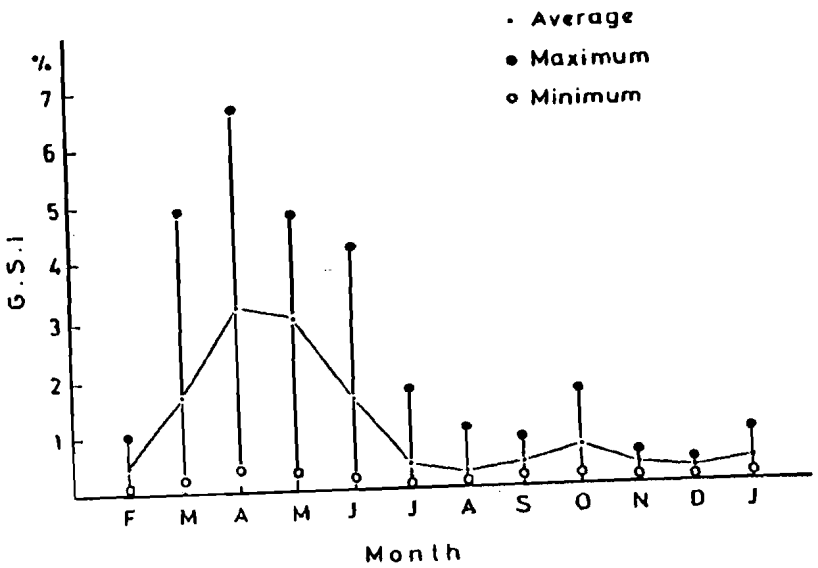


Fig. 11

Monthly variations of Gonado - Somatic Index of males **Trachurus Mediterraneus** in the Egyptian Mediterranean Waters (1988 - 1989)

2- Pre-spawning stage :

This stage extends from late March to April. The testis is whitish and shows a marked increase in volume. During this period, spermatogensis is at its peak, the lobules showing germ cells in all stages of development, including primary germ cells, spermatogonia, spermatocytes and groups of ripe spermatozoa. This stage is characterized by the predominance of spermatocytes and spermatids (Fig. 5).

The sperms appear in the month of March, filling the lobules completely by the end of April, the spermatocytes being reduced considerably. The spermatogonia show a marked reduction in number and they lie singly or in small groups close to the lobule walls, where they remain in a resting state until the next spawing season begins (Figs. 5 and 6). Lobule boundary cells are easily distinguishable because of their elliplical granular appearance. They are localized at the periphery of lobules adjacent to the spermatogonia and they have large oval granular nucleoli (Fig. 6).

3- Spawning stage :

This stage extends from late April to early June. The testis is turgid and pinkish red and attains maximum weight and size. In this period, the testicular lobules attains their maximum size. The tunica propria is very thin.

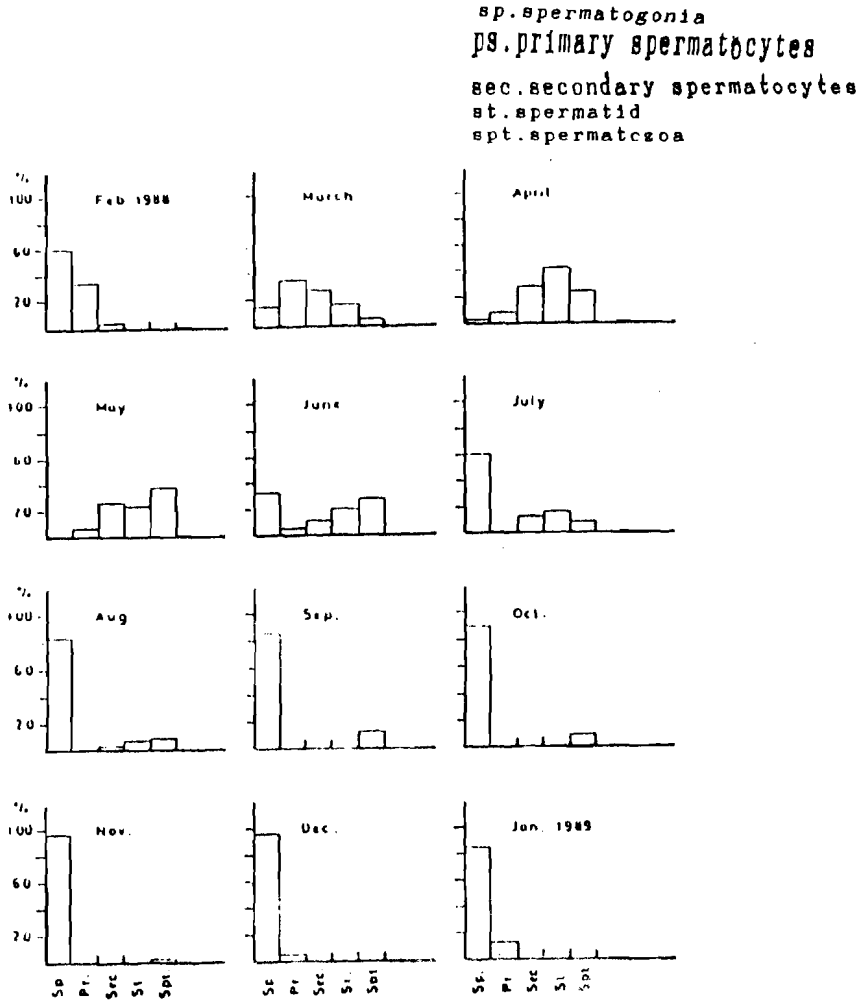


Fig. 12

Monthly variations of the state of spermatogenesis, expressed as the percentage proportion of the various stages of spermatogenesis in the functioning testicular part of the gonad of **Trachurus Mediterraneus** from Febtuary 1988 to January 1989

sp. spermatogonia
ps. primary spermatocytes
sec. secondary spermatocytes
st. spermatid
spt. spermatczoa

Spermatids and sperm are the common germ cells filling the thin walled lobules during this stage. There is a further reduction in the number of spermatocytes. Most of the lobules have a spacious lumen, with numerous free spermatozoa in the center and cysts of spermatids, secondary spermatocytes, and isolated spermatogonia at the periphery (Fig. 7). The number of spermatozoa in the lobules increases, up to the end of this stage, until the lobules are packed exclusively with mature sperm cells. The degree of distention with spermatozoa increases towards the center of the testis. The spermatic duct is also distended with spermatozoa and its lumen is continuous with the lumen of the seminiferous lobules in the innermost part of the testis (Fig. 9). Towards the periphery, the seminiferous lobules are formed mainly of cysts composed of spermatozoa.

However, few primordial germ cells and resting spermatogonia are present at the margin of the lobules. The spermatozoa are discharged gradually from the seminiferous lobules. The first to be released are the sperm cells, in the innermost part of the testes followed by those lying externally next to them and so on.

4- Post-spawning stage:

This stage lasts from June to July. The weight is reduced considerably due to discharging the spermatozoa. The testes become thick, flaccid, and pinkish white. In between the lobules interstitial tissue increased in thickness, the germ cells increase in number by multiplication and from the dominant germ cells in the lobules of the periphery. Although a few spermatids are present, the absence of spermatocytes indicates cessation of spermatogensis. Some residual sperms are present in a few lobules and in the lumen of the sperm duct (Fig. 10). The residual sperms finally disappear.

5- Resting stage :

This stage extends from August to February. The testes are slender, thin, translucent. Almost all the lobules are now packed with cysts of germ cells. Some of the germ cells are seen in a state of division, forming spermatogonia, the latter are characterized by deeply stained unclei. The interlobular spaces are densely filled with loose connective tissue, blood capillaries and a few interstitial cells.

DISCUSSION

There is no sexual dimorphism in **Trachurus mediterraneus** S. and it is not possible to identify the sex without opening the abdomen. However, during the breeding period the male milt running on slight pressure on the belly, and the female has abulging abdomen due to greatly enlarged ovaries.

With the approach of the breeding period, the testes of **Trachurus mediterraneus** present a lobulated appearance, these lobules becoming quite prominent during the breeding period. Such a lobulated condition of the testes has also been reported in **Myslus seenghala** (Sathyanesan, 1959), **Barbus tor** (Rai, 1965) and **Clarias batrachus** - (Lehri, 1967 and Allam, 1979).

The germ cells are believed to originate from the undifferentiated germ cells designated as dormant germ cells, reserve germ cells and resting germ cells (Suzuki, 1939 and Jones, 1940. Several investigators consider the formation of germ cells by the devision of resting germ cells within the lobules (Stenger, 1959; Henderson, 1962; Barr, 1963; Rai, 1965; Khanna and pant, 1966, Lehri, 1967; Bisht 1974 and Ramadan et al., 1987). In **Coesius plumbeus** Ahsan (1966) has stated that the spermatogonia are formed by the division of primary germ cells, and that they are also derived from certain migratory cells. Rai (1965) in **Barbus tor** has described the formation of a new generation of sex cells by mitotic division of the resting germ cells, which are present throughout the year. In **Trachurus mediterraneus**, the germ cells are present throughout the year but their number is greatly reduced during the breeding period. As the resting germ cells are present throughout the year and are seen in a state of division after spermatogenesis is over in the lobule, it is possible that the new crop of spermatogonia arises by the devision of the existing germ cells.

The testis of **Trachurus mediterraneus** S. passes through the successive stages of growth, maturation stage, pre-spawning stage, spawning stage, post-spawning stage and resting stage.

The spermatogenic activity starts at different times of the year in **Esox lucius** (Lofts and Marshall, 1957); **Plecoglossus altivilis** (Honma and Tamura, 1962); **Heteropneustes fossilis** (Nair, 1965); **Barbus tor** (Rai, 1965); **Glyptosternum pectinopterm** (Khanna and Pant, 1966; **Clarias batrachus** (Lehri, 1967); **Channa gachua** (Sanwal and Khanna, 1972) and **Sparus aurata** (Ramadan et al., 1987); **Melanogrammus aeglefinus** (Clay, 1989) and **Pleuronectes platessa** (Rijnsdorp, 1989). These variations are probably due to the local physiological factors. Swarup (1958) has reported an interesting feature in the testes of **Gasterosteus aculeatus** in which he found the testes in sexual mature condition at any time of the year, but the functional maturity is attained only during April and May (breeding period). Henderson (1962) did not find any period of quiescence in the testes of **Salvelinus fontinalis**. Ahsan (1966) has related the cyclic changes in the testes of **Coesius plumbeus** to the changing environmental factors.

In the present fish **Trachurus mediterraneus**, the testes undergo regular cyclic changes. The spermatogenic activity starts in late February, gradually increasing till March, reaching its maximum in the month of April, when the lobules

are full of spermatids and sperm. From then onwards, the process of spermatogenesis considerably slows down and almost ceases by July. The testes pass through a period of rest from August to early February. A quiescent period was also reported on Esox lucius (Lofts and Marshall, 1957), Barbus tor (Rai, 1965), Schizothorax richardsonii (Bisht, 1974) and Garra gotyla (Shrestha and Khanna, 1978). However, Henderson (1962) and Dadzie (1969) found no quiescent period in the testis of Salvelinus fontinalis and Tilapia mossambica respectively; spermatogonial proliferation began as soon as the period of functional maturity terminated and continued during winter and summer months.

Allam (1979) found that Trachurus mediterraneus spawns once a year and its spawning takes place in spring time.

In some teleosts, cells of a new type, probably with endocrine function and characterized by cyclic activity, were observed, e.g. in the pike Esox lucius (Marshall and Lofts, 1956); they did not arise in the interstices, but occurred in the lobule wall (lobule boundary cells). Similarly, Henderson (1963) reported the absence of interstitial cells and the presence of ;obule boundary cells in Salvelinus fontinalis.

According to Hoar (1957), the interstitial cells and lobule boundary cells are morphologically similar and they may be presumed to have similar functions.

On the other, hand, Hardisty et al.(1967) demonstrated interstitial cells together with lobule boundary cells in the river lamprey, Lampetra fluviatilis. Both interstitial and lobule boundary cells are likewise present in Barbus tor (Rai 1965), Schizothorax richardsonii (Bisht, 1974) and Garra gotyla (Shrestha and Khanna, 1978). This is supported by the present findings in Sparus aurata, in which a few large lobule boundary cells with a characteristic fusiform shape and acentral granular nucleus were found scattered among resting spermatogonia in the wall of the lobules.

REFERENCES

Ahsan, S.N., 1966. Cyclic changes in the testicular activity of the Lake Chub, Coussius plumbeus. **Canad. J. Zool.**, 44: 149-159.

Allam, S.M., 1979. **Biological studies of fish stocks belonging to genus Trachurus rafinesque in the Egyptian Mediterranean waters.** M. Sc. Thesis, Faculty of Science, Alexandria University; 208 pp.

Bancroft, J.D. and A. Stevens, 1977. **Theory and practice of histological techniques.** Churchill Livingstone, Edinburgh, London and New York.

Barr, W.A., 1963. The endocrine control of the sexual cycle in the plaice, **Pleuronectes platessa** (L). III. The endocrine control of spermatogenesis. **Gen. Comp. Endocr.**, 3: 205-215.

Bisht, J.S., 1974. Seasonal histological changes in the testes of hill-stream teleost, **Schizothorax richardsonii** (Gray and Hard). **Acta Anat.**, 88: 398-410.

Bowers, A.B. and F.G.T. Holliday, 1961. Histological changes in the gonad associated with the reproductive cycle of the herring (**Clupea harengus** L.). **Scotland Dept. Agr. Fish. Marine Res.**, 5: 1-16.

Clay, D., 1989. Oogenesis and fecundity of haddok (Melanogrammus aeglefinus L.) from the Nova Scotia shelf. **J. Cons. Int. Explor. Mer.**, 46: 24-34.

Dadzie, S., 1969. Spermatogenesis and stages of maturation in the male cichlid fish **Tilapia mossambica. J. Zool., Lond.**, 159: 399-403.

Gatenby, J.B. and H.W. Beams, 1950. **The microtomist's Vade-Mecum (Bolles Lee).** Eleventh Edn., Churchill, London.

Hardisty, M.W.; B. Rothwell and K. Steele, 1967. The interstitial tissue of testes of river lamprey **Lampetra fluviatilis .J. Zool. Lond.**, 152: 9-18.

Henderson, N.E., 1962. The annual cycle in the testis of the eastern brook trout, **Salvelinus fontinalis** (Mitchell). **Can. J. Zool.**, 40: 631-645.

Hoar, W.S., 1957. **The gonads and reproduction; in Brown, the physiology of Fishes**, 1: 289-321. Academic press; New York.

Honma, Y. and E. Tamura, 1962. Seasonal changes in the gonads of the land locked salmonid fish, Ko-auyu, Plecoglossus altivelis temminck et Schlegel. **Jap. J. Ichthyol.**, 1-6 : 136-152.

Jones, J.W., 1940. Histological changes in the testis in the sexual cycle of male salmon parr (Salmo salar L. Juv.). **Proc. Roy. Soc. B**, 128: 499-509.

Khanna, S.S. and M.C. Pant, 1966. Structure and seasonal changes in the testes of a hill stream fish **Glyptosternum pectinopterum. Jap. J. Ichthyol.**, 14: 110-119.

Latif, A.F. and B.F. Saady, 1973. Reproduction in the Nile Bolti Tilapia **nilotica** L. Sexual cycle of the male. **Bull. Inst. Ocean. & Fish.**, 3: 119-142.

Lehri, G.K., 1967. The annual cycle in the testis of the catfish **Clarias batrachus L. Acta Anat.**, 67: 135-154.

Lofts, B. and A.J. Marshall, 1957. Cyclical changes in the distribution of the testis lipids of a teleost fish, **Esox lucius. Quart. J. Micros. Sci.**, 98: 79-88.

Marshall, A.J. and B. Lofts, 1956. The Leydig cell homologue in certain teleost fishes. **Nature**, 117: 704-705.

Nair, P.V., 1965. Studies on the male reproductive system of some siluroid fishes. **Ind. J. Zool.**, 4: 1-35.

Rai, B.P., 1965. Cyclic changes in the testes of the mahaseer, Barbus tor (Tor tor). **Acta Anat.**, 62: 461-475.

Rijnsdorp, A.D., 1989. Maturation of male and female North Sea plaice (**Pleuronectes platessa** L.). **J. Cons. Int. Explor. Mar**, 46: 35-51.

Ramadan, A.A.; A.A. Ezzat; E.M. Khadre; N.A. Meguid and S.H. Abdel Aziz, 1987. Spermatogenesis in the marine Hermaphrodite teleost fish **Sparus aurata. Folia Morphol.**, 35: 154-160.

Sanwal, R. and S.S. Khanna, 1972. Seasonal changes in the testes of a fresh water fish, **Channa gachua, Acta Anat.**, 83: 139-148.

Sathyanesan, A.G., 1959. Seasonal cycle in the gonads of **Cirrhina reba** (Hamilton). **Indian J. Vet. Sci. Anim. Hust.**, 29: 27-34.

Shrestha, T.K. and S.S. Khanna, 1978. Seasonal changes in the testes of a hill stream teleost, Garra gotyla (Gray). Acta Anat., 100: 210-220.

Stenger, A.H., 1959. A study of the structure and development of certain reproductive tissues of Mugil cephalus. Zoologica, 44: 53-70.

Suzuki, K., 1939. Regeneration of gonad in Plecoglossus altivelis after spawning season. Cytologia, 10: 113-126.

Swarup, H., 1958. The reproductive cycle and development of the gonads in Gasterosteus aculeatus L. Proc. Zool. Soc. Bengal., 11: 47-60.