The reproductive system and the reproductive cycle of *Mystus vittatus* (Bloch) and *Channa punctatus* (Bloch).
Introduction

For the first time, the morphological aspects of fish gonad were studied in gold fish by Cavolini in 1792. Hyrtl (1855) and Brock (1878) were the other earlier pioneer workers who extended the knowledge of the structure of the fish gonads.

Morphological studies on the teleostean ovary have been contributed by many workers since the middle of the 20th century. Sufficient literature exists in this field of study. Workers like Brock (1878) and Calderwood (1892) described the general structure of the ovary. The histology of the gonads has been described in detail in some elasmobranch fish (Wallace, 1903), in Perca (Turner, 1919), in Gambusia affinis (Geiser, 1921 and 1924), in Xiphophorous helleri (von Oordt, 1925), in Gasterosteus aculeatus (Craig-Bennett, 1931 and Swarup, 1938), in Fundulus heteroclitus (Matthews, 1938), in Cymatogaster aggregatus (Turner, 1938), in Phoxinus laevis (Bullough, 1939), in Onchorhynchus nerka (Weisel, 1943), in Lepomis macrochirus and Huro salmoides (James, 1946), in Rhodeus amarus (Bretschneider and Duyvven de Wit, 1947), in Pomoxis nigromaculatus and P. annularis (Cooper, 1952), in Liopsetta obscura (Yamamoto, 1955).
in Gadus merlangus and G. esmarkii (Gokhale, 1937), in Scomber scomber (Bera, 1960), in Ophiocephalus (Channa) punctatus (Belsare, 1962), in Glyptosternum pectinopterum (Khanna and Pant, 1967) and in Amphilophus cuchia (Sinha and Rastogi, 1967).

Study on reproduction in the teleost fishes has drawn much attention by various workers in the field of reproductive physiology. The literature on the reproduction of teleost fishes has been reviewed by the authors like Hoar (1953 and 1957), Ball (1960) and others.

Seasonal cycle of the reproductive organs has been studied by a number of workers. The seasonal cycle of the female gonads in fishes has been studied in Cottus bairdii (Hann, 1927), in Gasterosteus aculeatus (Craig-Bennett, 1931 and Swarup, 1958), in Merluccius merluccius (Hickling, 1935), in Fundulus heteroclitus (Matthews, 1938), in Cymatogaster aggregatus and Brachyprion episcopi (Turner, 1938 a, b), in Phoxinus levis (Bullough, 1939), in Neotoca bilineata (Mendoza, 1943), in Lepomis macrochirus and Huo salmoides (James, 1946), in Leopsetta obscura (Yamamoto, 1956), in Gadus merlangus and G. esmarkii (Gokhale, 1957), in Carassius auratus (Beach, 1939), in Scomber scomber (Bera, 1960), Barbus stigma and Mystus seenghala (Sathyanesan, 1961 and 1962), Ophiocephalus punctatus (Belsare, 1962), in Glyptosternum pectinopterum

Hubbard (1894) for the first time studied the yolk-nucleus of Balbiani in the oocytes of \textit{Cymatogaster aggregatus}. The form and function of yolk-nucleus have been worked out by Cunningham (1898), Wheeler (1924), Nairn (1939 and 1951), Subramaniam and Aiyar (1935), Mendoza (1943), Chaudhry (1932), Yamamoto (1956), Sathyanesan (1959), Stolk (1959), Barb (1960), Yamamoto and Yamazaki (1961), Nayyar (1964), Gopal Dutt (1964), Rastogi (1966), Lehri (1968), Bhargava and Saksena (1971b) and Malaviya (1973).

Noteworthy studies in connection with piscine corpora arretica (Corpus lutea, or pre-ovulatory follicles or Atretic follicles) are described frequently such as in \textit{Lebistes reticulatus} (Jaski, 1939), in \textit{Neotoca bilineata} (Mendoza, 1943) in \textit{Rhodeus amarus} (Bretschneider and Duyvène de Wit, 1947) in \textit{Mystus seenghala} (Dixit, 1956 and Sathyanesan, 1961), in \textit{Wallago attu} (Dixit, 1956), in \textit{Gadus morhua} and \textit{G. osmerkii} (Gokhale, 1957), in \textit{Carassius auratus} (Beach, 1959).
and Yamamoto and Yamazaki, 1961), in *Scomber scomber* (Bara, 1960), in *Plecoglossus altivelis* (Honna, 1961), in *Ophiocephalus punctatus* (Belsare, 1962), in *Gobius giuris* (Rajalakshmi, 1966), in *Xenentodon cancila* (Rastogi, 1966b), in *Clarias batrachus* (Lehri, 1968), in *Rasbora daniconius* (Raizada, 1971), in *Channa gachua* (Sanwal and Khanna, 1972b) and in *Glossogobius giuris* (Saksena and Bhargava, 1972). Tromp-Blom (1959) has shown in the fish *Gasterosteus aculeatus*, the presence of corpora atretica before and after spawning. Hoar (1955) used the term pre-ovulatory and post-ovulatory corpora lutea in his review on fish ovary. However, Ball (1965 and 1969) Bhargava (1965), Rajalakshmi (1966), Rastogi (1966), Sinha and Rastogi (1967), Lehri (1968), Sahai (1970), Raizada (1971), Malaviya (1972), Sanwal and Khanna (1972) and Saksena and Bhargava (1972) used the more appropriate term corpora atretica or atretic follicles instead of pre-ovulatory corpora lutea or follicles of immature and mature oocytes which fail to spawn and undergo oolysis.

The post-ovulatory follicles or ruptured follicles were studied in *Gasterosteus aculeatus* (Craig-Bennett, 1931 and Tromp-Blom, 1959), in *Fundulus heteroclitus* (Matthews, 1938), in *Notoca hilliacea* (Mendoza, 1943), in *Rhodeus amarus* (Bretschneider and Duyvone de Wit, 1947), in *Gadus morlanque* and *G. eymardii* (Gokhale, 1957), in
Plecoglossus altivelis (Hornma, 1961), in Heteropneustes fossilis (Nair, 1963), in Phoxinus phoxinus (Bhargava, 1966), in Gobius giurus (Rajalakhami, 1966), in Claris batrachus (Lehri, 1968; Malaviya, 1972), in Rasbora daniconius (Raizada, 1971), in Channa gachua (Sanwai and Khanna, 1972b), and in Glossogobius giurus (Saksera and Bhargava, 1972). Working on Xenentodon cancila, Rastogi (1966) gave the term evacuated follicles to the ovarian follicles after ovulation.

Based on the study of ova-diameter the spawning has been worked out in Sardina carulea (Clark, 1934), in hake, pilchard, herring and Lepidogaster (Hickling and Rutenberg, 1936), in some Jawa sea fishes (De Jong, 1939), in Thrissocles purava, Harpodon nephelus and Coilia d issumieri (Palekar and Karandikar, 1952a, b and 1953), in Pampusperca waigiensis, Therapon putileptolepis, Macrones vittatus, Cypselurus obliquolepis, Chirocentrus dorab and Stelophorous indicus (Prabhu, 1956), Chanos chanos (Tampi, 1957), in Cirrhina trigala and Barb us stigma (Sathyenasan, 1939 and 1952), in Amphipnous cucha and (Sinha and Rastogi, 1967) in Claris batrachus (Lehri, 1968; Malaviya, 1972). Bullough (1939) described the seasonal changes in minnow, Phoxinus laevis on quantitative basis showing the maturity and spawning period of the fish. Following Bullough (1939), the reproductive cycle in
fish and the spawning periodicity has been assessed on a statistical basis in *Ambassis ransei*, *Puntius ticto* and *Rohtee cotic* (*Kaur, 1968*), in *Oxygaster basilla*, *Garra gotyla gotyla*, *Nandus nandus* and *Rasbora daniconius* (*Raizada, 1969*), in *Clarisas batrachus* (*Malaviya, 1972*) and in *Glossogobius giuris* (*Saksena, 1974 and 1976*).

Poisilia reticulata (Pandey, 1969), in Rasbora daniconius (Raizada, 1970) and in Glossogobius giuris (Saksena, 1974).

The testicular cyclical changes have been worked out in Perca (Turner, 1919), in Gambusia affinis (Geiser, 1921), in Gasterostetes aculeatus (Craig-Bennett, 1931; Swarup, 1958), in Betta splendens (Bennington, 1936), in Fundulus heteroclitus (Matthews, 1939), in Phoxinus leavis (Bullough, 1939), in Salmo salar (Jones, 1941), in Oncorhyncus nereck (Weisel, 1943), in Lepomis macrochirus and Huro sanloides (James, 1946), in Astyanax mexicanus (Rasquin and Hafer, 1951), in Gadus (Gokhale, 1937), in Salvelinus fontinalis (Henderson, 1962), in Conesius plumbeus (Ahsan, 1965), in Sebastodes paucispinis (Moser, 1957), in Heteropneustes fossilis (Sundararaj, 1960), in Rasbora daniconius (Raizada, 1970), in Tilapia nigra (Hyder, 1970), in Heteropneustes fossilis (Nayyer and Sundararaj, 1970), in Eucalia inconstans (Ruby and Donald, 1970), and in Channa gachua (Sanwal and Khanna, 1972).

A study on the relative number of cell types in the process of spermatogenesis in different months of the year has been made in Ambassia range and Puntius ticto (Kaur, 1968), in Nandua nandua and Rasbora daniconius (Raizada, 1969 and 1970), in Clarias batrachus and Mastacembelus pancerus (Malaviya, 1972), in Glossogobius giuris (Saksena, 1974 1976) and in Gerra goyla goyla (Anant Prakash, 1976).
Correlation between the water temperature and gonad volume has been made in *Perca* (Turner, 1919), in *Gasterosteus aculeatus* (Craig-Bennett, 1931), in *Fundulus heteroclitus* (Matthews, 1938), in *Phoxinus laevis* (Bullough, 1939), in *Galeichthys felis* (Frederick, 1949), in *Lepomis macrochirus* (James, 1946), in *Gadus merlangus* and *G. esmarkii* (Gokhale, 1937), in *Ambassies ranga*, *Rothee cotio* and *Puntius ticto* (Kaur, 1968), in *Oxygaster bacaila*, *Garra fortyla fortyla*, *Nandus nandus* and *Rasbora daniconius* (Raizada, 1969) and in *Glossogobius giuris* (Saksena, 1974 and 1976). More precisely Sinha and Rastogi (1967) found that the fluctuation in water temperature seems to have significant correlation with the internal changes in the ovary of *Amphipnoous cuchia*. Working on the same fish they also observed the relationship between the gonosomatic index and water temperature.

The present work deals with the study of reproductive system and reproductive cycle in the Indian fresh-water catfish, *Mystus vittatus* (Bloch) and *Channa punctatus* (Bloch). The cyclic changes observed in the gonads have been confirmed by statistical assessment which is based on the work of Bullough (1939). Volume of gonads and gonosomatic index have been correlated with the temperature of water during the reproductive cycle.
Observations:

1. Morphological study of the reproductive organs:

*Mystus vittatus* is a sexually dimorphic fish. The males are differentiated from females in having a long pointed muscular pseudo-copulatory papilla noticed below the genital opening whereas the females have a spheroidal swelling near the urinogenital opening. The gonads present a typical teleostean pattern with a set of related organs in each sex.

A. The female reproductive organs:

In *Mystus vittatus* the ovaries are of cystovarian type. They are paired structures situated ventral to the kidney and air bladder and occupy approximately 3/4th of the body cavity. The ovaries hang in the coelom by a thin transparent membrane, the mesovarium. They are spindle-shaped in immature condition and appear to change in shape occasionally according to the degree of maturity. In mature condition the ovaries are irregular in shape. Their caudal ends continue posteriorly as oviducts which immediately unite to form the common oviduct. The common oviduct opens independently through a slit-like
Fig. 63: Diagrammatic sketch of the ovary of *Ayu* * Vittatus* to show its morphology.

Fig. 64: Diagrammatic sketch of the ovary of *Channa punctatus* to show its morphology.

Fig. 65: Diagrammatic sketch of the testis of *Ayu* * Vittatus* to show its morphology.

Fig. 66: Diagrammatic sketch of the testis of *Channa punctatus* to show its morphology.

**Abbreviations:**

- A - Anal aperture
- C.O.D. - Common oviduct
- C.S.D. - Common sp.-em duct
- O - Ovary
- O.D. - Oviduct
- R - Rectum
- S.D. - Spem-duct
- T - Testis
- U - Ureter
- U.A. - Urinary aperture
- U.B. - Urinary bladder
- U.G.A. - Urino-genital aperture
- U.G.P. - Urino-genital papilla.
opening in the spheroidal swelling infront of the urinary aperature. The common urinary duct runs below the oviduct, joins the urinary bladder and opens separately near the female genital pore (Fig. 63, p. 113) in a urino-genital sinus. The ovaries vary in colour, length and width according to their seasonal activity in the year. At maturity, when the ova bulge out, the smooth contour of the ovary gets disrupted. The mature ova are thus clearly visible through its transparent peritoneal membrane. The immature ovaries are feebly vascularised while the mature ones show a rich blood supply.

In Channa punctatus the ovaries are paired organs and are situated in the same position as found in case of Mystus vittatus. The ovary of the left side is larger than that of the right side. In their anterior 2/3rd region, the paired ovaries run separately while in their posterior 1/3rd region they fuse together and terminate posteriorly in a thin-walled oviduct which opens outside through the female genital pore (Fig. 64, p. 113). The ovaries of Channa are elongated in shape but they show seasonal changes in shape, size and colour according to the degree of maturity they have attained.

B. The male reproductive organs:

In Mystus vittatus the testes are paired structures situated in the same position as ovaries in the female fish.
The testes hang in the coelom dorsally by a well-marked thin membrane, the mesorchium. Each testis is composed of numerous finger-like seminiferous lobes which are free at their outer ends while at their inner margin they open in a longitudinal sperm duct. Thus the whole structure appears like a comb. The sperm duct of each testis runs posteriorly and before joining with the fellow of the opposite side receives 10 to 12 large, pointed finger-like structures, the seminal vesicle lobes (Fig. 65, p. 113). Just posterior to them, the paired sperm ducts join with each other near the urinary bladder and form a common sperm duct.

The seminal vesicles are specialised lobes of testes which can not be differentiated from them morphologically. However, they are different histologically and are filled with colloidal secretion which probably helps in the spermiation. The kidney ducts join the urinary bladder. The urinary bladder and the common sperm duct run closely within the pseudo-copulatory papilla and open to the exterior through a common urinogenital aperture. The pseudocopulatory papilla is an elongated structure which is broader at the base and pointed at its free end. The shape, size, colour and vascularisation of these testes vary in different periods of the year.

In Channa punctatus, the testes are paired structures situated in the posterior part of the body cavity. They are
thin structures attached dorsally by the mesorchia. They terminate posteriorly in the sperm ducts which run freely backward and then unite with each other to form a small common sperm duct (Fig. 66, p. 113). The latter opens outside by a urino-genital pore. The vascularisation, colour and size of the testis changes seasonally according to the state of maturity attained by it.

2. Histological study of the gonads :

A. Histological study of the ovaries :

In *Mytus vittatus*, each ovary is covered with a thin delicate peritoneal membrane, the tunica albuginea, below which lies the germinal epithelium. The peritoneum is a transparent membrane and consists of connective tissue, muscle fibres and blood capillaries. From the inner wall of the ovarian sac many finger-like processes hang freely in the ovarian lumen. They are called as ovigerous lamellae or folds containing oocytes of different stages of maturity (Fig. 67, p. 118). In the ovigerous lamellae the early oocytes are present towards the ovarian wall, while the mature ones are centrally placed. The folds are prominent during post- and pre-spawning periods but indistinguishable in the spawning period.

In *Channa punctatus* the ovary consists of an
ovarian wall and ovigerous lamellae. The ovarian wall is made up of outer tunica albuginea and inner germinal epithelium. The tunica albuginea consists of connective tissue cells and fibres. The ovarian wall is richly supplied with the blood vessels. Ovarian lumen is obliterated by the ovigerous lamellae which are formed by connective tissue fibres, blood capillaries and germinal epithelium. Ovigerous lamellae contain oocytes in different stages of their maturation (Fig. 69, p. 118).

The different stages of developing oocytes present in the ovary of Mystus vittatus and Channa punctatus are classified into eleven stages which are as follows:

1. Early chromatin-nucleolus stage:

   In Mystus, it is the earliest visible stage (Fig. 69, p. 118). It is oval or round in shape. It is small in size, with a thin accumulation of cytoplasm. The oocyte has an oval nucleus with a deeply-stained nucleolus situated on the chromatin mesh-work. The cytoplasm of the oocyte at this stage takes a light stain while the nucleus is deeply stained. The oocyte at this stage measures up to 15 μ and the nucleus up to 7 μ in diameter.

   In Channa, this stage is almost oval in shape with a thin accumulation of cytoplasm which is clear and lightly-stained. The oocyte has a centrally placed round
Photomicrograph of the section of the ovary –

Fig. 67 : showing ovarian wall and the ovigerous lamellae in *Myurus vittatus* (Haematoxylin - eosin)

Fig. 68 : showing ovarian wall and the ovigerous lamella in *Channa punctata* (Azan)

Fig. 69 : showing oocytes of early and late chromatin nucleolus stage in *Myurus vittatus* (Azan)

Fig. 70 : showing oocytes of early and late chromatin nucleolus and early peri-nucleolus stage in *Channa punctata* (Haematoxylin - eosin)

Fig. 71 : showing oocytes of early and late peri-nucleolus stage in *Myurus vittatus* (Haematoxylin - eosin)

Fig. 72 : showing oocyte of late peri-nucleolus stage in *Channa punctata* (Azan)

**Abbreviations** :

- **E.C.N.** – Early chromatin-nucleolus stage
- **E.P.N.** – Early peri-nucleolus stage
- **L.C.N.** – Late chromatin-nucleolus stage
- **L.P.N.** – Late peri-nucleolus stage
- **N.** – Nucleus
- **N.L.** – Nucleolus
- **O.L.** – Ovigerous lamella
- **O.W.** – Ovarian wall.
nucleus with a deeply stained nucleolus. The oocyte at this stage measures up to 13 μ and nucleus measures 6.5 μ in diameter (Fig. 70, p. 118).

2. Late chromatin-nucleolus stage:

In this stage (Fig. 69, p. 118) the cytoplasm takes a basophilic texture. The oocyte has a large centrally placed nucleus which contains a nucleolus eccentric in position. The chromatin reticulum shows a bunch of thick and deeply stained threads. The maximum diameter of the oocyte is found as 35 μ and that of the nucleus as 16 μ.

In Channa this stage is marked with an oval shape with basophilic cytoplasm (Fig. 70, p. 118). The nucleus is large with a single deeply stained nucleolus. The chromatin granules concentrate just below the nuclear membrane. The oocyte at this stage measures 32 μ and the nucleus measures 19 μ in diameter.

3. Early peri-nucleolus stage:

In Mystus this stage of oocyte shows deeply-stained cytoplasm. The follicular layer of epithelium begins to develop around the oocyte. The nucleus is large and is surrounded by a thin layer of cytoplasm (Fig. 71, p.18). The nucleolus breaks into two or three nucleoli which are strongly basophilic in nature. They are of different size.
and tend to remain peripheral in position. The oocyte measures up to 113 \( \mu \) and nucleus is 68 \( \mu \) in diameter.

In Channa, the follicular layer of epithelium begins to appear around the oocyte. The oocyte at this stage is oval in shape and the cytoplasm is basophilic in nature. The nucleus is oval in shape and centrally placed (Fig. 70, p. 116). The chromatin network is scanty and concentrated just below the nuclear membrane in a peripheral zone. The nucleolus breaks up into two or three small nucleoli which lie in the nucleoplasm at random. The nucleoli are compact in structure and deeply stained. The yolk-nucleus appears at this stage as a dot-like structure. The oocyte at this stage measures up to 124 \( \mu \) and nucleus up to 72 \( \mu \) in diameter.

4. Late peri-nucleolus stage:

In Mystus, the follicular epithelium is almost completely formed at this stage due to which the outline of the oocyte becomes distinct. With further development and growth of the oocyte the cytoplasm loses its affinity towards stain and is stained lightly with haemotoxylin. The yolk-nucleus appears for the first time at this stage as a small dot-like structure in the cytoplasm near the nucleus. The nucleus is large in size and centrally placed. The nucleoli increase in number and arrange themselves towards
Photomicrograph of the section of the ovary —

Fig. 73 : showing early yolk vesicle stage in *Myxatus vittatus* (Azan)

Fig. 74 : showing early yolk-vesicle stage in *Channa punctatus* (Azan)

Fig. 75 : showing late yolk-vesicle stage in *Myxatus vittatus* (Azan)

Fig. 76 : showing late yolk-vesicle stage in *Channa punctatus* (Azan)

Fig. 77 : showing primary yolk stage in *Myxatus vittatus* (Haematoxylin-eosin)

Fig. 78 : showing primary yolk stage in *Channa punctatus* (Azan)

Abbreviations:

E.YV. — Early yolk-vesicle stage
L.YV. — Late yolk-vesicle stage
O.W. — Ovarian wall
P.YS. — Primary yolk stage
V.M. — Vitelline membrane
Y.N. — Yolk-nucleus
Y.G. — Yolk granule
Y.V. — Yolk vesicle.
the periphery of the nucleus (Fig. 71, p. 118). They vary in shape but are usually rounded. The nucleoplasm becomes granular. The maximum diameter of the oocyte and reaches 178 \( \mu \) and that of the nucleus is 81 \( \mu \) respectively.

In Channa, the shape of the oocyte is oval and its cytoplasm stains feebly. The follicular layer of epithelium covers the oocyte completely. The nucleus is centrally placed and contains 12-16 nucleoli which are concentrated towards the periphery of the nucleus. The nuclear membrane is quite distinct. The yolk-nucleus increases in size and appears as a spheroidal structure. It migrates a little away from the nucleus towards the peripheral region of the oocyte (Fig. 72, p. 118). At this stage the oocyte measures 188 \( \mu \) and the nucleus is 90 \( \mu \) in diameter.

5. Early yolk-vesicle stage:

At this stage the oocyte of Mystus is polygonal in shape. The vitelline membrane makes its appearance as a very thin layer between the cytoplasm and the follicular layer. Theca layer is also noticed at this stage. Few small yolk-vesicles appear in the peripheral cytoplasm of the oocyte (Fig. 73, p. 121). The yolk-nucleus increases in size and migrates a little away from the nucleus towards the periphery of the oocyte in the form of a small vesicle. The nucleus is rounded, lies in the middle and stains lightly
with acid dyes. The oocyte at this stage measures up to 287 \( \mu \) and the nucleus up to 118 \( \mu \) in diameter.

In *Channa*, at this stage, a number of yolk-vesicles are noticed in the peripheral zone (Fig. 74, p. 121). There is no further change in the structure and position of the yolk-nucleus at this stage in comparison to the preceding stage.

The oocyte is round in shape with a well-defined follicular layer. The cytoplasm is stained lightly. The nucleus is centrally located. The nuclear membrane is distinctly marked. The oocyte at this stage measures up to 206 \( \mu \) and its nucleus 102 \( \mu \) in diameter.

6. Late yolk-vesicle stage

In *Mystus*, the yolk-vesicles formed in the early yolk-vesicle stage are now larger in size and fresh yolk-vesicles appear in the cytoplasm towards the nucleus giving a mosaic appearance (Fig. 75, p. 121). The yolk-nucleus is not found in this stage and in the other following stages. The follicular layer is now completely formed and is quite distinct. The chromatin material as well as the nucleoli take a deep stain. The maximum diameter of the oocyte and nucleus reaches 347 \( \mu \) and 148 \( \mu \) respectively.

In *Channa*, the oocytes are also filled with large yolk-vesicles which are fully formed. The follicular layer
of the oocyte is now well marked (Fig. 75, p. 121). The oocyte is stained lightly with the basic dyes. The chromatin material as well as the nucleoli are still deeply stained. The yolk-nucleus is seen towards the periphery of the oocyte in the process of disintegration. The maximum diameter of the developing oocyte, at this stage measure 262μ and that of the nucleus as 117μ.

7. Primary yolk stage :

In Mystus, the follicular layer and vitelline membrane are more prominent in this stage than in the former stage. The formation of yolk in the form of small granules in the yolk-vesicle is recognised as the primary yolk stage (Fig. 77, p. 121). The oocytes look as pentagonal or hexagonal in shape due to heavy pressure of the oocytes on one another. The yolk granules are at first stained lightly basophilic but later on when they become large respond to acidic dyes. The nucleus is large and nucleoli are scattered in the nucleoplasm irregularly and their peripheral arrangement is disrupted. At this stage the maximum diameter of the oocyte reaches 447μ and that of the nucleus as 162μ.

In Channa, the oocyte is covered with an outer theca, middle follicular layer and inner vitelline membrane (Fig. 78, p. 121). The oocytes are now polygonal in shape. The yolk, in the form of large globules, appears within the yolk-vesicles which lie towards the periphery of the oocyte.
Photomicrograph of the section of ovary —

Fig. 79: showing secondary yolk stage in *Lysis vittata* (Haematoxylin-eosin)

Fig. 80: showing secondary yolk stage in *Channa punctatus* (Azan)

Fig. 81: showing tertiary yolk stage in *Lysis vittata* (Haematoxylin-eosin)

Fig. 82: showing tertiary yolk stage in *Channa punctatus* (Azan)

**Abbreviations**

S.YS.  —  Secondary yolk stage  
T.YS.  —  Tertiary yolk stage  
V.M.  —  Vitelline membrane  
Y.G.  —  Yolk granule  
Y.V.  —  Yolk vesicle.
The yolk globules respond strongly to basic dyes. The nucleus begins to shrink in size and the nucleoli are now arranged below the nuclear membrane. At this stage the maximum diameter of the oocyte and nucleus reaches 345\(\mu\) and 142\(\mu\) respectively.

8. **Secondary yolk stage**

At this stage, in *Mystus*, the vitelline membrane and follicular layer become thick. The yolk granules increase in size and they are now acidophilic in nature. The nucleus is situated centrally but the nucleoli are dispersed irregularly in the nucleoplasm (Fig. 79, p. 125). The maximum diameter of the oocyte at this stage is 436\(\mu\) and that of the nucleus is 174\(\mu\).

In *Channa*, the oocyte bears an irregular shape. The theca, follicular layer and vitelline membrane are quite distinct. Apart from the peripheral yolk-vesicles, the yolk-globules also appear in the other yolk-vesicles towards the nucleus. The yolk globules are of two types, some of them being large while others small in size (Fig. 80, p. 125). They stain deeply with acid dyes. The nucleus loses its distinct figure and becomes irregular in shape. The maximum diameter reached by the oocyte and the nucleus at this stage is 466\(\mu\) and 168\(\mu\) respectively.

9. **Tertiary yolk stage**

In *Mystus*, at this stage, the oocyte becomes large.
Photomicrograph of the section of ovary —

Fig. 83: showing prematuration stage in *Mytus vittatus* (Azan)

Fig. 84: showing prematuration stage in *Channa punctatus* (Haematoxylin-eosin)

Fig. 85: showing mature stage in *Mytus vittatus* (Azan)

Fig. 86: showing mature stage in *Channa punctatus* (Azan)

Abbreviations:

- M.S. = Mature stage
- P.P.S. = Prematuration stage
- V.M. = Vitelline membrane
- Y.G. = Yolk granules
- Y.V. = Yolk vesicles
in size and the vitelline membrane increases in thickness. The theca layer disappears while the follicular layer becomes well marked. The cytoplasm is filled with two types of yolk granules. A small zone of homogeneous yolk also surrounds the nucleus. All the yolk granules are acidophilic in nature. The nucleus is round in shape and is placed centrally. The nucleoli are arranged towards the periphery of the nucleoplasm. At this stage the oocyte measures 605 μ and the nucleus 260 μ in the maximum diameter (Fig. 81, p. 125).

In Channa, the oocytes are polygonal in shape. They are bounded by an outer theca, the follicular layer and the vitelline membrane. At this stage, almost every yolk-vesicle present in the oocyte contains yolk globules which are acidophilic in nature. These yolk globules are mostly of large and medium size. However, few small yolk globules are also noticed (Fig. 82, p. 125). The nucleus is centrally placed and loses its outline. The nucleoli are scattered in the nucleoplasm. The oocyte and the nucleus, at this stage measure 835 μ and 248 μ respectively in their maximum diameter.

10. Pre-maturation stage:

In Mystus, at this stage, the vitelline membrane and follicular layer are well-developed. The oocyte is filled with acidophilic yolk granules which now increase in size. The nucleus is somewhat eccentric in position and appears
as a clear body. The nucleoli are well marked and scattered towards the periphery of the nucleus. The maximum diameter of the oocyte, at this stage, measures 648 μ and that of nucleus as 243 μ (Fig. 83, p. 127).

In C. anna, the vitelline membrane increases in thickness at this stage. The yolk globules increase in size and in number and the oocyte is packed with yolk globules. The yolk-vesicles, however, persist in large number near the nucleus with broken outlines (Fig. 84, p. 127). The nucleus at this stage is a little eccentric and has one or two faintly-stained nucleoli. At this stage the maximum diameter of the oocyte reaches 733 μ that of the nucleus 252 μ.

11. Mature stage:

In C. mystax the vitelline membrane shows radial striations while the follicular layer disappears. The oocyte is filled with closely packed acidophilic yolk granules. At this stage the nucleus becomes clear and shrunken (Fig. 85, p. 127). After extruding out the polar body formed by maturation division, the oocyte is now ready to spawn. The mature oocyte and its nucleus reach 688 μ and 226 μ in their diameter.

In C. anna, the vitelline membrane acquires radial striations. The oocyte is now filled with acidophilic yolk globules. The yolk-vesicles still persist near the nucleus with
broken outlines (Fig. 86, p. 127). The nucleus gets shrunk and the nuclear membrane disappears. Few nucleoli are noticed in the nucleoplasm. At this stage the oocyte measures up to 848 μ and its nucleus up to 230 μ.

Corpora atretica and post-ovulatory follicles:

Many oocytes undergo resorption and are called as atretic oocytes. The immature oocytes which fail to attain maturity and mature oocytes which fail to spawn ultimately undergo resorption (atresia) and are called as corpora-atretica. Degeneration of the immature oocytes is not as common as that of the mature oocytes. In Channa punctatus, the process of formation and resorption of corpora atretica may continue throughout the year, but in Mystus vittatus the corpora atretica are seen from June to November.

Formation of corpora atretica and the process of atresia:

Bretschneider and Duyvende de Wit (1947) have described the process of follicular atresia in Rhodeus amarus and the changes undergone by the degenerating oocytes have been divided in four different stages. In the present study also the process of follicular atresia has been described in four stages in Mystus and Channa as follows:
Stage I:

Mystus:

The nucleus of the oocyte gets disorganised and is not seen. The follicular layer of the oocyte loses its syncytial nature to become hypertrophic and assumes a characteristic cellular appearance. The small yolk granules unite together to form bigger spherical globules. The vitelline membrane gets deflected off from the follicular layer and breaks up at many places, due to which the hypertrophied follicular cells invade the yolk with greater ease. Small granules with similar tinctorial behaviour to that of yolk granules are noticeable within the follicular cells, which probably correspond to the disintegrating ferments of Rhodeus amarus (Bretschneider and Duyviiene de Wit, 1949) (Fig. 87, p. 133).

Channa:

In this stage the follicular layer begins to hypertrophy and its cells show distinct outlines. The follicular cells are mostly columnar showing nuclei of different shape at their base. Vacuoles appear in some of the follicular cells. The vitelline membrane becomes folded and gets broken at various places. The colloidal particles of yolk appear between the vitelline membrane and follicular layer (Fig. 88, p. 133).
Stage II:

Mystus:

This stage shows further hypotrophy of the follicular cells. A large number of granules (ferments) are seen within the follicular cells. Some of the follicular cells migrate to the ooplasm and engulf the yolk by their phagocytic activity. The vitelline membrane disintegrates and now only its traces are noticeable in some cases. Most of the yolk globules present now liquify. Some of the follicular cells also begin to degenerate (Fig. 89, p. 133).

Channa:

Further hypertrophy of the follicular cells takes place. The vitelline membrane breaks further at various places and becomes very thin and the follicular cells begin to invade the yolk through the ruptured vitelline membrane. The oocyte begins to lose its shape. The yolk globules are less in number than in the preceding stage (Fig. 90, p. 133).

Stage III:

Mystus:

At this stage the follicular cells become reduced in size. A number of follicular cells are now seen inside the oocyte. The so-called granules (ferments) begin to diminish.
Photomicrograph of the sections of the ovary of *Anatina vittata* and *Channa punctatus* showing the stages in the formation of corpora atretica (Azan)

Fig. 87 : Stage I of *Anatina vittata*

Fig. 88 : Stage I of *Channa punctatus*

Fig. 89 : Stage II of *Anatina vittata*

Fig. 90 : Stage II of *Channa punctatus*

Abbreviations :

D.F. = Disintegrating ferments
F.L. = Follicular layer
I.C. = Invaded follicle cells
V.M. = Vitelline membrane
Y.G. = Yolk granules
Y.V. = Yolk vesicles.
in the follicular cells and ultimately disappear in the subsequent stage. The vitelline membrane gets completely disintegrated and lost at this stage. Most of the yolk globules undergo liquifaction and much of the liquified yolk is occupied by the follicle cells throughout the oocyte. Very few yolk globules are thus left at this stage and they lie scattered in the oocyte (Fig. 91, p. 135).

**Channa**

The follicular cells increase in size and number. The vitelline membrane is completely disintegrated and few traces of it are noticeable. Most of the yolk globules are replaced by the follicular cells and the oocyte loses its shape (Fig. 92, p. 135).

**Stage IV**

**Mystus**

At this stage the liquified yolk is completely exhausted. The cavity of the oocyte is now filled with the invaded follicular cells and large number of vacuoles. The contraction of the corpora atretica starts until it becomes intermingled with the connective tissue of the stroma of the ovary. The oocyte undergoing oolysis is, thus, ultimately lost in the ovarian stroma (Fig. 92, p. 135).
Photomicrograph of the sections of the ovary of *Mystus vittatus* and *Channa punctatus* showing the stages in the formation of corpora lutea (Azan)

Fig. 91 - Stage III of *Mystus vittatus*

Fig. 92 - Stage III of *Channa punctatus*

Fig. 93 - Stage IV of *Mystus vittatus*

Fig. 94 - Stage IV of *Channa punctatus*

Abbreviations

V.M. - Vitelline membrane
V.O. - Vacuoles
Y.G. - Yolk granules
This stage is characterised by the complete absence of yolk. The oocyte is filled with the follicular cells, vacuoles and a few blood vessels. There is a contraction of the corpora atretica and the entire mass becomes more compact and finally lost in the ovarian stroma (Fig. 94, p. 135).

The post-ovulatory follicle and its fate:

After the discharge of the mature eggs from the ovarian follicle, the follicular layer is left behind. Whole structure thus left is called as the post-ovulatory follicle, evacuated follicle, empty follicle, ruptured follicle or ovulation scar (Fig. 95, p. 137). A fresh post-ovulatory follicle contains a clear central space. The ruptured follicular layer in many cases acquires continuity with the outer germinal epithelium. The cells of the follicular layer do not show any hypertrophy but undergo proliferation. The follicular layer thus gets thickened and the internal space of the post-ovulatory follicle is reduced gradually. These masses of the follicle cells undergo gradual degeneration and finally get absorbed in the ovarian tissue.

In Channa, the post-ovulatory follicles form in the same way as in Mystus. The post-ovulatory follicles have a distorted shape. The follicular cells become loose and their
Photomicrograph of the section of the ovary showing post-ovulatory follicles in -

Fig. 93 - *Mystus vittatus* (Azan)

Fig. 96 - *Channa punctatus* (Azan)

Abbreviation:

P.F. - Post-ovulatory follicles
nuclei including nucleoli and the chromatin are not recognizable. These cells proliferate and occupy the space inside the follicle. The follicles then gradually shrink and undergo degenerative changes and are ultimately absorbed in the ovarian stroma (Fig. 96, p. 137).

B. Histological study of the testes:

In Mystus and Channa the testes are of typical cyprinoid type (According to Brock's (1878) classification). The testes are covered over by a thin layer of peritoneal membrane below which lies a comparatively thick layer of tunica albuginea composed of epithelial cells, fibrous connective tissue (Figs. 98, 99, p. 141) and blood vessels. In Mystus each testis consists of numerous branching and anastomosing lobes which are closely connected together but separated by the stroma tissue. The stroma tissue consists of loose connective tissue, blood capillaries and interstitial cells. In Channa, the testis is divided into a number of seminiferous tubules. The seminiferous tubules are separated by the interstitial tissue having interstitial cells, connective tissue and blood vessels. They vary in shape and size and contain various stages of spermatogenesis. In Mystus there is a seriation in the process of spermatogenesis from the periphery to the centre of the tubules. In Channa, the tubules contain all the spermatogenetic stages without seriation. Each cyst or tubule contains various
Fig. 97 - Camera lucida sketches in the different stages of spermatogenesis in —

I. - Mystus vittatus

II. - Channa punctatus

Abbreviations:

A. - Primary germ cells
B. - Spermatogonia
C. - Primary spermatocytes
D. - Secondary spermatocytes
E. - Spermatids
F. - Spermatozoa
FIG. 97
cells in different stages of division. In both fish
different stages of spermatogenesis are recognised as the
primary germ cells, spermatogonia, primary spermatocytes,
secondary spermatocytes, spermatids and spermatzoas.

1. Primary germ cells:

In *Mystus* the primary germ cells are situated
along the peripheral region of the tubules (Figs. 97, 100,
p. 139, 141). They are the largest of all the stages of
spermatogenesis and are found throughout the year. Each
germ cell is round in shape with a well marked cell boundary.
It contains a large round nucleolus with centrally-placed
nucleolus. A small quantity of clear cytoplasm surrounds
the nucleus in the form of a thin layer. The chromatin
granules are concentrated just under the nuclear membrane.
The cytoplasm stains light blue with *H*èhrl*ich's* haemotoxylin.
The nuclear membrane stains deep blue to high concentration
of chromatin granules, while the nucleus stain bright blue.
The maximum nuclear diameter is $18.2 \mu$.

In *Channa* also, the primary germ cells are largest in
size of all the stages of spermatogenesis. They are arranged
along the outer region of the seminiferous lobules (Figs. 97,
101, pp. 139, 141). They are spherical in shape with well-
marked cell boundary and contain a large nucleus with a
definite nuclear membrane. The cytoplasm stains light blue
Photomicrographs of the section of testis

Fig. 98 - Showing different stages of spermatogenesis in *Mystus vittatus* (Azan)

Fig. 99 - Showing different stages of spermatogenesis in *Channa punctatus* (Azan)

Fig. 100 - Showing seminiferous tubules containing primary germ cells and spermatogonia in *Mystus vittatus* (Haematoxylin - eosin)

Fig. 101 - Showing seminiferous tubules containing primary germ cells and primary and secondary spermatocytes in *Channa punctatus* (Azan)

Abbreviations:

- **P** - Peritonium
- **P.G.C.** - Primary germ cells
- **P.SPC.** - Primary spermatocytes
- **S.** - Spermatids
- **S.P.G.** - Spermatogium
- **S.SPC.** - Secondary spermatocytes
- **T.A.** - Tunica albuginea
with Ehrlich's haematoxylin while nucleoplasm stains deep blue due to the presence of the chromatin granules. The maximum nuclear diameter of the primary germ cell is 18.6 μ.

2. **Spermatoagonia**:

In *Mystus*, the spermatoagonia (Figs. 97, 98, pp. 139, 141) are formed by the mitotic division of the primary germ cells. They are smaller in size than the primary germ cells, and the cell outline is distinct. The spermatoagonia are more deeply stained with basic dyes. The nucleus is centrally placed. The spermatoagonia divide mitotically and give rise to the primary spermatocytes. The nuclear diameter of the spermatoagonium is 16.3 μ.

In *Channa*, the spermatoagonia are formed as in *Mystus* by the mitotic division of the primary germ cells. They are smaller in size. The cell boundary is inconspicuous, while the nuclear membrane is clearly visible and stained with basic dyes. The nucleus and a single nucleolus are noticed in the centre (Figs. 97, 101, pp. 139, 141). The spermatoagonia divide mitotically following a short growth phase and the daughter cells give rise to the primary spermatocytes. The nuclear diameter of the spermatoagonium is reduced to 16.7 μ.

3. **Primary spermatocytes**:

In *Mystus*, the primary spermatocytes are resulted by the mitotic division of spermatoagonia. The cell boundary is
Photomicrograph of the section of testis

Fig. 102 - Showing primary and secondary spermatocytes and spermatids in *Mystus vittatus* (Haematoxylin - eosin)

Fig. 103 - Showing primary germ cells, spermatogonia and primary spermatocytes in *Channa punctatus* (Azan)

Fig. 104 - Showing spermatozoa in *Mystus vittatus* (Azan)

Fig. 105 - Showing interstitial cells, spermatids and spermatozoa in *Channa punctatus* (Azan)

Abbreviations:

- P.G.C.  - Primary germ cells
- S.      - Spermatids
- S.P.    - Spermatogonia
- S.SPC   - Secondary spermatocytes
- T.A.    - Tunica albuginea
not recognised in the stage and hence this stage is only
recognised by its nucleus (Figs. 97, 102, pp. 139, 143).
The nuclei of primary spermatocytes are smaller than that of
spermatogonia. They are deeply stained and exhibit various
mitotic figures. After this stage all other stages of
spermatogenesis are recognised by the structure of their
nuclei. The chromatin network is now thickened. The nuclear
diameter of primary spermatocyte is 11.2 μ.

In Channa, also the primary spermatocytes are resulted
by the mitotic division of spermatogonia. The cell boundary
is conspicuous, the nuclear material gets clumped in the centre
of the cell (Figs. 97, 103, pp. 139, 143) and stained deeply
by the Ehrlich's haematoxylin. The nuclear diameter of primary
spermatocyte is reduced to 12.3 μ.

4. Secondary spermatocyte :

In Mystus after first maturation or reduction division
of primary spermatocytes, the secondary spermatocytes are
formed. The size and shape of the nucleus of secondary
spermatocyte is further reduced. These cells are very short-
lived and undergo mitosis forming spermatids (Figs. 97, 102,
pp. 139, 143). The nuclei contain a thick clump of chromatin
matter, the chromatin of secondary spermatocyte is deeply
basophilic and bears a mottled appearance. The nuclear
diameter of the secondary spermatocyte is reduced to 8.3 μ.
In Channa, the secondary spermatocytes are formed in the same manner as in Mystus (Fig. 97, p. 139). The nuclear diameter of the secondary spermatocyte is 8.3 μ.

5. The spermatids:

The division of secondary spermatocytes results in the formation of spermatids (Figs. 97, 102, 103, pp. 139, 143) in both the fishes. They show further reduction in size. The nuclei are spherical and stain more deeply. The nuclear diameter of the spermatids in Mystus and Channa is 4.4 μ and 4.3 μ respectively.

6. The spermatozoa:

A further contraction of the nuclear mass gives rise to spermatozoa from the spermatids. They are smallest in size (Figs. 97, 104, 105, pp. 139, 143) and are differentiated into a distinct head and tail. The head, representing the nucleus, shows great affinity for stain. The nuclear diameter of the spermatozoa in Mystus and Channa is 2.32 μ and 2.56 μ respectively.

3. Seasonal changes in the gonads:

The gonads of Mystus vittatus show well marked changes in their morphology (shape, size and volume), histology and cytology during different months of the
reproductive cycle. A quantitative study of the gonads has also been attempted in order to study their seasonal changes and to confirm the results from the studies of their morphological, histological and cytological aspects.

A. Morphological changes:

(1) Ovaries:

In *Mystus* the ovaries in October, November, December and January become smaller in size. They are pale yellow in colour with smooth texture. Their anterior end is broader than the posterior end. The ovaries are feebly vascularised. In February, March, April and May the ovaries show sudden changes in their size, shape and volume. They grow larger in size and occupy greater space comparatively in the body cavity. The ovaries of June, July, August and September attain a larger volume and occupy all the available space of the body cavity. The translucent peritoneal membrane is white in colour (the mature ova bulge out through the thin peritonal membrane and can be extruded out by making a slight pressure on its belly). The ovaries of these months are highly vascularised.

In *Channa* the ovaries in the month of October are small and shrunken in appearance. In November and December they are still smaller in size and brownish in colour with poor vascularisation. The ovaries, in January and February, are small in size and tubular in shape. They are poor in vascularisation. In March, April, May and June, the ovaries become larger and are sac-like in appearance. They are yellow in colour with a high
degree of vascularisation. The ova can be seen through a transparent peritoneal membrane. In July, the ovaries get slightly shrunken, become somewhat smaller in size with a poor blood supply. In August, the ovaries again become large and show beaded appearance on their surface. In September the ovaries gradually shrink in appearance.

(ii) Testes:

In Mystus, the testes in the months of October, November, December and January are gradually reduced to thread-like in appearance and creamy-white in colour with a small comb-edge. Vascularisation is poor during this period. During the months of February, March, April and May they tend to increase in size and become whitish in colour. A rich network of blood vessels is seen on the surface of the testes. In June, July, August and September the testes attain a larger size. The comb-edge becomes prominent. An increase in the blood supply is visible and they become dark yellowish brown in colour.

During the months of October, November and December, the testes of Channa are small in size and leaf-like in appearance. They are white in colour with poor vascularisation. The testes of January and February are small in size, oval in shape and white in colour. In March, April the testes grow in size but in May a sudden change is observed in their morphology and the testes attain a good size with prominent kidney-like
structure and high degree of vascularisation. They are pinkish in colour. In June, July, August and September the testes attain a large size reaching a maximum condition in August. Their shape and colour is like that of the month of May while their vascularisation increases.

B. Histological changes :

(i) Ovaries :

In Mystus, during the month of October the ovarian wall is thick, inter follicular space and connective tissue of stroma is prominent and ovigerous lamellae are clearly marked out which contain numerous oocytes of chromatin-nucleolus stage and peri-nucleolus stage along with a few oocytes of various other developing stages including mature ones (Fig. 106, p. 149). The corpora atretica and post-ovulatory follicles in their various stages of resorption, are observed very frequently in between different stages of developing oocytes. During November, December and January the ovarian wall is comparatively thin and shows a poor vascularisation. Ovaries are small and the ovigerous lamellae are indistinct having various stages along with a few early peri-nucleolus stages are seen. Very few corpora atretica and post-ovulatory follicles are seen during these months. In February and March the ovigerous lamellae are prominent. The oocytes develop further and a few oocytes of late
Photomicrographs of the section of the ovary of *Mystus vittatus* showing seasonal variations in different months

**Fig. 106** – Ovary of October fish showing different stages of developing oocytes with corpora atretica (*Haematoxylin - eosin*)

**Fig. 107** – Ovary of January fish showing ovigenous lamellae and early stages of oocytes (*Azan*)

**Fig. 108** – Ovary of June fish showing oocytes of tertiary yolk stage and prematuration stage (*Azan*)

**Fig. 109** – Ovary of August fish showing mature oocytes and post-ovulatory follicles (*Azan*)

**Abbreviations**:

- C.A. – Corpora atretica
- L.P.N. – Late perinucleolus stage
- M.O. – Mature ovum
- P.P. – Post-ovulatory follicles
- P.M.S. – Prematuration stage
- P.Y.S. – Primary yolk stage
- T.Y.S. – Tertiary yolk stage
peri-nucleolus stage are also seen along with the early stages of oocytes (Fig. 107, p. 149). The corpora atretica and post-ovulatory follicles are entirely absent. In April the ovarian wall is thin with numerous blood vessels in it. The ovigerous lamellae are not well marked. The oocytes of peri-nucleolus stage are abundant while a few oocytes of early yolk-vesicle stage are also seen. Corpora atretica and post-ovulatory follicle are absent. In May the inter-follicular space is reduced and the ovigerous lamellae become obliterated. The oocytes develop further and along with other earlier stages oocytes of late yolk-vesicle and primary and secondary yolk stage are also found. Corpora atretica and post-ovulatory follicles are not seen. In June the interfollicular space is further reduced. Further development of oocytes takes place and most of the oocytes are noticed in tertiary yolk stage or pre-maturation stage along with a few earlier stages (Fig. 108, p. 149). Few corpora atretica are present in different phases of resorption. In July, August and September sudden changes are observed in the histology of the ovary. The ovigerous lamellae get disorganised as the ovary becomes packed with the maturing stages of oocytes along with mature oocytes. A few early stages of oocytes are also present in between the maturing and mature oocytes. The corpora atretica and post-ovulatory follicles are numerous which are in various stages of their resorption (Fig. 109, p. 149).
In Channa during the months of October, November and December the ovarian wall is thick, the ovigerous lamellae are well formed and the interfollicular and interlamellar spaces are quite distinct. Along with a few mature oocytes, those of chromatin-nucleolus and peri-nucleolus stages are also present in good number. A good number of corpora atretica are present in October (Fig. 110, p. 152) while they are few in November and December (Fig. 111, p. 152). Again the post-ovulatory follicles in October are good in number, in November they are few while in December they are not observed. During the months of January and February the ovarian wall is thick with a few blood vessels in it, well defined ovigerous lamellae extend from the ovarian wall towards the lumen of the ovary (Fig. 112, p. 152). The chromatin-nucleolus and peri-nucleolus stages are noticed in a greater proportion. The interfollicular space, ovigerous lamellae and interlamellar spaces are prominent. Few corpora atretica are noticed but more in February. In March and April, sudden change is observed and the oocytes become larger in size. The ovarian wall becomes thick and highly vascularised. In March, along with earlier stages of oocytes, the early and late yolk-vesicle stages are abundantly present (Fig. 113, p. 152). During April the interfollicular spaces and interlamellar spaces are comparatively reduced than those of the earlier months. Oocytes of primary, secondary and tertiary yolk-stages are abundantly present along with comparatively few pre-maturation and mature
Photomicrographs of the section of ovary of *Channa punctatus* showing seasonal variations in different months

**Fig. 110** - Ovary of October fish showing early stages of oocytes alongwith corpora atretica (Azan)

**Fig. 111** - Ovary of December fish showing early stages of oocytes with few corpora atretica (Azan)

**Fig. 112** - Ovary of February fish showing prominent ovigenous lamellae with early stages of oocytes (Azan)

**Fig. 113** - Ovary of March fish showing yolk-vesicle stages alongwith earlier stages of oocytes (Azan)

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.A.</td>
<td>Corpora atretica</td>
</tr>
<tr>
<td>C.N.</td>
<td>Chromatin-nucleolus stages</td>
</tr>
<tr>
<td>O.L.</td>
<td>Ovigenous lamella</td>
</tr>
<tr>
<td>P.N.</td>
<td>Peri-nucleolus stage</td>
</tr>
<tr>
<td>Y.V.S.</td>
<td>Yolk vesicle stage</td>
</tr>
</tbody>
</table>
Photomicrograph of the section of ovary of *Channa punctatus* showing seasonal variations in different months

**Fig. 114** - Ovary of April fish showing different yolk stages (Azan)

**Fig. 115** - Ovary of June fish showing mature stages oocyte (Azan)

**Fig. 116** - Ovary of July fish showing corpora atretica and post-ovulatory follicles (Azan)

**Fig. 117** - Ovary of August fish showing post-ovulatory follicles and corpora atretica (Azan)

**Abbreviations:**

- C.A. - Corpora atretica
- E.Y.V. - Early yolk vesicle stage
- L.P.N. - Late sperinucleolus stage
- N. - Nucleus
- D.L. - Digerose lamelle
- P.F. - Post-ovulatory follicles
- P.Y.S. - Primary yolk stage
- Y.G. - Yolk granules
- Y.V. - Yolk vesicles
stages. Greater number of corpora atretica are also observed while the post-ovulatory follicles are completely absent (Fig. 114, p. 153). In May, the ovarian wall is thin and interfollicular spaces are obliterated due to further maturation of eggs. A good number of mature oocytes are present with a few other developing oocytes surrounding them. Maximum number of corpora atretica along with the post-ovulatory follicle are present during this month. In June, a good number of mature oocytes, oocytes of primary, secondary and tertiary yolk stage with other oocytes of earlier developing stages are found (Fig. 115, p. 153). The corpora atretica and post-ovulatory follicles are fewer in number than in May. In July and August mostly oocytes of mature stage are present along with some other developing stages of oocytes. Corpora atretica are present in good number as in June.

In July, post-ovulatory follicles are not observed (Fig. 116, p. 153) while in the month of August some post-ovulatory follicles are again seen (Fig. 117, p. 153). In the month of September the mature oocytes and some oocytes of other developing stages are seen. Interfollicular spaces are completely obliterated by the oocytes. The corpora atretica and post-ovulatory follicles are large in number in comparison with that of August.

(ii) Testes:

In Mystus, during October, November and December the
testicular wall is thin and the testis consists of well marked seminiferous tubules. The seminiferous tubules are surrounded by a thin layer of connective tissue, blood vessels and some interstitial cells. In October many seminiferous tubules possess a good number of primary and secondary spermatocytes and spermatids and a large percentage of spermatozoa while the earlier stages of spermatogenesis i.e. primary germ cells and spermatogonia are comparatively few (Fig. 118, p. 156). In November and December the tubules are mainly composed of spermatozoa primary germ cells and fewer spermatogonia. During these months, the primary germ cells are present in the periphery of the seminiferous tubules, while the spermatozoa are found in the centre of the seminiferous tubules (Fig. 119, p. 156). In January and February the testes show some advancement over those of previous months. Considerable histological changes are exhibited during these months. In January, with the mitotic division of the primary cells, a large number of spermatogonia are formed. The primary germ cells are situated at the periphery of the tubules while the spermatogonia are placed in the centre. In February the testes show differentiation of some primary spermatocytes. In the centre of the testes a few tubules are seen filled entirely with spermatozoa. The interstitial cells are numerous and lie in the thick connective tissue in between the tubules. The testes have a rich vascular supply. In March, the testes are large in calibre. Seminiferous tubules are separated by thick connective tissue. In most of
Photomicrographs of the section of testis of *Mystus vittatus* showing seasonal variations in different months

**Fig. 118** - Testis of October fish showing spermatogonia, spermatocytes, spermatids and spermatozoa (Haematoxylin - eosin)

**Fig. 119** - Testis of December fish showing primary germ cells, spermatogonia and a few spermatozoa (Azan)

**Fig. 120** - Testis of March fish showing various stages of spermatogenesis (Azan)

**Fig. 121** - Testis of May fish showing tubules filled with spermatozoa (Haematoxylin - eosin)

**Fig. 122** - Testis of July fish showing seminiferous tubules partially filled with spermatozoa (Azan)

**Fig. 123** - Testis of September fish showing empty seminiferous tubules (Azan)

**Abbreviations:**

- P.G.C. - Primary germ cells
- S. - Spermatids
- SP. - Spermatozoa
- SPG. - Spermatogonia
- S.SPC - Secondary spermatocytes
- T.A. - Tunica albuginea
the seminiferous tubules primary germ cells, spermatogonia, primary spermatocytes and secondary spermatocytes are found in such a way that the primary germ cells are located in the peripheral region and the spermatocytes in the central region (Fig. 120, p. 156). Besides these tubules, a few tubules are also present in the centre of the testis filled entirely with spermatozoa. During the months of April, May and June significant histological changes are noticed in the testes. All the stages of spermatogenesis are present. The intertubular connective tissue gets narrower due to a significant increase in the diameter of seminiferous tubules. The interstitial cells increase in their size. In April, the rate of spermatogenesis is slow, but it is accelerated in May and June as is evident by the presence of large number of spermatocytes, spermatids and spermatozoa in these months (Fig. 121, p. 156). During the months of July, August and September, the seminiferous tubules attain their maximum size. The connective tissue is very thin with a few interstitial cells and rich blood supply. During these months the spermatogenetic activity is lowered down as the tubules are filled with an overwhelming majority of spermatozoa as compared to other earlier stages (Fig. 122, p. 156). In all these months there is a gradual tendency for the increasing percentage of later stages and a decreasing percentage of earlier stages of spermatogenesis as one passes from July to September or even October. In September many tubules without
spermatozoa are also observed (Fig. 123, p. 156) which is suggestive of the peak period of spawning.

It may be concluded that the testes during the months of November, December, January and February begin to show rebuilding process marked by a rapid rate of spermatogenesis resulting in the formation of large number of spermatogonia and a comparatively lesser number of primary spermatocytes in February. In the months of March, April, May and June the spermatogenetic activity increases due to which all the later stages of spermatogenesis are present in an increasing number, (However in March the spermatids are not differentiated). During July, August and September the testes attain functional maturity and again get depleted after the month of October. It is interesting to note that the spermatozoa are maintained in the tubules throughout the year. However, their number declines up to April after which they again increase thus indicating the formation of a fresh crop of sperms.

In Channa, during the month of October the seminiferous tubules are large in calibre. The intertubular connective tissue, in which the interstitial cells are scattered, is highly vascularised. The seminiferous tubules are filled with all the spermatogenetic stages. In November and December, the testes show distinct histological changes (Fig. 124, p. 159). The seminiferous tubules are small in size. The connective tissue gets thickened while the interstitial cells are conspicuous. The
Photomicrographs of the section of testis of *Channa punctatus* showing seasonal variations in different months

**Fig. 124** - Testis of December fish showing seminiferous tubules containing primary germ cells, spermatogonia and primary spermatocytes (Hae'matoxylin - eosin)

**Fig. 125** - Testis of February fish showing seminiferous tubules containing secondary spermatocytes with other earlier stages of spermatogenesis (Azan)

**Fig. 126** - Testis of March fish showing spermatids and spermatocytes with other earlier stages of spermatogenesis (Azan)

**Fig. 127** - Testis of June fish showing seminiferous tubules partially filled with spermatozoa (Azan)

**Fig. 128** - Testis of August fish showing a number of seminiferous tubules filled with spermatozoa (Azan)

**Fig. 129** - Testis of September fish showing empty seminiferous tubules (Azan)

**Abbreviations:**

- **E.L.** - Empty lobules
- **I.C.** - Interstitial cells
- **P.G.C.** - Primary germ cells
- **S.** - Spermatids
- **SP.** - Spermatozoa
- **S.SPC.** - Secondary spermatocytes
- **S.PG.** - Spermatogonia
- **T.A.** - Tunica albuginea
Seminiferous tubules are filled with a large number of primary germ cells, spermatogonia, primary and secondary spermatocytes, spermatids, and a comparatively lesser number of spermatozoa than in October. During the months of January and February, the seminiferous tubules are small in diameter and connective tissue in between them is thick with conspicuous interstitial cells. The seminiferous tubules are packed with different spermatogenetic stages i.e., the primary germ cells are peripheral in position while the spermatogonia and spermatocytes are directed towards the centre of the tubules (Fig. 125, p. 159). In a few tubules even the spermatids are also seen. During the months of March and April the tubules are larger as compared to those of January and February. Thick connective tissue containing interstitial cells is present in between the tubules which has a rich vascular supply. A fast rate of spermatogenesis takes place during this period as a result of which a large number of primary and secondary spermatocytes, spermatids, and spermatozoa are found along with early stages of spermatogenesis (Fig. 126, p. 159). In May, the seminiferous tubules are much larger in width. The testicular wall is thin and the thin connective tissue, situated in between the tubules, contains prominent interstitial cells and is highly vascularised. The tubules consist of all the spermatogenetic stages. They are filled with fewer primary germ cells and spermatogonia, large number of primary and secondary spermatocytes, and a very high percentage of spermatids and centrally placed spermatozoa.
During the months of June and July the testes show marked changes. The tubules exhibit rapid spermatogenesis. Most of the tubules are filled with all the spermatogenetic stages while spent tubules are also present (Fig. 127, p. 159). The primary germ cells and spermatogonia, which are peripheral in position in the tubules, are smaller in number than the primary and secondary spermatocytes which show a high percentage. During this period there is a low percentage of spermatozoa as they are found only in certain tubules.

During the months of August and September the testes show distinct histological changes. The intertubular connective tissue is very rich in blood vessels and the interstitial cells are scattered in them. In August, the testes indicate a rapid spermatogenesis resulting in the formation of a large number of later stages particularly spermatozoa (Fig. 128, p. 159). The testes exhibit functional maturity as this is the peak period in the formation of spermatozoa. In September, the seminiferous tubules are filled with all the spermatogenetic stages greater in number except spermatozoa in comparison with August condition. Few empty tubules are also present in this month (Fig. 129, p. 159).

It may be concluded that the testes from mid-October/November are in their rebuilding process. From March to mid-April, the testes become mature. From mid-April to September or mid-October, the testes remain in an active state with
varying number of stages of spermatogenesis.

Based on the above mentioned morphological and histological changes in the ovary and testis during different periods of the year and considering the date of collection, the reproductive cycle of *Mystus vittatus* and *Channa punctatus* has been divided into the following periods:

*Mystus vittatus*:

- Post-spawning period: Mid-October to January.
- Pre-spawning period: February to May.
- Spawning period: June to mid-October.

*Channa punctatus*:

- Post-spawning period: November to February.
- Pre-spawning period: March to mid-April.
- I Spawning period: Mid-April to May.
- Preparatory period: June to mid-July.
- II Spawning period: Mid-July to October.

C. Quantitative study of the reproductive cycle:

In *Mystus vittatus* and *Channa punctatus*, the quantitative study of the reproductive cycle has been made in respect of gonadal volume and gonosomatic index during the different periods of reproductive cycle. Though, these calculations regarding the volume and weight of the gonads...
are approximate, but they are still helpful in understanding the seasonal changes in the gonads. The reproductive cycle has also been statistically assessed on the basis of different cell types of spermatogenesis in the testes and the relative number of the oocytes of different diameter and relative number of corpora atretica and post-ovulatory follicles in the ovary during different periods of the year.

(1) The gonad volume and gono-somatic index:

The volume and weight of both male and female gonads and the weight of the fish were noted every month throughout the annual cycle (monthly collection of Mystus vittatus was done in the middle of each month while that of Channa punctatus was done in the first week of each month). The weight of the fish and gonad (fixed material) was taken after blotting away extra fixative. The gonad volume was measured by liquid displacement method (Bullough, 1939). The volume of the liquid (water) displaced by gonads (testes or ovaries) of approximately thirty fish was measured separately and the average volume of testes and ovary was deduced separately for each month for both the sexes of both the fishes.

In Mystus and Channa, the gono-somatic index has been calculated in male and female separately by the following formula.

\[
\text{Gono-somatic index} = \frac{\text{Weight of the gonad}}{\text{Total weight of the fish}} \times 100
\]
<table>
<thead>
<tr>
<th>Months</th>
<th>Date of Collection</th>
<th>Water Temperature in °C</th>
<th>No. of Male Fish Collected</th>
<th>Range of Length of Male Fish in cm</th>
<th>Average Weight of Male Fish in gms</th>
<th>Average Volume of Testes in ml</th>
<th>Average Weight of Testes in gms</th>
<th>Average Gonosomal Index</th>
<th>No. of Female Fish Collected</th>
<th>Range of Length of Female Fish in cm</th>
<th>Average Weight of Female Fish in gms</th>
<th>Average Volume of Ovaries in ml</th>
<th>Average Gonosomal Index</th>
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<td>22.0</td>
<td>14</td>
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<td>0.23</td>
<td>0.126</td>
<td>0.35</td>
<td>15</td>
<td>15.0-17.0</td>
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</tr>
<tr>
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<td>16</td>
<td>6.8-9.6</td>
<td>4.57</td>
<td>0.13</td>
<td>0.044</td>
<td>0.96</td>
<td>19</td>
<td>7.1-9.9</td>
<td>5.41</td>
<td>0.20</td>
<td>0.141</td>
</tr>
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<td>7.5-9.7</td>
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<td>0.017</td>
<td>0.35</td>
<td>19</td>
<td>8.0-10.3</td>
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<td>0.050</td>
</tr>
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<td>9.0-12.5</td>
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<td>0.45</td>
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<td>3.5-11.5</td>
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<td>0.035</td>
<td>0.53</td>
<td>19</td>
<td>9.0-10.4</td>
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<td>0.23</td>
<td>0.095</td>
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<td>3.5-11.0</td>
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</tr>
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<td>9.2-16.5</td>
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<td>12.1-17.7</td>
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<td>17.0-19.0</td>
<td>33.30</td>
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</table>

**Explanation**: Table showing the range of body length and average weight of the fish, average weight and average volume of gonads and the gonosomal index of *Systius vittatus* along with the water temperature and dates of collection during different months of a year.
### Table 4

<table>
<thead>
<tr>
<th>Months</th>
<th>Date of collection</th>
<th>Water temperature in °C</th>
<th>No. of male fish collected</th>
<th>Range of length of male fish in cm</th>
<th>Average weight of male fish in g</th>
<th>Average volume of testes in ml</th>
<th>Average gonoosomatic index</th>
<th>No. of female fish collected</th>
<th>Range of length of female fish in cm</th>
<th>Average weight of female fish in g</th>
<th>Average volume of ovaries in ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>October</td>
<td>3.10.68</td>
<td>22.0</td>
<td>15</td>
<td>11.0–13.6</td>
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<td>0.45</td>
<td>0.105</td>
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<td>10.3–15.8</td>
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<tr>
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<td>2.11.68</td>
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<td>13.0–22.6</td>
<td>33.34</td>
<td>0.29</td>
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<td>13.0–16.7</td>
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<tr>
<td>December</td>
<td>3.12.68</td>
<td>21.0</td>
<td>15</td>
<td>12.5–20.5</td>
<td>27.32</td>
<td>0.11</td>
<td>0.045</td>
<td>0.16</td>
<td>15</td>
<td>12.6–19.4</td>
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<td>2.1.69</td>
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<td>10.0–15.4</td>
<td>23.61</td>
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<td>11.2–18.9</td>
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<td>April</td>
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<td>15</td>
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</tr>
<tr>
<td>May</td>
<td>2.5.69</td>
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<td>June</td>
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<td>July</td>
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<td>0.141</td>
<td>0.48</td>
<td>15</td>
<td>12.2–16.2</td>
<td>32.63</td>
</tr>
</tbody>
</table>

**Explanation:** Table showing the range of body length and average weight of the fish, average weight and average volume of gonads and the gonoosomatic index of *Chaunax punctatus* along with the water temperature and dates of collection during different months of a year.
The average volume was deduced for each month throughout the reproductive cycle. The average volume of the gonads, the average gonosomatic index and the water temperature have been recorded throughout the year on a monthly basis and are shown in the Tables 3, 4, pp. 164, 165; Figs. 130, 131, 132, 133, pp. 167, 169.

(a) The changes in the ovary volume and gonosomatic index of the female fish:

In Myxus, during the post-spawning period (mid-October to January), the volume and gonosomatic index indicate a declining trend up to December which then show a gradual improvement from December to January (Fig. 130, p. 167). This is justified in view of the fact that the ovaries shrink after spawning and then undergo rebuilding process. During pre-spawning period (February to May) the volume and the gonosomatic index of the ovaries for these months are in an ascending order which in the month of May show a sudden increase in the two values (Table 3, p. 164). During the spawning period (June to mid-October) the volume of the ovaries increases until September when it reaches the maximum value (3.40 ml). However, the gonosomatic index value is found maximum in July being the lowest in September during this period. This difference may be accounted for the fish sample of September ranging between 17.0 to 19.0 cm.
Fig. 130 - Curves showing the volume and gono-somatic index of ovaries of *Mystus vittatus* in relation to water temperature during different months of the year.

Fig. 131 - Curves showing the volume and gono-somatic index of ovaries of *Channa punctatus* in relation to water temperature during different months of the year.
In *Channa*, during post-spawning period (November to February) the volume and the gono-somatic index show a declining trend in their values until December/January after which there is some improvement in the values in February. This indicates shrinking in the ovaries after spawning followed by their rebuilding process starting in February (which is really January in view of the date of collection) (Table 4, p. 165). In the pre-spawning period (March to mid-April) the ovary volume and the gono-somatic index indicate a gradual increasing trend thus pointing a corresponding increase in the metabolic activity of the fish during this period. In the first spawning period (mid-April to May) the volume of the ovaries and the gono-somatic index values increase further until May after which in the preparatory period (June to mid-July) there is a slight fall in the values (Fig. 131, p. 167). During this period the fish prepares for the second spawning period (mid-July to October) which lasts for a longer period. During the latter period the values again increase attaining the highest values in August/September when maximum spawning takes place, though extending up to October. In November the values show a sudden decline and the fish enters the post-spawning period.

(b) The changes in the testis volume and gono-somatic index of the male fish:

In *Mystus*, during the post-spawning period (mid-October to January) the values of the testis volume and gono-
Fig. 132 - Curves showing the volume and gonad-somatic
index of testes of *Mystus vittatus* in
relation to water temperature during
different months of the year

Fig. 133 - Curves showing the volume and gonad-somatic
index of testes of *Channa punctatus* in
relation to water temperature during
different months of the year
somatic index of the fish show a gradual declining trend (Table 3, p. 164) which justifies the gradual shrinkage in the gonad during this period. Thus a low metabolic activity of the fish during this period is also indicated. During the pre-spawning period (February to May) the volume as well as the gono-somatic index are in an increasing order which shows an active state of gonad and general metabolic activity of the fish during this period. In the spawning period (June to mid-October) the average testis volume and the gono-somatic index values are high up to September which shows that the rhythm of spawning is maximum from July to September (Fig. 132, p. 169). From October (i.e., mid-October) these values tend to decline in the post-spawning period.

In Channa, during the post-spawning period (November to February) the values of the testis volume and the gono-somatic index in the male fish are strikingly less in November as compared to the earlier period after which there is a slow increase in the values (Table 4, p. 165). This is indicative of the fact that during this period the general metabolic activity of the fish and the gonad in particular, after the spawning, is at a low ebb. In the pre-spawning period (March to mid-April) the values for both the volume and gono-somatic index are in an increasing order (Table 4, p. 165). This shows that the testes are now preparing to spawn. During first spawning period (mid-April to May), the values of the testis volume and gono-somatic index respectively are quite
high justifying the spawning process (Fig. 133, p. 169). During the preparatory period (June to mid-July) the values for testis volume and gono-somatic index are almost static and a little lower than those of May. The data thus indicates the maturation of the gonad during this period and the preparation of the fish for second spawning process (Table 4, p. 165). In the second spawning period (mid-July to October) the gonad volume and gono-somatic index values are again very high, reaching highest figure in the month of August followed by September (Fig. 133, p. 169). In October the values decline though spawning continues during this period. The values for gonad volume and the gono-somatic index of the fish and water temperature has also been found to show a close relationship between them (Fig. 133, p. 169).

(ii) Quantitative study of the relative number of the oocytes of different diameter.

In order to study the reproductive cycle more precisely, the quantitative assessment in respect of relative number of oocytes of different diameter in the ovaries of Mystus vittatus and Channa punctatus during different months of a year has been made. For this study the ovaries (insections) of eight specimens were taken into account in every month. The oocytes were measured across their maximum diameter with the help of an ocular micrometer. In this way a large number of oocytes (approximately 4000 in number) were measured every month in this study. The
<table>
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<tr>
<th></th>
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<th>January</th>
<th>February</th>
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Explanation: Table showing the relative number of eggs of different diameters in the ovaries of *Hyasus vitatus* in different months of the year.
Fig. 134 - Graphs showing the relative number of eggs of different diameters in the ovaries of *Mystus vittatus* for different months of the year.
histograms were plotted for the various months with the
diameter of the oocyte taken as abscissa and the relative
number of the oocyte as the ordinate. The results are
summarised in the Tables 5, 6, pp. 172, 173 and shown in
Figs. 134, 135, p. 173, 176.

In Mystus, during the post-spawning period (mid-
October to January) the oocytes of small diameter are larger
in number. With further development and growth the oocytes
tend to increase in diameter. By the end of the post-spawning
period i.e., in the month of January, the oocytes grow to a
diameter up to 174 μ.

During the pre-spawning period, the oocytes increase
further in their diameter but a good number of small oocytes
are also present. In the months of April and May, however,
a marked reduction in the small oocytes is observed. The
maximum diameter of the oocytes at the end of this period,
i.e., in the month of May reaches to 605 μ.

With the start of the spawning period (June to mid-
October), a further increase in the diameter of the oocytes
is observed. In the months of July, August and September the
oocytes grow further in diameter. However, the early stages
of oocytes are also present during this period. In October
the number of small oocytes tends to increase (Table 5,
p. 172). This indicates the development of new crop of the
oocytes with a corresponding reduction in the number of
Fig. 135 - Graphs showing the relative number of eggs of different diameters in the ovaries of Channa punctatus for different months of the year
<table>
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<th>February</th>
<th>March</th>
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**Explanation**: Table showing the relative number of eggs of different diameters in the ovaries of Chauna punctatus in different months of the year.

**Note**: In view of the date of collection of Chauna punctatus, the values for the month indicated in the table would almost hold true for the proceeding month as this point has been kept under consideration in dividing the reproductive cycle into different periods.
oocytes of larger diameter. Thus, on the basis of oocyte diameter, the highest rhythm of spawning takes place from July to September with September being the peak month of spawning period.

In Channa, during the post-spawning period (November to February) the oocytes are mostly of small size in diameter with the exception of early post-spawning period i.e., November when, in addition to early stages, other larger stages of oocyte are also present. These larger stages in November may represent those oocytes which are left over after spawning.

The oocytes further increase in their diameter in the pre-spawning period (March to mid-April) when a good number of oocytes of smaller diameter are also present along with some oocytes of larger diameter particularly in the month of April (Table 6, p. 175). In April, the maximum diameter reaches up to 573 µ.

In the first spawning period (mid-April to May) the oocytes increase further in diameter. In the month of May the maximum diameter of oocytes is attained i.e., 850 µ. The oocytes representing the early stages are also present in good number.

During the preparatory period (June to mid-July) the number of oocytes of larger diameter show a slight fall in June but in July they again start increasing thus preparing
for the second spawning. However, the ovaries rebuild themselves during the preparatory period and fresh crop of oocytes of small diameter appear (Table 6, p. 175).

During the second spawning period (mid-July to October) the oocytes of larger diameter are again frequently present. The maximum diameter of oocytes in August, September and October reaches up to 830 μ, indicating the peak period of second spawning from mid-July to September.

Thus on the basis of diameter of oocytes there is every indication of maximum spawning taking place during September followed by August.

(iii) Quantitative study in respect of the corpora atretica and post-ovulatory follicles during the reproductive cycle:

The corpora atretica and post-ovulatory follicles were also studied quantitatively in order to assess the spawning periodicity of the female fishes more precisely. For this study sections of ovaries of eight fish were taken into account in every month. The corpora atretica and post-ovulatory follicles were counted from a random selection of twenty to twenty-five sections representing various areas of the ovaries of monthly sample. The results obtained are given in the Table 7, p. 179). The quantitative study indicates that a good number of corpora atretica are present during the spawning
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</tr>
<tr>
<td>September</td>
<td>35</td>
<td>74</td>
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</tbody>
</table>

Explanation: Table showing the number of Corpora atretica and Post-ovulatory follicles counted from 2+ sections from the ovaries of *Nystus vittatus* and *Channa punctatus* in different months of the year.

Note: In view of the date of collection of *Channa punctatus*, the values for the month indicated in the table would almost hold time for the preceding month and this point has been kept under consideration in dividing the reproductive cycle into different periods.
period, and just a few in the post-spawning period in *Mystus*. On the other hand they are present throughout the year in *Channa*. Thus the occurrence of corpora atretica can be regarded to be related with the spawning periodicity of the fish as they are present abundantly throughout the spawning period. However, the number and the presence of corpora atretica is not good enough for a precise determination of the spawning periodicity of the fish.

On the other hand the presence and variation in the number of the post-ovulatory follicles in the ovaries provides a more reliable evidence in determining the correct spawning periodicity of the fish which is based on the fact that the post-ovulatory follicles would be present in the ovary soon after the spawning has really begun. In the present work the samples of the fish, *Mystus vittatus* were collected in the middle of each month while the samples of *Channa punctatus* were collected in the first week of each month. Thus the appearance of the post-ovulatory follicles in the ovaries of a particular monthly sample of *Mystus* would mean that the spawning had taken place earlier in the same month or in the preceding month while the appearance of post-ovulatory follicle in *Channa* in the ovaries of a particular month would mean that the spawning had taken place in the preceding month more likely than the same month. The appearance of the post-ovulatory follicles in different months would thus indicate the duration of the spawning period. The post-ovulatory follicles are generally
absorbed within a month after their appearance and as such this factor has to be borne in mind while dividing the reproductive cycle into different periods and in assessing the spawning periodicity.

In *Mystus vittatus*, the post-ovulatory follicles appear in July indicating that the spawning had started earlier in the preceding month. Their subsequent presence in August, September and October shows the duration of spawning period up to middle of October (the time of sample collection) and presence of a few ruptured follicles even in November. The number of the post-ovulatory follicles in different months reveals that the rhythm of spawning is highest in the month of September followed by August, July, June and mid-October (Table 7, p. 179).

In *Channa punctatus*, the post-ovulatory follicles are present in May and June (time of collection being the first week) and then in August, September and October (Table 7, p. 179) which indicate that the fish breeds twice in a year from mid-April to May and from mid-July to October with a short preparatory period between the two spawning periods.

(iv) **Quantitative study of different spermatogenetic stages in testes**

The quantitative study in the testes of *Mystus vittatus* and *Channa punctatus* is based on a similar study made in *Phoxinus phoxinus* (Bullock, 1939). About eight male specimens were taken into account as a monthly sample to
count the relative number of different spermatogonetic cell types in the testes in both the fishes during the reproductive cycle. A whipple oculometer was used which is divided into a number of squares for counting the number of different cell types present in the testes. Different developmental stages of spermatogenesis totalling approximately 10,000 in number were counted from the seminiferous tubules selected at random (from the sections of testes) from each monthly sample. At a time only those cells were counted from the section which came within the limits of the whipple oculometer. The area of testes (in section) employed for the purpose of counting was kept constant throughout the reproductive cycle. The results are summarised in Tables 8,9, pp. 183, 187 and Figs. 136, 137, pp. 184, 188.

In Mystus, during the post-spawning period (mid-October to January) a large number of primary germ cells, spermatogonia and spermatocytes (presumably the left over the preceding reproductive cycle) are present from November to January. However, in October the earlier stages are very few comparatively and this indicates the continuation of spawning period up to mid-October.

In the pre-spawning period (February to May) all the stages of spermatogenesis are present in April and May with greater percentage of later stages and corresponding reduction of earlier stages of spermatogenesis. On the other hand in February and March there is relatively greater number of
<table>
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<tr>
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<td>280</td>
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<td>342</td>
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<td>4.84%</td>
<td>3.70%</td>
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<td>52.14%</td>
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</tbody>
</table>

Explanations: Table showing the relative number and percentage of the different cell types in the testes of Mystus vitatusin different months of the year.
Fig. 136 - Graphs showing the relative number of different stages of spermatogenesis in the testes of *Mystus vittatus* for different months of the year.
earlier stages and lesser number of later stages i.e. in February the secondary spermatocytes and spermatids and in March the spermatids are absent. However, in both these months and in April the spermatozoa are present in large number though in a descending order. From May the percentage of spermatozoa increases again and this indicates that new crop of spermatozoa is formed from May onwards while in the earlier months the spermatozoa represent the left over of the earlier spermatogenesis (Table 8, p. 183).

During the spawning period (June to mid-October) all the stages of spermatogenesis are present (Fig. 136, p. 184). While the primary germ cells, spermatogonia, primary and secondary spermatocytes decrease gradually in their number, there is a gradual increase in the number of spermatids and spermatozoa (Table 8, p. 183). The data shows that during this period the spermatogenesis is very active leading to the formation of the spermatozoa which are constantly released throughout this period. The spermatozoa are found even in the post-spawning period as left over of the preceding reproductive cycle and as a matter of fact in Mystus the spermatozoa are thus continued throughout the reproductive cycle (Table 8, p. 183).

In Channa, all the stages of spermatogenesis are present throughout the year. During post-spawning period (November to February) the primary germ cells increase from October and their number reaches the maximum figure in the month of December.
(Fig. 137, p. 188). Other stages of spermatogenesis are also present in large numbers except the spermatozoa, the leftovers, which dwindle in number by the end of this period (Table 9, p. 187).

In the pre-spawning period (March to mid-April) the gonads mature and become ready for the spawning period. This is clear from the percentage of spermatozoa in the month of May (Table 9, p. 187).

During first spawning period (mid-April to May) the primary germ cells and spermatagonia gradually decrease in their number while spermatocytes and spermatids increase in number which shows that the process of spermatogenesis is enhanced. The spermatozoa are 40.70% in the May (this figure really speaks of April) and this percentage is continued as 15.92% in June which indicates elaboration and release of sperms during this period.

During the preparatory period (June to mid-July) the spermatids are maximum (June 38.58%, July 40.95%) compared to other stages of spermatogenesis. This indicates the preparation of the testes during this period for the subsequent II spawning period.

Again in the second spawning period (mid-July to October) the spermatogenesis seems to be very active because leaving aside spermatids and spermatozoa other stages are present in a high percentage (Table 9, p. 187). The process
<table>
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<tr>
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<td>1610</td>
<td>812</td>
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<td>1937</td>
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<td>15.82%</td>
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<td>1.11%</td>
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<td>1.90%</td>
<td>2.01%</td>
<td>40.70%</td>
<td>15.92%</td>
<td>9.11%</td>
<td>42.80%</td>
<td>17.48%</td>
</tr>
</tbody>
</table>

**Explanation**: Table showing the relative number and percentage of the different cell types in the testes of *Channa punctatus* in different months of the year.

**Note**: In view of the date of collection of *Channa punctatus*, the values for the month indicated in the table would almost hold time for the preceding month and this point has been kept under consideration in dividing the reproductive cycle into different periods.
Fig. 137 - Graphs showing the relative number of different stages of spermatogenesis in the testes of Channa punctatus for different months of the year.
of release of spermatozoa in the month of August and September seems to be more in comparison to their release in July and October.

Discussion:

The reproductive organs in *Mystus vittatus* and *Channa punctatus* exhibit typical teleostean disposition with a set of related paired organs. Sexual dimorphism, as shown in *Clarias batrachus* (Mookerjee and Majumdar, 1950; Malaviya, 1972), *Wallago attu* (Sinha, 1961), *Mystus tengara* (Rastogi, 1966), *Glossogobius giuris* (Saksena, 1971) is also seen in *Mystus vittatus* (Bais, 1972). In the male fish a prominent urogenital papilla is present while in female there is a distinct spheroidal swelling near the urinary aperture. The urinogenital papilla (the so-called pseudocopulatory papilla) is more prominent, during the breeding season. In *Channa* no such structure is noticed.

Considerable literature exists regarding the morphology of the ovary. Earlier workers like Brock (1878) and Calderwood (1892), confined their studies to the general structure of the ovary. A detailed study of the histology of the ovary has been made in teleost such as in *Cottus bairdi* (Hann, 1927), in *Gasterosteus aculeatus* (Craig-Bennett, 1931 and Swarup, 1958), in *Mallotus merluccius* (Hickling, 1935), in *Fundulus heteroclitus* (Matthews, 1938), in *Cymogaster*

The origin of the new crop of the oocytes has been the subject of different opinions and the observations in this direction show great variations. In the opinion of Wheeler (1924), Yamamoto (1956) and Andrew and Pinto (1957), the origin of the new crop of germ cell has been observed from the follicle cells of the empty follicle left behind after the release of the mature oocytes. Stuhlmann (1887), Cunningham (1898), Wallace (1903), Franz (1909), Mendoza (1943), Tromp-Bolm (1959), Bara (1960), Khanna and Pant (1967), Sinha and Rastogi (1967), Raizada (1971), and Saksena (1974 and 1976) have observed that the new crop of oocytes are formed as a result of proliferation of germinal epithelium. Hann (1927),
Craig-Bennett (1931), Eggert (1931), Stenger (1959), Homma (1961), Belsare (1962) and Lehri (1968) are of the view that the origin of new crop of the oocytes takes place from the pre-existing oogonia. Bullough (1939) indicated that the oogonia germinating directly from the germinal epithelium are transformed into the oocytes following a short period of rest. He further pointed out that shortly after spawning new oogonia are also formed from the cells of discharged follicles in which mitotic division are commonly observe. Bhargava (1966) in Phoxinus phoxinus has confirmed the view that the oogonia are developed from the post-ovulatory follicle. Unlike that of some species of Gobiformes (Eggert, 1931), in Rasbora daniconius (Rezada, 1972), in Clarias batrachus and Masta cembelus punghis (Malaviya, 1972), in Glossogobius giuris (Saksena, 1974, 1976 a), in Clarias batrachus and Garra gotyla gotyla (Anant Prakash, 1976) and in Mystus vittatus and Channa punctatus also the early chromatin-nucleolus stages seem to be derived from the germinal epithelium as they are always found associated with the germinal layer.

Regarding the role of the nucleoli, its origin and extrusion, Eggert (1931), Stromsten (1931) and Chaudhry (1951) are of the opinion that a single nucleolus of the oogonium divides by fragmentation and division into a number of nucleoli but the fragmentation has not been seen by Yamamoto.
(1936) and Bara (1960) and others. Bara (1960) has pointed out that the peripheral nucleoli are fused bodies emerging from the peripheral cytoplasm. Arndt (1960) has not observed budding (division) during the formation of a nucleoli. A syneezisis stage has been described in *Phoxinus laevis* (Bullough, 1939) when the chromatin-material is accumulated at one side of the nucleus during the formation of the primary oocytes. No syneezisis stage has been observed in the present study. Many workers have observed the nuclear extrusion but their role during the oogenesis is obscure. Malhotra (1963) has observed its role in vitellogenesis but Eggert (1931), Narain (1937) and Chaudhry (1932) held a different view. However, Yamamoto (1956) and Bara (1960) have assigned them to be playing an active role in cell metabolism. In *Mystus vittatus* and *Channa punctatus*, the first visible stage is early chromatin-nucleolus stage in which the nucleus consists of deeply-stained single nucleolus embedded on the mesh-work of chromatin reticulum. In the early peri-nucleolus stage one or two large true nucleoli are present which give rise to many nucleoli arranged themselves below the nuclear membrane. The oocyte attains maturity often passing through different oogenetic stages and the size of nucleoli decreases as the oocyte approaches maturity. The extrusion of nucleoli has not been observed at any stage of oogenesis in the present study.

Many workers have described a mass of cytoplasmic body in the vicinity of the nucleus in the oogenetic stage of various
groups of animals and terms like yolk-nucleus, centrosphere, archoplasm, corp vitelline and balbaini body have been used to designate it. The yolk-nucleus was first described by Hubbard (1894) in the teleost Cymatogaster as a crescentic mass capping the nucleus, which later on migrates towards the peripheral zone of the ovum. Similar structures have been reported by Cunningham (1898), Franz (1909), Wheeler (1924), Narain (1937 and 1951), Mendoza (1943), Chaudhry (1952), Yamamoto (1956), Sathyanesan (1959), Bara (1960) and Yamamoto and Yamazaki (1961). Recently a comparative study of the yolk-nucleus in eight different fishes has been made by Nayyar in (1964) who inferred that the yolk-nucleus appears as a mass of lipid granules which later on migrates towards the periphery of oocyte. In Anabas scandens (Gopal Dutt, 1964), three crescentic zones of the yolk-nucleus have been recognised with probable role in vitellogenesis. Similar crescentic zones have also been found in the yolk-nucleus of Clarias batrachus by Malaviya (1973). Hubbard (1894), Wheeler (1924), Subramaniam and Gopal Dutt (1964) believed the yolk-nucleus to be designated as the organelle of nuclear region which passes through the nuclear membrane into the cytoplasm. Narain (1937 and 1951), Chaudhry (1952), Nayyar (1964) and Lehri (1968) have observed its cytoplasmic origin and have recognised a relationship between the yolk-nucleus, Golgi and mitochondrial elements. Bara (1960) has also observed an association between
the yolk-nucleus and mitochondria. Bhargava and Saksena (1971), Malviya (1973), Bais (1973) and Anant Prakash (1975) have also reported the presence of yolk-nucleus in their study. In Mystus, the yolk-nucleus of Balbaini appears 'do novo' in the cytoplasm juxta-nuclear in position as a vesicle with granules in it very close to nuclear membrane. In Channa, the yolk-nucleus of Balbaini appears for the first time in the early peri-nucleolus stage. In Mystus it persists in the early yolk-vesicle stage without showing any remarkable change in its form, structure and position while in Channa it disappears at the late peri-nucleolus stage or early yolk-vesicle stage.

Various views regarding the functional significance of the yolk-nucleus have been put forth without a final answer so far. According to Wallace (1904) the yolk-nucleus is concerned in forming yolk in the oocyte but Wheeler (1924) pointed out that yolk-nucleus has nothing to do with yolk formation. Its role in the regulation of growth, distribution and disposal of all the important cellular inclusions has been pointed out by Chaudhry (1952). Nayyar (1964) is of the view that the yolk-nucleus initiates the synthesis of lipids. Lehri (1968) pointed out that migration of yolk nucleus towards the periphery is associated with the process of yolk formation. However, Bhargava and Saksena (1971), Malaviya (1973) and Anant Prakash (1976) found no visible role in vitellogenesis but assigned some indirect role in the
vitellogenesis. Bais (1973), in Mystus and Channa also pointed out its indirect significance in vitellogenesis.

The process of formation of fish yolk is generally similar in all teleosts (Marza et al., 1937; Mattchewa, 1938; James, 1946; Malone and Hisaka, 1963 and Lal, 1964). The formation of fish egg with reference to vitellogenesis and histochemistry of yolk granules has been studied by Yamamoto (1956, 1957 and 1958). Chopra (1958), in Ophiocephalus punctatus, has described the intravacuolar and extravacuolar yolk granules containing proteins and carbohydrates and proteins and lipoproteins respectively. In Mystus and Channa the yolk formation starts at the primary yolk stage of oocyte as a small granule of yolk inside each yolk vesicle. The small yolk granules are at first lightly basophilic in nature. Later on they fuse to form yolk globules which are strongly acidophilic. The yolk granules or globules are of different shapes and sizes and show differential staining property to dyes at various stages of their formation.

A controversy exists on account of different names used for the egg membrane. This led to the use of different nomenclature for intra-ovarian and ripe (Scharff, 1887). In order to avoid unnecessary confusion in the present investigation, the nomenclature has been used after Bretschneider and Duyvende de Wit (1947). They described the
ovarian follicle to be surrounded by an outer theca layer and an inner granulosa layer. According to them these two layers are difficult to recognise and the wall of the follicle often seems to be made up of a single investment. In Mystus vittatus and Channa punctatus the oocytes are covered with an outmost single-called theca layer, of Breitschneider and Duyvene de Wit (1947), a follicular layer below which lies an innermost vitelline membrane like the condition present in Rasbora daniconius (Raizada, 1971) and Glossogobius giuris (Saksena, 1974, 1976a).

The corpora atretica or atretic follicles in fishes have long been referred as corpora lutea, though they are not comparable physiologically with these structures of the higher vertebrates. Hoar (1955, 1957 and 1969) has described that these structures in fish ovary have been termed as corpora lutea because they develop from the theca and granulosa cells as in higher vertebrates. However, he has given the two terms, pre - and post-ovulatory corpora-lutea, the former developing from the oolysis of immature oocytes and mature oocytes which fail to spawn and the latter resulting from the follicles after the discharge of the ripe ova. However, Dixit (1956), Ball (1960 and 1963), Bhargava (1963), Rajalakshmi (1966), Rastogi (1966b and 1967), Lehri (1968), Kaur (1968), Raizada (1969 and 1971), Malaviya (1972), Bais (1973), Saksena (1974, 1976a) and Anant Prakash (1976) have given
the terms corpora atretica or atretic follicles and post-
ovulatory follicles or ruptured follicles for pre- and post-
ovulatory corpora lutea respectively.

About the formation of corpora atretica, Cunningham
(1898) described that the cells forming the connective tissue
of the follicles proliferate and invade the contents of the
oocyte to effect its resorption. Jaski (1939), however, has
another opinion regarding their formation. He states that a
suitable 'coupling' is released by sexually mature males in
water which after reaching in the blood (gills) of the females
affects the ovary via hypophysis (pituitary) and causes the
formation of corpora lutea. Mendoza (1943) has found that the
granulosa layer takes a key part in resorption of oocytes in
Neotoca bilineata. Bretschneider and Duyvend de Wit (1947)
distinguished four stages in the formation of corpora lutea or
corpora atretica in Rhodeus amarus as in Gadus (Gokhale, 1957),
Carassius (Beach, 1959), Philocephalus punctatus (Balsare, 1962),
Gobius giuris (Rajalakshmi, 1965; Bhargava and Saksena, 1972).
Similar stages in the process of resorption of oocyte have also
been distinguished and described in the present work on Mystus
and Channa. However, in Gasterosteus aculeatus (Tromp-Blom, 1959),
Scomber scomber (Basa, 1960), Plecoglossus altivelis (Honma, 1961),
Carassius auratus (Yamamoto and Yamazaki, 1961), Mystus seenghala
(Sathyansan, 1961) and Heteropneustes fossilis (Nair, 1963)
several stages in the process of resorption have been described.

There remains much dispute regarding the fate of the
atretic follicles. Cunningham (1898) in Zearias compared the structure formed due to the hypertrophy of the granulosa cells with the mammalian corpora lutea. Wallace (1904) and Mendoza (1943) also agreed for such a similarity. Wallace (1904), however, has pointed that whether or not corpora lutea are formed, resorption of the riping ova is a constant feature in the teleosts. Bretschneider and Duyvene de Wit (1947) described phagocytosis of the yolk by the hypertrophied granulosa cells in resorption of the ovum. According to Bretschneider and Duyvene de Wit (1947), Hoar (1955) and Ball (1960) the pre-ovulatory corpora lutea or atretic follicles, which are universally present in the fish ovaries form the important endocrine tissue of the teleost ovary. However, according to Pickford and Atz (1937) the chemical nature and site of production of hormone remain unknown. The hyperpophied granulosa cells are regarded as the possible source of the ovarian hormones (Hoar, 1955). In the present work the endocrine function of the corpora atretica could not be seen histologically except that the 'disintegrating ferments', as seen by Bretschneider and Duyvene de Wit (1947) in Rhodeus, are also seen in the hypertrophied cells of follicular layers in both the fishes.

In goldfish, Beach (1959) observed that the so called corpora lutea (corpora atretica) are apparently associated with the removal of yolk and this has also been supported by
Sathyanesan (1961) in *Mystus senghala*. Like *Plecoglossus altivelis* (Honma, 1961), *Phiocephalus punctatus* (Belsare, 1962), *Xenentodon cancila* (Rastogi, 1966), *Clarias batrachus* (Lehri, 1968), *Rasbora daniconius* (Raizada, 1971) and *Glossogobius giuris* (Saksena and Bhargava, 1972), in *Mystus vittatus* and *Channa punctatus* (Bais, 1973) also the granulosa cells take part in the resorption of the yolk and the yolk of oocyte is digested by their phago-enzymatic activity. The granules inside the granulosa cells, referred as 'disintegrating ferments' by Bretschneider and Duyvene de Wit (1947) are also found in the present fish as in *Clarias batrachus* (Lehri, 1968). These granules are prominent and present abundantly in the earlier stages as compared to later stage when they diminish in number or disappear. Like *Glossogobius giuris* (Saksena and Bhargava, 1972) the presence of the granules in *Mystus* and *Channa* seems to be associated with the removal of yolk substance by their phago-enzymatic activity.

The occurrence of post-ovulation hypertrophy in the cells of follicular layer is a constant feature among the fishes as a whole. In stickleback a short-lived corpus luteum (post-ovulatory follicle) has been described by Craig-Bennett (1931). In *Fundulus heteroclitus* (Matthews, 1938), *Rhodeus amarus* (Bretschneider and Duyvene de Wit, 1947), *Plecoglossus altivelis* (Honma, 1961) and *Heteropneustes fossilis* (Nair, 1963) also the corpora lutea are reported
to be formed by the hypertrophy as well as proliferation of the follicle cells after ovulation. In Xenentodon cancila (Rastogi, 1966) the follicular layer after ovulation becomes hypertrophic and multiplies to form a corpus-luteum-like structure which later on gets disorganised and intermingled into the ovarian stroma. A similar structure has also been described in Glossogobius giuris (Saksena and Bhargava, 1972). In the present study on Mystus and Channa the proliferation of the granulosa cells has been found to occur after ovulation to form a well developed cellular mass which later on gets disorganised and absorbed in the ovarian stroma (Baiz, 1973).

Craig-Bennett (1931) has compared fish post-ovulatory follicles with the mammalian corpora lutea. However, Mendoza (1943), Hoar (1935, 1937 and 1969), Gokhale (1957), Tromp-Blom (1959), Stenger (1959), Bara (1961), Bhargava (1966), Lehri (1968), Raza (1971) and Saksena and Bhargava (1972) do not regard them comparable in any respect with mammalian corpora lutea. In Mystus and Channa the post-ovulatory follicles undergo resorption in the ovarian stroma thus confirming the views of Rajalakshmi (1966), Bhargava (1966), Raza (1971), Saksena and Bhargava (1972), Malaviya (1972) and Anant Prakash (1976). In the present study it has been observed that the post-ovulatory follicle cells undergo hypertrophy and proliferation but the whole mass later on gets disorganised and intermingled in the ovarian stroma. Histochemical study if carried could reveal the functional nature of this tissue.
Many workers (Hann, 1927; Craig-Bennett, 1931; Matthews, 1938; Bullough, 1939; Mendoza, 1943; James, 1946; Ghosh and Kar, 1952; Yamamoto, 1956; Gokhale, 1957; Swarup, 1958; Beach, 1959; Bara, 1960; Yamamoto and Yamazaki, 1961; Sathyanesan, 1961; Belsare, 1962; Sinha and Rastogi, 1967; Lehri, 1968; Raizada, 1971; Khanna and Sanwal, 1971; Malaviya, 1972; Saksena, 1974, 1976 a; and Anant Prakash, 1976) have studied the seasonal changes in the ovary of several fishes.

In the present work on *Mystus* and *Channa* the seasonal changes in the ovary have been studied on the basis of morphological and histological changes, rise and fall in its volume, its gonosomatic index and the duration of spawning period by the ova diameter measurements like that of work of Clark (1934), Hickling and Rutenberg (1937), De Jong (1939), Gokhale (1957), Bara (1960), Jhingran (1961), Sathyanesan (1959, 1960 a and b), Belsare (1952), Sinha and Rastogi (1967), Lehri (1968), Raizada (1971), Malaviya (1972), Saksena (1974, 1976 a) and Anant Prakash (1976). Such a study has been useful in determining the different periods of the reproductive cycle. Marza (1938) has classified the rhythm of the maturation of oocytes into the total synchronism, the group synchronism, and the asynchronism. The present work on *Mystus vittatus* shows that the fish belongs to the group synchronism as a clear distinction is found in the general stock and mature oocyte in the ovary. Prabhu (1936) has studied the spawning periodicities of several species and found difference in the
spawning periods in various species. He identified four types of spawning viz., spawning once in a year with short duration, spawning once in a year with longer duration, spawning twice in a year and spawning throughout the year. Jhingran (1961), however, found that in *Setipinna phase* there is no such spawning periodicity. *Mystus vittatus* thus belongs to the second category of Prabhu (1956) and the fish spawns only once in a year, the spawning period extending from June to mid-October. On the other hand in *Channa punctatus* the spawning periodicity belongs to the third category of Prabhu (1956) since two different groups of maturing ova are present in addition to mature ova. Thus the fish breeds twice a year from mid-April to May and from mid-July to October.

The significance of the corpora atretica and post-ovulatory follicles in the determination of reproductive cycle of the fish has been pointed out in *Phoxinus phoxinus* (Bhargava, 1966), *Ambassis ranga*, *Puntius ticto* and *Rothee cotic* (Kaur, 1968), *Rasbora daniconius* (Raizada, 1971), *Glossogobius giuris* (Saksena and Bhargava, 1972), *Clarias batrachus* (Malaviya, 1972) and *Garra gotyla gotyla* (Anant Prakash, 1976). In the present work, the quantitative study of corpora atretica and post-ovulatory follicles clearly shows variation in different months of the year which is quite significant in explaining the precise nature of periodicity of the fish. In *Mystus*, the higher number of corpora atretica and the appearance of post-ovulatory follicles
in July indicates that spawning has started in the preceding or the same month and their subsequent presence in August, September and October shows the duration of spawning period. In *Channa* also, the presence of post-ovulatory follicles in the month of May would mean that the 1st spawning had started from mid-April and subsequent presence in June shows the duration of 1st spawning up to May. Similarly, the presence of corpora atretica and post-ovulatory follicles in the month of August would mean that the 2nd spawning had started in mid-July and their subsequent presence in the month of September, October and to some extent in November shows that spawning period for the 2nd spawning extended up to October. Thus, the importance of taking at least the presence of post-ovulatory follicles in the determination of the spawning periodicity more precisely is further established by the present work.

Like most teleostean fishes, the testes in *Mystus* and *Channa* are lined by a thin peritoneal membrane below which lies a layer of tunica albuginea composed of connective tissue. The germinal epithelium is dispersed into many finger-like seminiferous tubules in *Mystus*. In *Channa* the seminiferous tubules radiate from the periphery towards the centre. The intercellular spaces are filled with connective tissue. According to Brock's (1978) classification the testes of *Mystus* are typical teleostean acinous
in nature with complex anastomoses of seminiferous tubules which are attached alterrally lengthwise with the vas deferens or spermatid, while in *Channa* they exhibit a typical teleostean pattern.

There have been varied opinions in connection with the presence and possible function of the interstitial cells in the teleost fishes. Marshall and Lofte (1936) have denied the presence of true interstitium in *Erca lucius*, *Salvelinus willughbii* and *Labes* sp., but they have reported certain cells at the tubular boundary as leydig cells and located them homologous to the vertebrate male endocrine cells. Robertson (1958) in *Salmo salar* and Raj (1965a) in *Tor* (Sarbus) tor have mentioned the presence of lobular and interstitial cells respectively. The typical vertebrate endocrine interstitial cell (leydig cells) are not present in *Salvelinus fontinalis* (Henderson, 1962) and in *Eucalea inconspicua* (Ruby and Donald, 1970).

Craig-Bennett (1931) found leydig cells easily recognisable in the testes of *Gasterosteus aculeatus* and also correlated with the reproductive cycle. Courrier (1921), in Callionymus, has correlated the changes in the interstitial cells of the testes with the secondary sexual character and these observations have been confirmed in *Gasterosteus* (Craig-Bennett, 1931). However, such relationship has been denied by Essenberc (1923) and Champy (1923), Champy and Gley (1923) and von Oordt (1923) in cyprinodonts. Matthews (1938) could not observe any secretory activity in the interstitial cells. In *Gadus*, Gokhale (1957) found vacuolisation in the interstitial cells after spawning and suggested that their contents play a nutritive role for the developing germ cells. Sathyanesan
(1959) has also noticed the vacuolisation in the interstitial cells but has not discussed their role. In the _Tor_ (Barbua) for the vacuolisation and disintegration of these cells (lobule boundary cells) during the active phase has been reported by Rai (1965). Restogi (1968) in _Amphiprionous euchla_, has suggested the formation of germ cells by the interstitial cells. In the present study in _Myatus_ and _Channa_ the interstitial cells show typical vertebrate pattern in their arrangement. However, they do not show any change during the reproductive cycle.

Seminal vesicles form an important part of the male reproductive system in catfishes, _Gallicthys_ (Weisek, 1949), _Heteropneustes fossilis_ (Sundararaj, 1959), _Clarias lazera_ (Nawar, 1959), _Myatus acrochala_ (Sathyanesan, 1959), and _Clarias batrachus_ (Lahiri, 1967; Malaviya, 1972). The seminal vesicles of _Myatus vittatus_ have seminal vesicular lamellae. They are not morphologically distinguishable from the testes. They are characterised by the presence of inter-communicating lamellae and the presence of vesicular fluid. The function of the seminal vesicular fluid is a matter of dispute (Sundararaj, 1959). But the seminal vesicular fluid helps in the spermiation in the natural condition and fluid is extruded out as a part of milt in the spawning period (Sundararaj, 1958). The storage of the spermatocytes which is obviously seen in _Heteropneustes fossilis_ (Sundararaj, 1958) is not observed in _Myatus_.
Regarding the formation of the new crop of germ cells, the literature is full of diversing views. In *Gottus bairdii* (Hann, 1927), *Betta splendens* (Bennington, 1936), *Fundulus heteroclitus* (Matthews, 1938), *Phoxinus laevis* (Bullough, 1939), *Oncorhynchus nerka* (Weisel, 1943), *Magil cephalus* (Stenger, 1959), *Hyphus acenophala* (Sathyanesan, 1959), *Salvelinus fontinalis* (Henderson, 1962), *Pleuronectes platessa* (Barr, 1963), *Tor* (Barbus) *tor* (Rai, 1965), *Clarias batrachus* (Lehri, 1967), *Rasbora daniconius* (Raizada, 1970) and *Glossogobius giuris* (1974, 1976b) a reserve stock of dormant cells are present throughout the year which gives rise to a new crop of spermatogonia. Turner (1919), Foley (1926), Lofts and Marshall (1937) and Rastogi (1966) are of the views that migratory cells originating from some part of the testes produce a new crop of germ cell. However, in *Perca flavescens*, Turner (1919) and in *Xenentodon cancila*, Rastogi (1966) have suggested the origin of such cells from parts other than testes. In Amphibious cichlas also the formation of new crop of germ cells is considered from migratory cells (Rastogi, 1969). In *Hyphus* and *Channa* a reserve stock of germ cells is present in tubules throughout the year and it seems that the seasonal supply of germ cells is maintained through them.

Cyclical changes of the testes have been worked out by many workers (refer page 110). The duration of the spawning period is described to be variable in different
fishes and this has been ascribed to the climatic factors mainly the water temperature (Craig-Bennett, 1931; Bullough, 1939 and Lofts and Marshall, 1957).

In Mystus and Channa, unlike the presence of quiescent period during the testicular cycle (Turner, 1919; Foley, 1926; Craig-Bennett, 1931; Bullough, 1939; Lofts and Marshall, 1957; Rai, 1962 and Ahaan, 1966), there is no quiescent period in the gonadal cycle because the formation of different stages of spermatogenesis takes place in different periods of the reproductive cycle. The spermatogonial proliferation in Mystus is rapid in the months of November, December and January, but from the February it slows down. The formation of primary spermatocytes occur at a slow rate during December, January and February. In March, April and May the process becomes rapid. The secondary spermatocytes are quite ephemeral and present relatively in higher percentage during May, June and July. The formation of spermatids is relatively higher in August and September. The spermatids are the final product of spermatogenesis and are formed by the direct transformation of the spermatids. The spermatids are present in higher percentage in the month of July and August. Like Mystus, Channa also exhibits same type of spermatogenesis. The spermatocytes are higher in the months of March and April. Here the spermatogenesis is faster than in the Mystus, the sperms are developed in the month of mid-April and
first lot of spermatogenesis is shed in the same month. In mid-June and July the spermatogenesis is in its resting phase, but in late July the tubules are again filled with sperms and continue up to October and November month. The process of spermatogenesis is same as observed in *Myxus*. In *Channa*, all the spermatogenetic stages are noticed throughout the year similar to the results obtained in *Gasterosteus aculeatus* (Swarup, 1938), *Heteropneustes fossilis* (Sundararaj, 1960) in *Salvelinus fontinalis* (Henderson, 1962) in *Xenentodon cancila* (Rastogi, 1965), and in *Glossocephalus giuris* (Saksena, 1974).

The testes of *Myxus vittatus* always possess a good number of spermatogonia in the tubules throughout the year as found in blue gill and large mouth bass (James, 1946), *Heteropneustes fossilis* (Sundararaj, 1960) and in *Nandus nandus* (Raizada, 1973). However, there seems that all the spermatogonia are not released in the spawning period and as such the spermatogonia are left over after spawning.

Bullough (1939), Raizada (1970 and 1971), Saksena (1974, 1976a) and Anant Prakash (1976) have described the seasonal changes of gonads by counting the relative number of oocytes of different diameters in the ovaries and relative number of spermatogenetic stages in the testes. Following these studies the seasonal changes of the gonads of *Myxus vittatus* and *Channa punctatus* have been described.
quantitatively and it has been found to be helpful in dividing the reproductive cycle in different periods.

It has been observed in the case of many fishes that the spermatogenetic activity (Craig-Bennett, 1931; Frederick, 1941; James, 1946; Gokhale, 1957; Lehri, 1968; Raizada, 1970 and 1975; Saksena, 1974, 1976b and Anant Prakash, 1976) and also the increase in the size of spermatocytes (Matthews, 1938; Guerbilsky, 1939; James, 1946; Gokhale, 1957; Sinha and Rastogi, 1967; Lehri, 1968, Raizada, 1971 and Saksena, 1974, 1976a) is correlated with an increase in the temperature of water. In *Ayu* and *Channa*, the volume of gonads is found to be directly related to the water temperature with an increase in the average volume of gonads in summer and a corresponding decrease in the average volume of the gonads in winter.