Bull. Nat. Inst. Oceanogr. & Fish., ARE, 14 (2), 1988 : 55 - 69.

HISTOMORPHOLOGY OF OVARIAN CHANGES DURING THE REPRODUCTIVE CYCLE OF TRACHURUS MEDITERRANEUS (TELEOSTEI, CARANGIDAE)

M. M. EL-GHARABAWY* AND S. H. ABDEL-AZIZ**

*National Institute of Oceanography and Fisheries, Alexandria, Egypt.
**Oceanography Dept., Faculty of Science, Alexandria University, Egypt.

ABSTRACT

Seasonal changes in ovaries of **Trachurus mediterraneus** are described. The ovary of this fish is surrounded by vascularized longitudinal and circular muscle layers. Six developmental stages are described in detail. In each stage, the nucleus and cytoplasm are described as the diameter, affinity to stain and distribution of nucleoli. Lampbrush chromosomes are observed during the maturation and prespawning periods.

Histological analysis reveals that the oocyte passes through two successive phases before it becomes a ripe egg, migration of the nucleus to the animal pole and liquefaction of the yolk spheres.

Oogenetic activity begins in late March and attains the maximum in April, Along quiescent period (August to February) was recorded.

INTRODUCTION

Since the end of the last century, the study of oogenesis in fishes has attracted the attention of many investigators (Chandhery, 1952, Yamamoto, 1956; Bara, 1960; Rizkalla, 1970; El-Agamy, 1974; Kamel et al., 1974; El-Agamy et al., 1983; 1987; Abdin, 1986; Ramadan et al., 1978, Clay 1989). They were mainly interested in the morphological and histological changes which took place in the course of oocyte growth. The gonads of bony fishes have clear cyclic changes, on the basis of which the sexual cycle is characterized.

The arbitrary maturity stages, in teleost fishes were described histologically by many authors (Hickling, 1930, 1935; La Haye, 1960; 1962; Rastogi, 1968; Latif and Saady, 1973; Ramadan et al., 1978; El-Agamy et al., 1987).

Trachurus mediterraneus. (S) is a widely distributed and widely important fish in the Mediterranean waters.

The present work investigates the ovarian cycle and spawning season of this imporatnt fish.

MATERIAL AND METHODS

Trachurus mediterraneus (S) speciemens were obtained fresh from fishermen working Alexandria fishing grounds. Histological studies were carried out with fresh gonads at different stages of development (I-VI) after fixation in Bouin's fluid, and 10% neutral formalin. Sections 5 Um thick were stained with Heidenhain's azan (HA), Masson's trichrome stain (MTS); and Heidenhain's iron alum haematoxylin (HH) (Pantin, 1966; Gatenby and Beams; 1950). To determine G.S.I. (gonadosomatic index), gonad weight was recorded as a percentage of the gutted body weight.

RESULTS

Cyclic Changes in the Ovary

The ovaries of **T. mediterraneus** have been arbitrarly placed in six stages according to the anatomical and histological examination of the ovaries, the monthly variation of the GSI, and mean egg diameter.

I- Immature Stage (Fig. 1)

a- The immature thread (Fish less than 10 cm in length) this stage is present the whole year round. The gonads are transparent and occupy less than

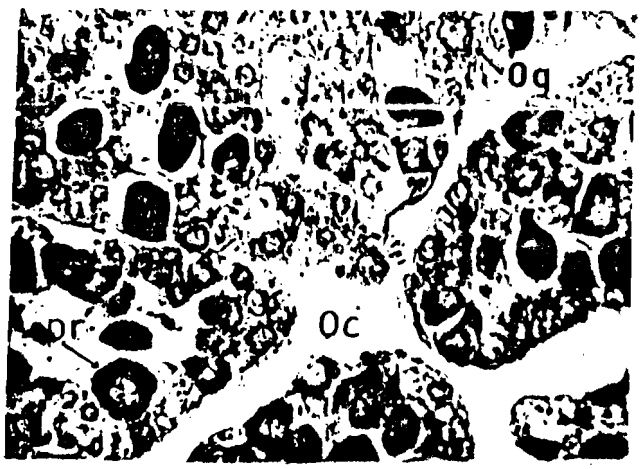

Fig. (1)
Immature stage (thread) 7 cm nests of oogonia (og) and premiotic oocyte (pr). HH, 380 X.

one third of the cavity. Sections show the ovary to be composed of clusters of oogonia and a few scattered premeiotic oocytes. The oogonia are small spherical cells with a thin, indistinct peripheral zone of mildly basophilic cytoplasm and a large nucleus (7-19 Um) with a single large nucleus. The premeiotic oocyte is mostly polygonal and is characterized by a large, spherical nucleus (12-82 Um) which occupies the greater part of the cell and is surrounded by a layer of homogeneous cytoplasm which accepts all the given stains. Two or three large strongly basophilic nucleoli are frequently present.

b- The immature virgin (first caught from Jan. to March, body length 10-15 cm, Fig. 2). The ovary occupy about one third of the body cavity. It is small, translucent and faintly flesh-coloured. In transverse sections, ovary is composed of "nests" of premeiotic and previtellogenic oocytes of varying sizes. The nests are separated by layers of connective tissue supplied with blood vessels. In addition to premeiotic oocytes with homogenous cytoplasm (cells 28-86 & nuclei 15-17 Um in diameter), there are some previtellogenic oocytes which are larger (cells 88-141 & nuclei 58-80 Um in diameter) and have finely granular cytoplasm. The number of nucleoli increases and they begin to approach the nuclear membrane. Some of the nucleoli can be seen to cross the nuclear envelope. These nucleolar extrusions finally disintegrate and disapear in the ooplasm. In such places the nuclear envelope is often seen to be indistinct or discontinuous.

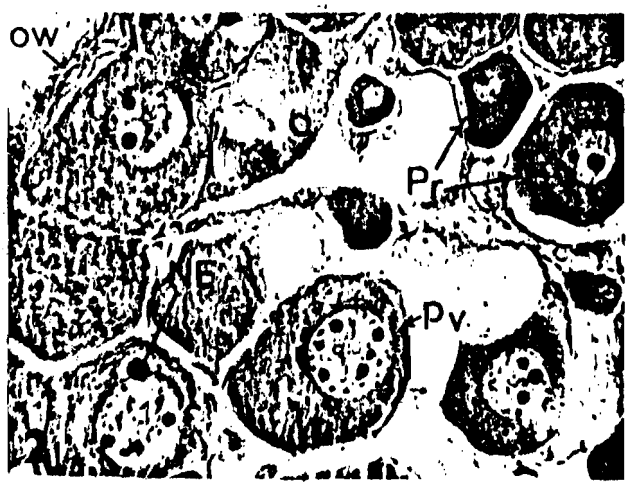

Fig. (2)
Immature stage (virgin) 12 cm. premiotic (pr) and previtillogenic oocyte (pv), nuclear extrusion (NE), nucleoli (Nu) and ovarian wall (OW). HA, 380 X.

2- Maturation Stage (Fig. 3, 4)

This stage lasts from February to late March and comprises fish with total length of over 20 cm. The ovary is pink, owing to the large number of blood vessels, and occupies almost half the body cavity. In sections, the ovary is filled with premeiotic, previtellogenic and yolk vesicle oocytes, the ovarian wall is thick (30-42 µm) and consists of an outer circular and an inner longitudinal muscle layer (Fig. 2). During this period of development, more oocytes are formed.

This stage represents a period of protoplasmic growth, since the oocyte shows an increase in cytoplasm and nuclear volume, together with changes in the affinity of the oocyte for stains, the distribution of the nucleoli, the appearance of the yolk nucleus, the egg wall and the yolk vesicles.

In HH preparations, minute vacuoles or vesicles start to appear at the periphery of the oocytes (cell diameter 150-235 µm, nuclear diameter 58-87 µm). The marginal vesicles are at first few and empty, but with growth of the oocyte their number and size (7-10 µm) increase.

The wall of oocytes containing yolk vesicles is fully formed with a zona radiata, externally to which lies the follicular epithelial layer (Fig. 6). Lampbrush chromosomes are also clearly discernible at this stages (Figs. 4 and 7).

The maturation phase is also characterized by the presence of one or two distinct yolk nucleus (YN) in the oocytes (cell diameter 154 µm, nuclear diameter 20 µm). The YN adheres to one side of the nucleus appears to

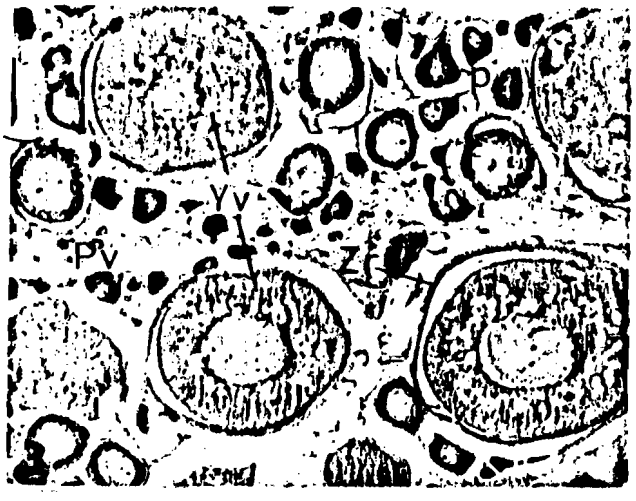

Fig. (3)
Maturation stage 15 cm. Yolk vesicle oocytes (YV), previtellogenic oocytes (PV) and premeiotic oocytes. 96 x.

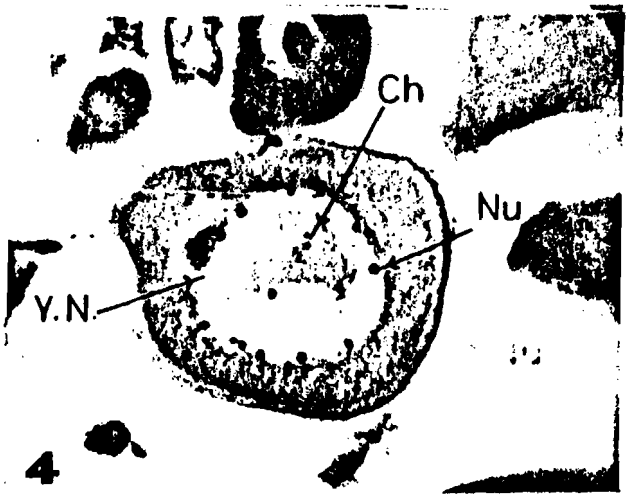

Fig. (4)
Magnified part from Fig. 3 . Yolk nucleus (YN) and Nucleoli (NU).
MTS. 380 X.

consist of two distinct zones: an inner intensely staining zone, sharply marked from the outer clear but faintly stained zone surrounding it (Fig. 4). With further growth of the oocyte, the YN breaks away from the nucleus and migrates towards the periphery, preparatory to its disintegration.

3- Prespawning Stage (through April), (Fig. 5, 6, 7)

In this stage the ovaries turn yellow and occupy more than half the body cavity. The growing oocytes are seen as a granular protuberances on the surface of the ovary. In sections, the ovary is filled with oocytes in different stages of yolk deposition. The deposition of yolk spheres starts at the marginal regions of the maturing oocytes (cells 220-261); nuclei 83-107 µm in diameter); then spread centripetally untill the entire central cytoplasm of the oocytes (cells 410-451, nuclei 91-110 µm in diameter) is full of yolk spheres (protein yolk), which are interspersed with comparatively large, empty vacuoles (fatty yolk), (Fig. 5). The yolk spheres increase further in both size (up to 45 µm) and number, pushing the peripheral yolk vesicles more and more towards the periphery. At the onset of this stage, the nucleus occupies a central position (Fig. 5). As yolk deposition increases, its shape becomes irregular and the nuclear envelope indistincts, (Fig. 7). The large nucleoli are situated at the periphery, but their number (10-18 per section) is smaller than in the preceeding stage. The egg wall, which becomes thicker in this stage, is composed of a comparatively large, striated inner layer (ZR) surrounded by a thicker layer of follicular cells, (Fig. 6). Lampbrush chromosomes are still present until this stage, (Fig. 7).

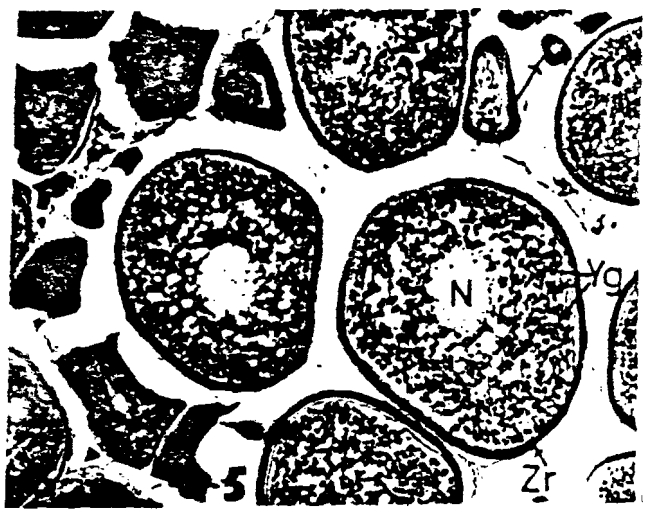

Fig. (5)
Oocytes in different stage of yolk deposition (YG), previtellogenic (PV), zona radiata (ZR) and Nucleoli (N). HH. 96 X.

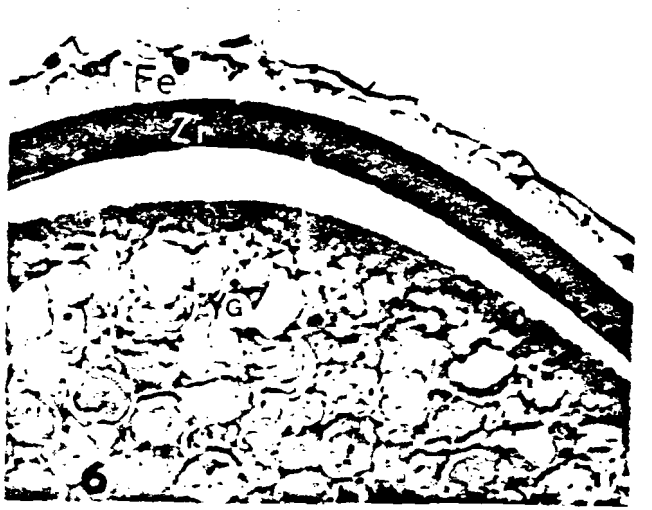

Fig. (6)
Magnified part from the previous figure, showing yolk globule (YG), striated zona radiata (ZR) and thicker follicular epithelium (FE). MTS. 1040 X.

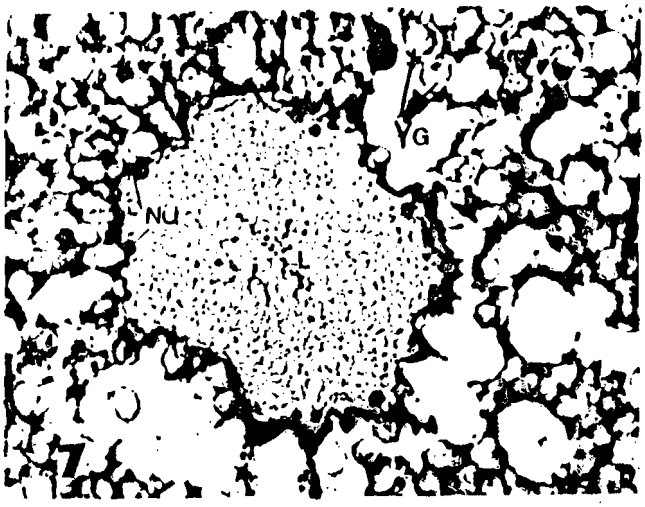

Fig. (7)
Magnified part from figure 5, showing lampbrush chromosomes (LP) and nucleoli (NU). HA. 1040 X.

4- Spawning Stage (late April to late June), (Fig. 8)

Anatomically, the ovaries occupy almost the entire length of the body cavity. They are typically orange yellow and appear turgid, owing to the presence of several ripe eggs ready for ovulation.

During this stage, the gonadal wall is thin, about (13 µm). The ovary is filled with oocytes in different stages of yolk deposition, ripe egg ready for ovulation and few dispersed previtellogenic oocytes, (Fig. 8).

As the oocytes ripen, they undergo morphological and functional changes. The yolk spheres undergo liquifaction, with a concomitant increase in size. The nucleus can be seen to shift to the surface of the oocyte, which now measures about 520 µm, (Fig. 8). The number of nucleoli decrease (5-7) and they shift from the periphery of the nucleus to the center. During liquifaction of the yolk the zona radiata becomes thinner, (Fig. 8), because it stretches following the increase in the size of the cell. The follicular membrane ruptures and the egg is released into the ovarian cavity.

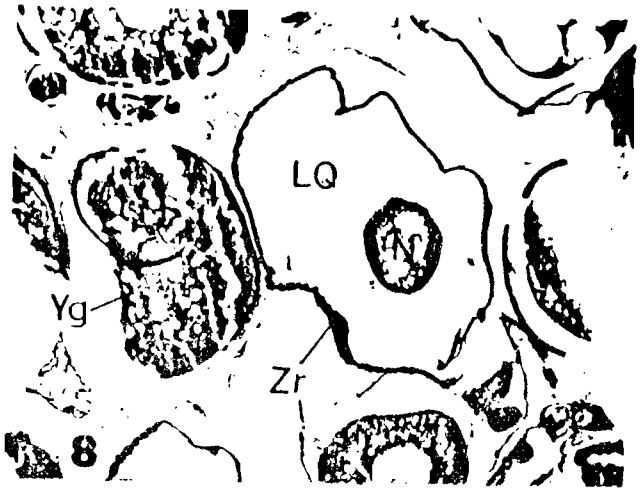

Fig. (8)
Spawning stage 19 cm. Ripe ova showing liquified yolk (LQ), nucleus (N) and zona radiata (ZR). HH. 96 X.

5 Post Spawning Stage (through July, Figs. 9 and 10)

The ovaries are severely shrunken, flaccid and collapsed, and being highly vascularized they are reddish. Sections of the post-spawning ovary show it to be full of discharged follicles atretic oocytes, un-ovulated mature yolky eggs in the process of resorption and large number of premeiotic oocytes, (Fig. 9). The completely depleted ovary contains large numbers of premeiotic and previtellogenic oocytes. Wall thickened and folded, (Fig. 10).

6 - Resting Stage (August to early February, Fig. 11)

The ovary is translucent and yellowish and accounts for over half the size of the gonad. In transverse section, the ovary has well developed ovarian lamellae and consists of premeiotic and some previtellogenic oocytes (diameter of 21 to 92 µm). From our observation of different stages, we noticed that ovary sections showed the continuous presence of oogonia premeiotic and previtellogenic oocytes the whole year round.

Gonadosomatic Index:

The monthly variation of the GSI for females gonad shows five phases, (Table 1, Fig. 12):

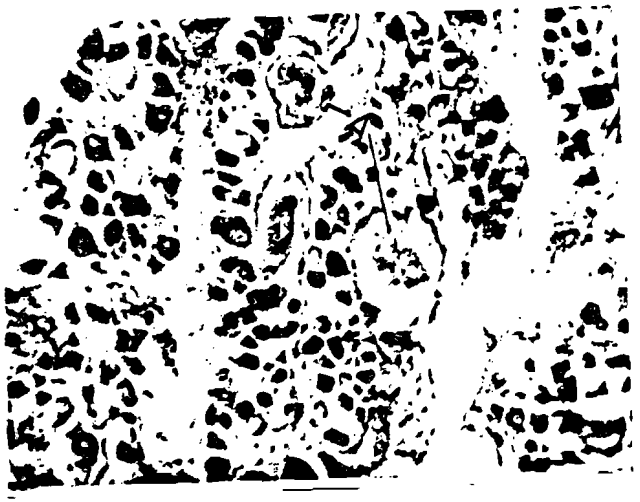

Fig. (9)
Post spawning stage 22.2 cm. Atretic oocytes (A) and premeiotic oocyte (Pr). HA. 96 X.

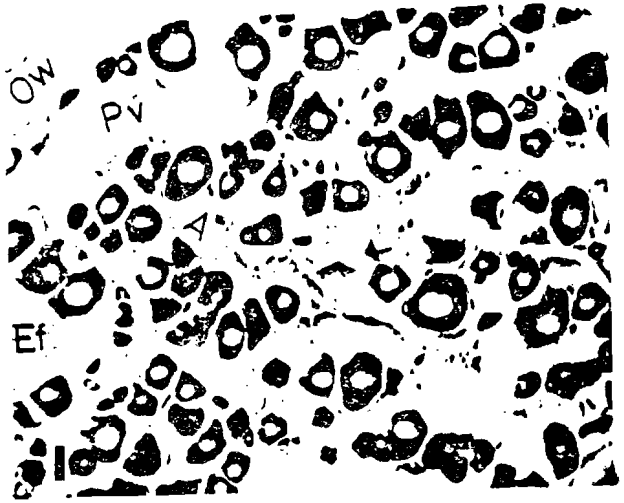

Fig. (10)
Post spawning stage 18.8 cm, showing empty follicle (EF), thick ovarian wall (OW) and previtellogenic oocyte (PV). HH. 96 X.

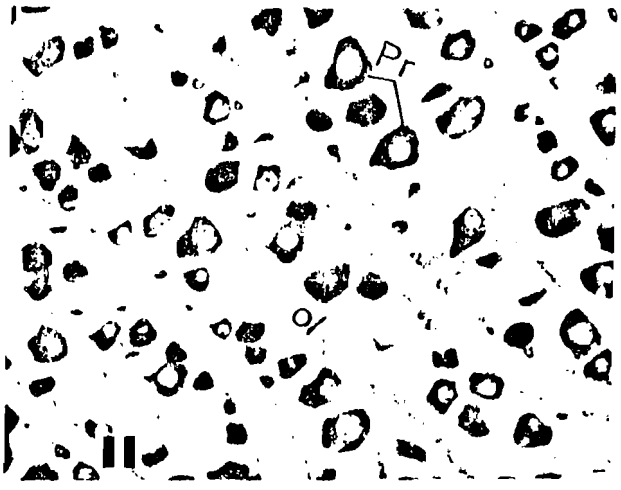

Fig. (11)
Resting stage 23.6 cm. Ovarian lamellae (OL) with premeiotic (Pr) and previtellogenic oocyte (PV). MTS. 96 X.

Table (1)
Monthly variation of GSI for females **T. mediterraneus** in The Egyptian Mediterranean waters.

Month	Number of fish examined	Mean GSI
Dec.	7	0.4112
Jan.	21	o.5917
Feb.	15	0.6238
March	30	2.6215
April	74	3.9211
May	98	3.4452
June	101	2.4991
July	50	0.6808
August	30	0.4811
September	10	0.5911
October	40	0.7214
November	21	0.4200

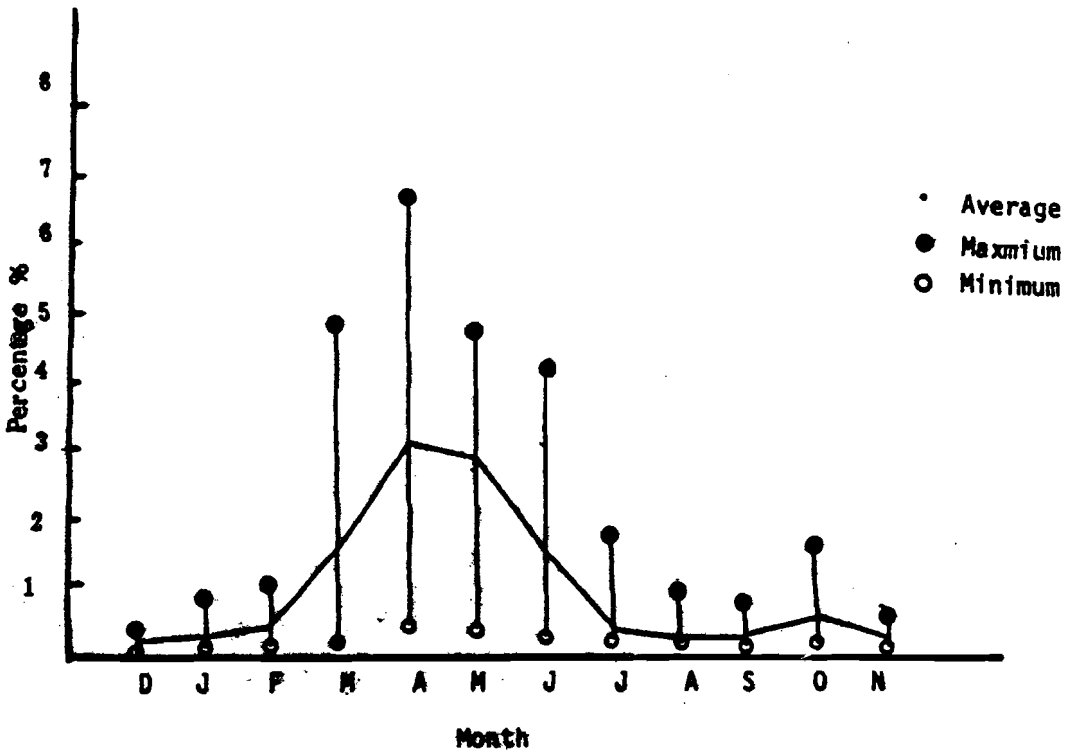

Fig. (12)
monthly variations of Gonado Somatic Index of female
Trachurus mediterraneus in the Egyptian
Mediterranean waters.

1- Maturation phase; starting from February to late March in which the weight of the gonad increases due to active oogenesis.
2 - Pre-spawning phase, extending from late March to early April, at the end of which the GSI reaches its maximum.
3 - Spawning phase, extending from late April to late June, during which the GSI decreases due to expulsion of the genital products.
4 - Post-spawning phase, during July, in which GSI reaches its minimum value.
5 - Resting phase, extending from August to early February, in which GSI nearly constant.

From the monthly variations in GSI, the spawning period of **T. mediterraneus** in Egyptian waters takes place from late April to late June.

DISCUSSION

Marza (1983) placed the rhythm of maturation of the oocytes in three categories: a- Total synchrony, in which all the oocytes of the ovary developed synchronously as in **Oncorhynchus mason**(yamamoto et al. 1959;b) partial synchrony, when group of oocytes can be distinguished, indicating that spawning takes place once a year, with a short and definite season (Guraya et al. 1975 c) a synchrony, in which several batches of oocytes at different stages are present indicating that the spawning season is long, with several spawnings as in **Glyptosternum pectinopterum** (khanna and pant 1967) and **puntius sophore** (Dixit and Agrawala 1974).

In this study, *falls* in the third category (a synchrony), in which the same, full size ovary contains oocytes at various stages of development and maturity. Here young oocytes are converted continuously into developing and maturing stages.

The seasonal ovarian cycle of T. **mediterraneus.** is divided into six stages based on the morphology and histology of the ovary, the monthly variation in gonadosomatic index and mean egg diameter. They are ; the immature stage (thread and virgin), the maturation stage, the prespawning stage , the spawning stage, the postspawning stage and the resting stage.

Our observations in the immature stage, which is characterized by nests of oocytes with homogeneous, intensely staining cytoplasm, are exactly the same as the findings of Guraya et al. (1975), and correspond to the phase of pre-meiotic changes of Rastogi (1968), the synapsis period of Latif and Saady (1973) and the chromatin-nucleolus stage of Dixit and Agrawala (1974).

The maturation stage described in the present study corresponds to the pre-vitellogenesis stage of Rastogi (1968), the period of protoplasmic growth of Latif and Saady (1973), the perinucleolus stage and yolk vesicle stage of Dixit and Agrawala (1974), the preparatory virgin phase of Guraya et al. (1975) and the maturation and vaculation stage of Ramadan et al.

(1978). It is characterized by an increase in the relative cytoplasm and nuclear volume of the maturing oocytes, by changes involving the staining affinity of the cytoplasm, the distribution of the nucleoli, the appearance of the lampbrush chromosomes, the yolk nucleus, the yolk vesicles and the egg wall.

The lampbrush chromosomes in vitellogenic **T. mediterraneus**. oocytes are retain their individuality and staining with HH. This supports the findings of Bara (1960), who reported that lampbrush bivalents appeared in **Scomber scomber** in the perinucleolus stage and remained conspicuous until later stages of oogenesis.

In the fish oocyte, during the maturation phase, the yolk nucleus is differentiated gradually in the cytoplasm adjoining the nuclear envelope. It is generally a spherical and differentiated two separate zones of varying widths (Dutt 1964 in **Anabas scandens**, Ramadan et al. 1978 in **Merluccius merluccius** and in **T. Mediterraneus** used in this study). It eventually migrates towards the outer cytoplasm, where it disintegrates and disapear from view before yolk deposition takes place (Nayyar 1964, Latif and Saady 1973, Guraya et al. 1975, Ramadan et al.1978). In general, two layers enveloping fish eggs have been identified.

The follicular epithelium appear in early differentiated oocytes and the zona radiata makes its appearance in yolk, vesicle oocytes, increasing in thickness with oocyte growth, as many authors have reported in fishes, including the present species (Bowers and Holiday 1961, Latif and Saady, 1973, Guraya et al. 1975, Ramadan et al. 1978).

The present description of the pre-spawning phase, during which yolk deposition takes place in the maturing oocytes, corresponds to the phase of vitellogenesis (Rastogi 1968), the trophoplasmic growth period (Latif and Saady 1973) the maturing virgin phase and pre-spawning virgin phase (Guraya et al. 1975), the primary, secondary and tertiary yolk stages (Yamamoto et al. 1959, Dixit and Agrawala 1974 and the yolk formation stage, (Ramadan et al. 1978).

Present observations in the post-spawning stage, in which the remaining unspent eggs are resorbed, confirm previous findings on spent and recovering phases (Dixit and Agrawala 1974, Guraya et al. 1975).

In the present study, from the monthly variation in GSI and histological examination of the gonads, the spawning of **Trach. medit.** in the Egyptian Mediterranean waters takes place from April to June.

Allam (1979) reported the same finding and claimed that **T. mediterraneus** spawns once a year and its spawning takes place in Spring time (April and May).

REFERENCES

Abdin, A.Z.,1986 : The reproductive biology of a typical Cyprinid, Mampala macrolepidota (Van hasselt), from Zoo Negara Lake, Kuala Lumpur, Malaysia. J. Fish. Biol., 29, 381-391.

Allam, S.M., 1979 : Biological studies of fish stocks Belonging to genus Trahurus Rafinesque in the Egyptian Mediterranean waters. M. Sc. Thesis, Faculty of Science, Alexandria University; 208 pp.

Bara, G. ,1960 : Histological and cytological changes in ovaries of the Mackerel Scomber scomber during the annual cycle. Rev. Fac. Sci. Univ. Inst. Ser. Tome 25 Fasc. 1-2: pp. 49-91.

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

Chaudhry, M.S.,1952 : The yolk and nucleus of Balbiani in Teleosten fishes. Z.Zelforschg. Bd., 37: S. 455. p.

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

Dixit, P.K. and Agrawala, M.,1974 : Studies on the developmental rhythm in the oocyte of Puntius sophore (Ham.). Acta anat., 90: 133-144.

Dutt, N.H.,1964 : The yolk nucleus in the oocytes of Anabas scandens. Quart. J. micr. Sci., 105: 349-352.

El-Agamy, A.E.,1974. Fruschtbakeitsbestimmung Die Decapterus rhonchus (Geoffr.) Und die Dynamik dieser Bestande in Gewassern Von Norddwest Afrika. Dissser. Univ. Rostock.

El-Agamy, A.E., 1986. The fecundity of Gerres oyena forskal 1775 (Fam. Gerreidae) in Qatari waters of the Arabian Gulf. Qatar Univ. Sci. Bull., 6: 371-388.

El-Agamy, A.E., A.S. Al-Zahaby, Y.S. El-Assy and Z.A. Moustafa ,1983. Histological and cytological changes in the ovaries of Clarias lazera during the annual cycle. Bull. Fac. Sci. Zagazig Univ., 5 , 511-532.

El-Agamy, A.E. M.A. El-Shabaka and M.E. Mohallal, 1987. Ovarian cycle and spawning season of Lethrinus lentjan Lecepede in Qatari waters, Arabian Gulf. Bull. Fac. Sci. Zagazig Univ.;9, 672-692.

Guraya, S.S., R. Karur and P.K. Saxena, 1975. Morphology of ovarian changes during the reproductive cycle of the fish, Mystus tengara (Ham.). Acta Anat., 91: 222-260.

Hickling, C.F., 1930. The natural history of the hake. part III. Seasonal changes in the ovary of the immature hake Merluccius merluccius L. J. Mar. Biol. Ass., U.K., Plymouth, Vol. 20: 443-461.

Kamal, A., M.Rizkalla and E. Shahsheir, 1974. Morphology of the ovary of the Nile Teleost fish, Chrysichthys auratus Geoffr. Bull. Zool. Soc. Egypt, Vol. 26: 79-87.

Khanna, S.S. and H.C. Pant, 1967. Seasonal changes in the ovary of a Silurid Cat fish, Glypto sternum pectino pterum. Copia, 1:82-88.

Lahaye, J. , 1960. Croissan et maturation des ovocytes chez la femme totalement adaptic aux eaux douees I' Alosa macrocaima (Alosa alosa Linne). Rev.Trav.Inst.Pecches-Marit., Vol. 24: 499-511.

Latif, A.F.A. and B.E. Saady, 1973. Oogonesis in the Nile Bolti **Tilapia nilotica** L. **Bull. Inst. Ocean. & Fish .**, Vol. 3: 183-202.

Marza, V.D., 1983. Histophysiologie de L'ovogenese. C. Editeurs, paris.

Monique, M.M., 1977. Contribution to the histological study of the gonads particularly the ovary, in the **Box boops** and **Box salpa** (L.) (Teleost fish, Sparidae). New data on its hermaphroditism. **Invest. Pesq.** Vol. 41(1): 165-184.

Nayyare, R.P., 1964. The yolk nucleus of fish oocytes .**Quart J. micr. Sci.** Vol. 105: 353-358.

Norrevang, A., 1968. Electron microscopic morphology of oogenesis. **Inst. Rev. Cytol.**, Vol. 23: 157-164.

Ramadan, A.A; A.A. Ezzat and S.H. Aziz, 1978. Studies on fish oogenesis. I-Histomorphological changes in the oocyte of **Merluccius merluccius** Medit. **Folia Morphologica**, Vol. 1 : 8-15.

Rastogi, R.K., 1968. Studies on the fish oogenesis. I- Histomorphological and cytochemical changes in the oocyte nucleus of **Amphipnous cuchia** (Ham.). **Cytol.** Vol. 33, (3-4): 357-369.

Rizkalla, W., 1970. Studies on the gonads of the teleost Nile fish, **Clarias lazera**, with special refrence to their endocrine tissues. **Acta vetrinaria Scientiarum Hungaricae, Tomus**, Vol. 20(1); 1-12.

Stock, A, 1959. Development of the yolknucleus in the oocytes of some fishes. **Nature**, 1986: 1746.

Yamamoto, K., 1956. Studies on the formation of fish eggs. Annual cycle in the development of ovarian egg in the flounder, **Liposetta obscura. J. Fac. Sci., Hokkaido Univ. Ser. VI.**, Vol. 12 (3): 362-373.

Yamamoto, K.; H. KAI, and R. Ishida, 1959. A preliminary report on the formation of the egg of trout. **Oncorhynchus mason. Bull. Hokkaido Red. Fish. Res.** Vol. 20: 109-116.