The reproductive cycle in *Ambassis ranca* Cuv. and Val., *Puntius ticto* Ham. Bach., and *Rohitec cotic* Sykes.

1. Introduction:

The reproduction in teleost fish has been reviewed by Hoar, (1955 and 1957) and later by Ball, (1960). The reproductive cycle has been studied in a few teleosts including the minnow (Bullough, 1933), the viviparous teleost *Neotoca bilineata* (Mendoza, 1939 and 1943) and *Casterostus aculeatus* (Craig-Bennett, 1931). Seasonal changes in the gonads of some teleosts have been investigated in *Perca* (Turner, 1919), in *Merluccius merluccius* L. (Hickling, 1935); in *Fundulus heteroclitus* (Matthews, 1938); in *Lenomis macrochirus* Rafinesque and *Hyro salmoides* (Lacépéde) (James, 1946); in *Heteronemus fossilis* (Bloch.) (Chosh and Kar, 1952) in two species of *Catus* viz; *C. merlangus* L. and *C. asperkii* (Gokhale, 1957); in *Carassius auratus* (Ranch, 1959); in *Scomber scomber* L. (Berg, 1960); in *Onchopsephalus punctatus* (Bloch.) (Belsare, 1962); in *Mytus seenchala* (Sykes) (Sathyanesan, 1962) and most recently in a sisorid catfish *Clypeosternum pectinopterum* (Khanna and Pant, 1967).

The ovary to act as an indicator of the spawning period of fishes has been proved in the hake, haddock, pilchard and herring (Hickling and Rutenberg, 1936).

In a few cases maturity and spawning periods of fishes have been determined by diameter measurements of the oocytes.
e.g., in Thriassocles purava (Hem.) (Palekar and Karandikar, 1952) and in Pseudonorthus weirensis Cuv.; Theronon pus Cuv.; Theronon jurbus (Forskal); Palaetes quadrilineatus (Bleeker); Caranx (Seleroides) leptolepis Cuv. and Val.; Macrurus vittatus (Bloch); Cynsilurus olivolens (Bleeker); Chirocentrus dorab (Forskal) and Stolephorus indicus (v. Hess.), (Prabhu, 1956)

The ovaries of Gasterosteus aculeatus before, during and after the spawning period with special reference to the presence of corpus luteum has been studied by Tromp-Bloem (1959). The study of corpus luteum (corpora atretica or atresia of the oocytes) in the ovary of the fish dates from the earliest work on Rhodesus amarus by Bretschneider and Duyvène de Wit, (1947). Hoar, (1955) discussed the term corpus luteum as used in the fish ovary. Since then many workers, (Ball, 1960 and 1965; Bhargava, 1966, and Rajalakshm, 1966) have used the term corpora atretica or atresia for the unspawned ripe oocytes or the immature oocytes undergoing oolsis.

The present work includes a detailed study of the reproductive cycles of three teleosts Ambassis range, Puntius ticlo and Rohtee cotio. The seasonal gonadal changes on the basis of their morphology, histology and a quantitative assessment of the gonads in respect of their maturity, have been investigated.

2 A. Morphology of the gonads of Ambassis range, Puntius ticlo and Rohtee cotio;

In Ambassis range the gonads (Fig. 1a, p. 13.) are a pair
Fig. 1 - A. & B. To show the morphology of the gonads of *Ambassis ranza*, *Puntius ticto* and *Rohtee coticin*.

a. - Ovaries of *Ambassis*
b. - Ovaries of *Puntius*
c. - Ovaries of *Rohtee*
d. - Testes of *Ambassis*
e. - Testes of *Puntius*
f. - Testes of *Rohtee*

Abbreviations:

T. - Testis
SP.D. - Spermduct
OV. - Ovary
OV.D. - Oviduct
of elongated organs lying in the dorsal region of the body cavity, ventral to the air bladder. The gonads are narrow at both ends whereas in the middle, they are comparatively very broad in calibre. They remain separate throughout their length except for the posterior most parts which fuse to form a single duct opening separately from the kidney duct into a urogenital pit. The urogenital pit opens to the exterior, posterior to the anal opening.

The testes of *Ambassis ranza* are small leaf-like structures with a serrated edge on the outer margin. They are whitish in colour. The ovaries are of the cystoerian type and are larger structures with a cream colour and occupy a larger space in the peritoneal cavity. Sometimes the ovary of the left side is smaller than that of the right side. In a fully matured ovary, ova can be seen through the thin outer covering.

The gonads in *Puntius ticto* (Fig. 1bc p. 13) are a pair of elongated structures extending along the whole length of the body cavity. They are broader anteriorly but narrow down gradually towards the posterior end. In the young fishes the gonads are uniformly thick while in the adults they become broader anteriorly. They are separate throughout their length. The anterior part of both testis and ovary is lobulated. The testes are creamy in colour while the ovaries are light brown. Like *Ambassis* the ovary presents the cystoerian condition. In the male, the testes unite posteriorly to form a common sperm duct which opens in the urogenital pit, separately from the kidney duct, behind the anal opening. In the female also the oviducts fuse together and open into the urogenital pit,
separately from the duct of the kidneys, behind the anal opening.

In *Rhoitea cotic* the gonads occupy a position ventral to the air bladder as in the other two fishes. The testes (Fig. 1f, p. 12) are free throughout their length and are somewhat broader towards their anterior end only. They are creamy in colour. Like the other two fish the ovary presents a cysto-ovarian condition. The paired ovary (Fig. 1c, p. 12) is united for about three fourths of its length posteriorly leaving its remaining anterior part free. The ovaries are broadest in their anterior region but the free ends gradually taper anteriorly. Generally, the ovaries are yellow in colour with reddish or dark brown ova protruding all over to give them a granular surface. The gonads fuse at the posterior end and form a single genital duct which opens in the urogenital pit, separately from the kidney duct, posterior to the anal opening.

B. Histology of the gonads of *Ambassis renza*, *Puntius ticto* and *Rhoitea cotic*:

In basic features, the histology of the ovary and testis in all the three fishes is the same. There are some slight differences which will be pointed out in their individual descriptions.

**Histology of the ovaries**:

In *Ambassis renza* the ovary consists of an ovarian wall and ova enclosed by an outer thin peritoneal membrane. The wall of the ovary is made up of two layers, (Fig. 2A, p. 17)
Fig. 2. - Photomicrographs of the sections of the ovary of *Ambassis rozena*.

A. - Shows the ovarian wall and oviscrous lamellae (Azan)

B. - Shows oocytes and primary oocytes (Azan)

C. - Shows secondary oocytes, mature ova and post-ovulatory follicle (Azan)

Abbreviations:

B.V. - Blood vessel
C.T. - Connective tissue
M.O. - Mature ovum
O.C. - Oorion
O.L. - Oviscrous lamellae
O.W. - Ovarian wall
P.O. - Primary oocyte
P.O.F. - Post-ovulatory follicle
S.O. - Secondary oocyte
an outer and an inner. The outer layer is thin and is formed of connective tissue and blood vessels. The inner is the germinal epithelium consisting of the ova in different stages of development. At first, in the young stages a lumen is seen inside the ovary, but later on it gets obliterated by the development of the ovigerous lamellae which project inwards. The ovigerous lamellae consist of connective tissue and blood vessels, along with the ova in different stages of development since they are simply the infoldings in the ovarian wall. The ova are almost round in outline. The smaller ova are not always situated towards the periphery.

The oogonia (Fig. 2 B, 7a p. 17a 27) are the earliest egg stages found. They are characterised by their small size, presence of a large nucleus, a net work of chromatin fibres and a single centrally situated nucleolus. The cytoplasm is clear and lightly stained. The maximum diameter of the nucleus and oogonium is 8 μ and 12 μ respectively. The oogonia are never seen in a state of division.

The oogonia develop to form the primary oocyte (Fig. 2 B, 7b p. 17b 27). At this stage the oocyte and its nucleus become large. The chromatin breaks into small bits which arrange themselves towards the periphery of the nucleus beneath the nuclear membrane. This is the primary growth phase. The cytoplasm takes up a deeply staining appearance. The maximum nuclear diameter is 51 μ and that of the oocyte is 85 μ. The follicular epithelium begins to form but it is not complete.
The secondary growth phase begins and gives rise to the secondary oocyte and finally the mature ovum. The secondary oocyte (Fig. 2C, p. 1787) is characterised by its bigger size. The chromatin bits are present beneath the nuclear membrane. The cytoplasm takes up a comparatively lighter stain and darkly staining granules appear along the inner edge of the cell. Inner to these granules vacuoles are formed which later enlarge and multiply occupying almost the whole of the cytoplasm. The follicular epithelium is completely formed by the close of this phase. The maximum nuclear diameter at this stage is 119 \( \mu \) and that of the oocyte is 255 \( \mu \).

As the vacuoles enlarge and increase in number, the granules disappear. Yolk droplets then arise in association with these vacuoles. The vitelline membrane begins to form. The development of the yolk droplets is centripetal i.e. they develop first towards the periphery and then towards the centre of the cytoplasm. With the development of the oocyte, yolk droplets increase in number and spread throughout the cytoplasm along with the vacuoles. The vitelline membrane becomes completely formed. The nucleus becomes lobulated with a few chromatin bits inside, thus forming the mature ovum whose maximum diameter is 527 \( \mu \) and that of its nucleus being 122.5 \( \mu \) (Fig. 2C, p. 1787).

In *Puntius ticto*, the ovary is enclosed in a thin peritoneal membrane and is attached to the body cavity by the mesovarium. The outer layer of the ovary, as in *Ambassis ranga*, is made up of connective tissue and blood vessels.
Fig. 3  - Section of the ovary of *Puntius* showing the ovarian wall and oviscrous lamellae sitting of the ova in different stages of development (Azem)

Fig. 4  - Section of the ovary of *Puntius* showing oogonia and primary oocyte (Azem)

Abbreviations:

- B.V.  - Blood vessel
- C.T.  - Connective tissue
- O.C.  - Oocanium
- O.L.  - Oviscrous lamellae
- O.W.  - Ovarian wall
- P.O.  - Primary oocyte
- S.O.  - Secondary oocyte
The inner layer is made up of germinal epithelium and various stages of developing ova. (Fig. 3 p. 2). The oogonia and primary oocytes are round in shape, the secondary oocytes are somewhat irregular while the mature ova are again round in shape.

The oogonia (Fig. 4 p. 2) are small in size with a single nucleolus and chromatin fibres in the nucleus and clear cytoplasm. The maximum nuclear diameter is 6 μ and that of the oogonium is 12 μ.

In the next stage of development, the primary oocyte, the chromatin gets separated from the nuclear membrane at various places and bits of chromatin material occupy a peripheral position in the nucleus beneath the nuclear membrane (Fig. 4 p. 2). The formation of the follicular epithelium begins. The maximum diameter of the nucleus and the oocyte at this stage is 85 μ and 170 μ respectively.

The primary oocyte undergoes further changes to give rise to the secondary oocyte (Fig. 3 p. 2). With the beginning of the formation of the secondary oocyte the formation of the follicular epithelial layer is completed. Vitellogenesis begins with the development of a single layer of small vacuoles which get arranged in a ring-like fashion in the peripheral cytoplasm. Dark staining granules which appear before the formation of these vacuoles in Ambassis ranga are not seen in this fish. The cytoplasm is lightly stained and a large number of chromatin bits are present beneath the nuclear membrane occupying almost the entire
Fig. 5  - Section of the ovary of Rhoitea showing the ovarian wall and ovigerous lamellae (Haematoxylin eosin)

Fig. 6  - Section of the ovary of Rhoitea showing oogonia and primary oocytes (Haematoxylin eosin)

Abbreviations:
B.V.   - Bloodvessel
C.T.   - Connective tissue
O.G.   - Oogonia
O.L.   - Ovigerous lamellae
P.O.   - Primary oocyte
nucleus. In later stages the vacuoles enlarge and increase in number. Yolk droplets staining blue with Azen are seen inside these vacuoles towards the periphery. The maximum nuclear diameter of the secondary oocyte is 136 μ and that of the oocyte is 357 μ.

The secondary oocytes grow further to form the mature ova (Fig. 9d-p. 2957). With this change the yolk droplets increase in number and occupy whole of cytoplasm and gradually turn carminophil from the centre to the periphery. The nucleus becomes elongated. With the formation of the vitelline membrane the mature ovum is complete. The maximum diameter of the nucleus is 187 μ and that of the mature ovum is 1,088 μ.

In Rohtees cotio, as in the other two fishes, the ovary is enclosed in a peritoneal membrane which is attached to the body wall by the mesovarium. The wall of the ovary is made up of connective tissue and blood vessels. The inner layer of the ovarian wall is made up of the germinal epithelium consisting of ova in different stages of development (Fig. 5 p. 24), which are distinguishable on the basis of nuclear changes.

The earliest stage of the developing ova, the oogonia (Fig. 68a-p. 2427) are small in size and possess the same characteristics as in the other two fishes. The maximum nuclear diameter is 10 μ and that of the oogonium is 12 μ.

The primary oocyte (Fig. 68b-p. 2427) possesses the same characteristics as in Ambassia ranca and Puntius ticto. The maximum nuclear diameter is 93.5 μ and that of the primary
Fig. 7 - Camera lucida sketches showing the different stages of oogenesis in *Ambassia renza*.

a. - Oogonium  
b. - Primary oocyte  
c. - Secondary oocyte  
d. - Mature ovum

Fig. 8 - Camera lucida sketches showing the different stages of oogenesis in *Rheea rotic*.

a. - Oogonium  
b. - Primary oocyte  
c. - Secondary oocyte  
d. - Mature ovum

Abbreviations:

C. - Chromatin  
CY. - Cytoplasm  
F.EP. - Follicular epithelium  
N. - Nucleolus  
NU. - Nucleus  
V. - Vacuoles  
V.M. - Vitelline membrane
Fig. 9  - Camera lucida sketches showing the different stages of oogenesis in *Puntius ticto*.

a. - Oogonium  
b. - Primary oocyte  
c. - Secondary oocyte  
d. - Mature ovum

**Abbreviations:**

C.  - Chromatin  
CY.  - Cytoplasm  
F.EP.  - Follicular epithelium  
N.  - Nucleolus  
NU.  - Nucleus  
V.M.  - Vitelline membrane
oocyte is 170 μ. The follicular epithelial layer begins to form. This represents the close of the primary growth phase.

In the later stage of development vacuoles appear in the peripheral region of the cytoplasm. Yolk droplets also make their appearance. The nucleus elongates and the cytoplasm takes up a lighter stain. The follicular epithelial layer is completely laid down and this marks the formation of the secondary oocyte (Fig. 82, p. 274). The maximum diameter of the nucleus is 153 μ and that of the oocyte is 357 μ.

The changes which follow this stage are the increase in number of yolk droplets in the cytoplasm, formation of a vitelline membrane and elongation of the nucleus. The development of the yolk droplets is centripetal as in the other two fish. The maximum diameter of the nucleus is 187 μ and that of the mature ovum is 916 μ. (Fig. 83, p. 2748).

Corpora stratica and post-ovulatory follicle:

The ova which fail to attain ripeness or fail to spawn undergo resorption and are called the corpora stratica or stratic follicles. Jaski, (1939) proved the presence of corpora lutea in the ovary of a viviparous teleost Labistes raticulatus. Bretschneider and Duyvend de Wit, (1947) were the first to make an exhaustive study of the histology and endocrinology of these bodies in Rhodeus amarus. The term "corpus luteum" as used in the fish ovary has been discussed by Hoar, (1955 and 1957). He states that these bodies develop from the follicular epithelium and theca in fish in higher vertebrates and consequently referred to as corpora lutea.
Fig. 10 - Camera lucida sketches of the stages in the formation of corpora stricta in *Ambassis ranca*.

A. - Stage I
B. - State II
C. - Stage III
D. - Stage IV

Abbreviations:

- G.C. - Granulosa cells
- V. - Vacuole
- V.M. - Vitelline membrane
- Y.D. - Yolk droplet
FIG. 10
Fig. 11 - Photomicrographs of the stages in the formation of corpora atretica in *Amblyopsis ranga*.

A. - Stage I  
B. - Stage II  
C. - Stage III  
D. - Stage IV  

Abbreviations:

G.C. - Granulosa cells  
V. - Vacuole  
V.M. - Vitelline membrane  
Y.D. - Yolk droplet
He gave the terms pre- and post-ovulatory corpora lutea, the former resulting from the oolysis and resorption of unspawned ripe oocytes and the immature oocytes of the spent fish and the latter resulting from the follicles after the discharge of the ripe ova.

Recently, Ball (1960 and 1965); Dhargava, (1966) and Rajalakshmi, (1966) have used the term corpora atretica or atresia of the oocytes for the unspawned ripe oocytes or the immature oocytes undergoing oolysis. In the present work the term corpora atretica or atresia has been used for the unspawned ripe oocytes or immature oocytes undergoing oolysis.

Atresia of the oocytes: The process which leads to the formation of corpora atretica is more or less the same in all the three fishes except for minor differences. The formation of corpora atretica may be described under four stages (as given by Bretschneider and Duyvène de Wit, 1947).

In Ambassia ranza the stages are as follows:—

Stage I:— The granulose layer loses its smooth outline, breaks and gets distorted and its cells become loose. The cells have small round nuclei. Vitelline membrane (oolemma) becomes broken at various places. Yolk droplets are present in the cytoplasm along with a large number of vacuoles.

(Fig. 108a, p. 3234)

Stage II:— The vitelline membrane disappears and the hypertrophied granulose cells invade the yolk. The entire oocyte looses its original shape. A large number of vacuoles with a few yolk droplets are present. (Fig. 108b, p. 3234)
Fig. 12. - Camera lucida sketches of the stages in the formation of corpora strcta in *Puntius ticto*.

A. - Stage I
B. - Stage II
C. - Stage III
D. - Stage IV

Abbreviations:

C.C. - Granulosa cells
L.Y. - Liquified yolk
V.M. - Vitelline membrane
Y.D. - Yolk droplet
Fig. 13 - Photomicrographs of the stages in the formation of corpora alretica in Puntius ticto.

A. - Stage I
B. - Stage II
C. - Stage III
D. - Stage IV

Abbreviations:
C.C. - Granulose cells
L.Y. - Liquified yolk
V.M. - Vitelline membrane
Y.D. - Yolk droplet
Stage III: In this stage, the granulosa cells, along with the large number of vacuoles occupy almost the whole of the oocyte. Yolk droplets are completely absent. (Fig. 10.6, p. 3203).

Stage IV: In the fourth stage the whole of the oocyte is filled with enlarged vacuoles, very few number of granulosa cells and blood capillaries which are finally resorbed. (Fig. 10.6, p. 3203).

In Puntius ticto, in the first stage the granulosa layers gets hypertrophied and its cells become loose. The cells of the granulosa have small round nuclei. The vitelline membrane becomes folded at various place. Yolk droplets are present in the oocyte. Unlike Ambassia large vacuoles are absent in Puntius. (Fig. 12.3, p. 3743).

In the second stage the vitelline membrane ruptures at various places unlike Ambassia in which it completely disappears at this stage. The granulosa layer is much more hypertrophied and its cells begin to invade the yolk. (Fig. 12.3, p. 3743).

In the third stage most of the yolk is liquified. The vitelline membrane becomes completely disintegrated leaving its few remnants. The granulosa cells are present around the periphery of the liquified yolk. (Fig. 12.3, p. 3743).

In the fourth stage the yolk has almost completely liquified. The granulosa cells are present in the peripheral portion. The oocyte is finally resorbed. (Fig. 12.3, p. 3743).

In Rohitee cotic, the formation of corpora strerica passes through the same stages as seen in Ambassia and Puntius.
Fig. 14 - Camera lucida sketches of the stages in the formation of corpora stratica in *Rohdea japonica*.

A. - Stage I
B. - Stage II
C. - Stage III
D. - Stage IV

Abbreviations:

C.C. - Granulosa cells
L.Y. - Liquified yolk
V.M. - Vitelline membrane
Y.D. - Yolk droplet
Fig. 15 - Photomicrographs of the stages in the formation of corpora atertia in Rohtee cotico.

A. - Stage I
B. - Stage II
C. - Stage III
D. - Stage IV

Abbreviations:

C.C. - Granulose cells
L.Y. - Liquified yolk
V.M. - Vitelline membrane
Y.D. - Yolk droplet
FIG. 15
In the first stage the cells of the granulosa layer loosen and the vitelline membrane ruptures at a few places. Yolk droplets are present in the oocyte. (Fig. 14A, 15D, 42V44).

In the next stage the granulosa cells increase and invade the yolk which starts liquifying. (Fig. 14B, 15D, 42V44).

In the third stage the granulosa cells have occupied a larger portion and the yolk has almost completely liquified except for a few droplets. (Fig. 14C, 15D, 42V44).

In the last stage there is no yolk as it has completely liquified. The granulosa cells surround the liquified yolk and occupy the peripheral part of the strectic follicle. (Fig. 14D, 15D, 42V44).

Atresia of the immature oocyte :- In Ambassia some of the immature oocytes also undergo oalysis to form the corpora strectica. They are very few in number. The process is simple and involves a distortion in shape of the cell followed by a loss in the homogeneity of the cytoplasm.

Resorption of the ruptured or post-ovulatory follicle :-

In Ambassia rana the ruptured follicle has a clear central space once occupied by the extruded egg. (Fig. 2c, p. 17)

The follicle acquires a distorted shape. The cells become loose, the nuclei, chromatin and nucleolus are hardly distinguished. The follicle then shrinks and undergoes degenerative changes.

In Runtia ticto and Rohtee octio also the post-ovulatory follicle becomes distorted and its cells become loose. (Fig. 26, 17, p. 41). In Rohtee nuclei of the follicular cells are
Fig. 16 - Photomicrograph of the sections of ovary of *Echtes cotin* showing post-ovulatory follicles (Haematoxylin eosin)

**Abbreviation:**

PO,F. - Post-ovulatory follicle.
distinguishable unlike the other two fish. It then undergoes degenerative processes and is finally resorbed.

Structures resembling the post-ovulatory corpora lutea in *Rhodeus amarus* (Bretschneider and Duyvène de Wit, 1947) and those described by Hoar (1955, 1957), resulting from the changes taking place in the ruptured follicle, were not seen in these fish.

**Histology of the testes:**

In *Ambassis ranga* each testis is covered by a connective tissue sheath and consists of numerous tubules or follicles. The latter are separated from each other by an interstitial tissue. The tubules tend to converge towards the lumen of the testis as in perch (Turner, 1919). Within the follicles various stages of spermatogenesis are seen. These stages consist of the primary germ cells, the spermatogonia primary and secondary spermatocytes, spermatids and spermatocytes.

(Fig. 7A p. 52)

The primary germ cells (Fig. 31A p. 89) are found in groups towards the periphery of the follicles. Each of these cells is round in shape and has a clear nucleus, a single nucleolus and chromatin stretching from the nucleolus to the nuclear membrane. The cytoplasm of the cell takes up a very light stain. The maximum diameter of the primary germ cell is 12 μ.

The next stage in the development is a spermatagonium. It is characterised by its small size, the presence of a darkly staining nucleus, and the cell outline looses
distinctness. The diameter of the cell has reduced to 4 μ. The spermatogonia give rise to the primary spermatocytes which subsequently form the secondary spermatocytes. The primary spermatocytes are smaller in size and characterised by a darkly staining nucleus and the cell outline is not distinguishable. The diameter of the primary spermatocytes has reduced to 2.8 μ. As a result of the first maturation division the primary spermatocytes give rise to the secondary spermatocytes which are still smaller in size, the diameter being 2 μ. The secondary spermatocytes undergo second maturation division and give rise to the spermatids.

The spermatids are seen as deeply staining bodies and their diameter is reduced to 1.6 μ. The chromatin of the spermatids contracts further to produce a solid deeply stained round sperm head. With the formation of a slender tail the mature spermatozoon is formed. The diameter is further reduced and the sperm head measures 1.2 μ.

The testes of Puntius ticto are covered over by connective tissue inside which are found the follicles showing different stages of spermatogenesis. The follicles are irregular in shape and separated by thin interstitial tissue (Fig. 348 p. 94).

The primary germ cells are the largest in size among the different stages (Fig. 315 b, p. 52, 98). They have a definite cell outline, a nucleus and a single nucleolus. Their diameter is 8 μ.

The spermatogonia are smaller and possess a darkly staining nucleus and very little cytoplasm. The diameter of
the spermatogonium is reduced to 6 μ.

Both the primary and secondary spermatocytes are smaller in size than the spermatogonia. They have a deeply staining nucleus and the cell outline is not seen. The diameter is further reduced to 4 μ and 2.4 μ respectively (Fig. 17B, p. 52A).

Spermatids (Fig. 17B, p. 52A) are still smaller and measure 2 μ in diameter. Spermatozoa (Fig. 17B, p. 52A) appear as deeply staining granules. They are very minute and lie in the lumen of the follicle. The sperm head measures 1.6 μ in diameter.

The histological structure of the testes of Rohitee cotic (Fig. 17, p. 28) is essentially the same as in Ambassis ranca and Puntius ticto except that the interstitial tissue present between the follicles is comparatively lesser and the cell outline of the spermatogonia is almost indistinguishable. Spermatogenesis passes through the same stages as are described for the other two fishes (Fig. 36, p. 98). The diameter of the different stages of spermatogenesis is as follows:

<table>
<thead>
<tr>
<th>Stage</th>
<th>Diameter (μ)</th>
</tr>
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<tbody>
<tr>
<td>Primary germ cell</td>
<td>8</td>
</tr>
<tr>
<td>Spermatogonium</td>
<td>6</td>
</tr>
<tr>
<td>Primary spermatocyte</td>
<td>4</td>
</tr>
<tr>
<td>Secondary spermatocyte</td>
<td>3.2</td>
</tr>
<tr>
<td>Spermatids</td>
<td>2</td>
</tr>
<tr>
<td>Sperm head</td>
<td>1.2</td>
</tr>
</tbody>
</table>
Fig. 17. Camera lucida sketches showing the different stages of spermatogenesis.

A. *Ambassia ranza*

B. *Puntius ticto*

C. *Ehothee cotio*

Abbreviations:

- CY. = Cytoplasm
- N. = Nucleolus
- NU. = Nucleus
- P.G.C. = Primary germ cell
- P.S. = Primary spermatocyte
- S. = Spermatids
- SP. = Spermatozoa
- S.P.C. = Spermatogonie
- S.S. = Secondary spermatocyte
C. Seasonal changes in the gonads of *Ambassis ranica*,
*Puntius ticto* and *Pachydrilus cotic*:

The gonads of *Ambassis ranica*, *Puntius ticto* and *Pachydrilus cotic* undergo changes during the different phases of the reproductive cycle. These changes relate to morphological, (shape, size and volume) histological and cytological structures. Finally, in order to confirm the findings from the study of these aspects, a quantitative assessment of the seasonal changes in the gonads of *Ambassis* and *Puntius* has been attempted in order to know precisely the reproductive cycle in these fishes.

The seasonal changes based on the study from all these different aspects indicate that the breeding period in these fishes is different from one another. In *Ambassis ranica* and *Pachydrilus cotic*, spawning takes place twice a year. In *Puntius* also spawning occurs twice a year, with the exception of a few months which may be regarded to represent the intervening preparatory phase of the gonads.

Morphological changes in the gonads:

The reproductive cycle in these fish may be divided into the following four phases:

1. Post-spawning phase.
2. Pre-spawning phase.
4. Preparatory phase.

The post-spawning phase of the fish will be considered first as the fresh reproductive cycle begins at the end of the spawning period.
Fig. 18 - A. Showing the volume of the ovaries of
    Ambassia in relation to the temperature
during the different months.

B. - Showing the volume of the testes of Ambassia
    in relation to the temperature during the
different months.

Note: Temperature expressed in centigrade volume
      expressed in millilitres.
Seasonal changes in the ovary:

Ambassia ranza:

Post-spawning phase: (October-November)

In October the ovaries are small, elongated and pale. They occupy a small portion of the body cavity. Their average volume during this month is 1.62 ml. In November they are thin, small and pale. Their average volume is 1.01 ml., being most slight during the year (Table I p. 9 Fig. 18A).

Pre-spawning phase: (December-January)

During this period the ovaries are still elongated and small but they present a granular surface. They are pale yellow in colour. Their average volume has increased to 1.62 ml. and 1.46 ml. in December-January respectively. (Table I p. 9 Fig. 18A p. 55).

Spawning phase: (February-March and July-September)

In February the ovaries increase in size, become swollen and have a yellow colour. They occupy a larger space in the body cavity and are granular in appearance. Blood vessels are seen over the surface of the ovary. Their average volume has increased to 1.96 ml.

In March, the ovaries become very large and swollen. They have a deep yellow colour. The rich vascularisation can be seen externally. They have a highly granular surface on account of a large number of mature eggs protruding over the surface. Their average volume has increased to 3.99 ml.

In July, the ovaries again enlarge considerably. They are deep yellow in colour with a granular appearance. The
Fig. 19  - A. Showing the volume of the ovaries of *Puntius*
in relation to temperature during the
different months.

B. - Showing the volume of the testes of *Puntius*
in relation to temperature during the
different months.

Note: Temperature expressed in centigrade volume
expressed in millilitres.
FIG. 19
average volume i.e. 13.26 ml. (Table I p. 9, Fig. 14 Ap. 59).
Ovaries change from light brown to dark brown in colour. By December the ovaries are highly vascularised and the ova can be seen protruding externally. From the state of the ovaries in the month of December it is suggested that, in Puntius also the ripe egg bound ovaries are retained in the fish until a number of favourable factors for spawning are concurrent similar to the condition in some carps (Khan, 1924), and in Heteropneustes (Sunderaraj, 1959).

1st spawning phase: - (January to April)

During this period the ovaries are large and dark brown in colour. Large number of ripe eggs are present in the ovary which make the surface of the ovary highly granular. The volume for these months is shown in Table I p. 9, Fig. 14 Ap. 59.

2nd Preparatory phase: - (May-June)

During this period the ovaries are large and show profuse vascularisation. The volume somewhat increases as compared to that in April. (Table I p. 9, Fig. 14 Ap. 59)

2nd spawning phase: - (July to October)

During this period, in the beginning, there is an increase in volume (Table I p. 9, Fig. 14 Ap. 59) which later on decreases by October to 5.25 ml. The ovaries present a granular surface indicating the presence of mature eggs. The ovaries remain dark brown in colour as in the first spawning phase. (It appears that spawning takes place mostly in the month of July.)

Rohtee cotio:

In Rohtee cotio, as in Ambassis range, there are two spawning periods.
Fig. 20 - A. Showing the volume of the ovaries of *Rohtas* in relation to temperature during the different months.

B. Showing the volume of the testes of *Rohtas* in relation to temperature during the different months.

Note: Temperature expressed in centigrade. Volume expressed in millilitres.
FIG. 20
Post-spawning phase: (September-November)

In September the ovaries are deep yellow in colour, large and granular but their average volume is reduced to 6.80 ml. In October, the ovaries are pale in colour but their average volume is the same as that of September i.e. 6.80 ml. In November, the ovaries are small thin and leaf-like structures pale in colour. Their average volume is now reduced considerably to 1.25 ml. (Table I p. 9, Fig. 20Ap. 62)

Pre-spawning phase: (December-February)

In December, the ovaries are small and yellow in colour but their average volume has increased to 3.80 ml. In January and February the ovaries increase in size and become deep yellow in colour. Blood vessels and deep brown-coloured ova are seen scattered over the surface of the ovary. The average volume of the ovaries during these months is 1.30 ml. and 5.10 ml. respectively. (Table I p. 9, Fig. 20Ap. 62)

Spawning phase: (March-May and July-August)

In March, April and May the ovaries enlarge considerably occupying a large space in the body cavity. They are deep yellow in colour with dark brown ova scattered over the surface giving it a granular appearance. The vascularisation becomes conspicuous. The average volume of the ovaries during these months is 7.50 ml., 8.30 ml. and 7.90 ml. respectively.

In July and August the ovaries are like those of March, April and May except that their average volume has increased considerably, being 8.60 ml. and 10.70 ml. respectively. This indicates a greater spawning activity during this period. (Table I p. 9, Fig. 20Ap. 62)
Preparatory phase (June)

The month of June is the preparatory phase between the two spawning periods in Rohtee. In June, the ovaries are large and mature. Their average volume is increased to 8.20 ml. (Table I p. 9, Fig. 20 p. 62)

Seasonal changes in the testes:

Ambassia ranca:

Post-spawning phase (October-November)

The testes in this period are poorly developed. They are small and thin elongated structures white in a colour. The average volume is low being .07 ml. and .06 ml. respectively for October and November. (Table I p. 9, Fig. 18 p. 55)

Pre-spawning phase (December-January)

The testes do not show any marked change in their morphology and are more or less the same as they are in October and November. Their average volume in December and January is .05 ml. and .07 ml. respectively. (Table I p. 9, Fig. 18 p. 55)

Spawning phase (February-March and July-September)

In February and March the testes show a marked increase in size. They are elongated but thicker in calibre and possess a creamy colour. The average volume during these months increases to .09 ml. and .10 ml. respectively.

Again, in July, August and September, the testes do not exhibit any marked change. Their average volume, however, shows a decline during these months, being .10 ml., .06 ml. and .07 ml. respectively. (Table I p. 9, Fig. 18 p. 55).
Preparatory phase (April-June)

During April, May and June the testes increase and attain their maximum size. They are thick elongated structures with a creamy colour. The average volume during these months is .10 ml., .12 ml. and .10 ml. respectively. (Table I, p. 9, Fig. 18 B p. 55).

Puntius Ticto:

1st preparatory phase (November-December)

The testes are creamy in colour. During this phase the testes undergo rebuilding and increase in volume being 2.13 ml. in December. (Table I, p. 9, Fig. 19 A p. 59)

1st spawning phase (January-April).

During this period the testes are elongated structures and possess a creamy colour. The average volumes for these months are shown in Table I, p. 9, Fig. 19 B p. 59).

2nd preparatory phase (May-June)

Morphologically the testes are not much different from the breeding phases in colour and structure. However, their average volume is reduced as shown in Table I, p. 9, Fig. 19 B p. 59).

2nd spawning phase (July-October)

During this period the volume of testes fluctuates (Table I, p. 9, Fig. 19 B p. 59).

The morphological features, remain the same as in other phases of the reproductive cycle and like Ambassis, there is no well defined testicular seasonal cycle.

Rochea actio:

Post-spawning phase (September-November)

The testes, during these months, are thin elongated and
thread like structures. They are white in colour and their average volume is less. (Table I p. 9, Fig. 20B p. 62)

Pre-spawning phase (December-February)

In December, January and February the testes are like those of October and November morphologically and possess a creamy colour. Their average volume during these months increases gradually (Table I p. 9, Fig. 20B p. 62).

Spawning phase (March-May and July-August)

During this period, (March-May) the testes are creamy in colour and gradually increase in their average volume. (Table I p. 9, Fig. 20B p. 62).

In July and August the testes are like those of March, April and May except that their average volume is the largest in these months being .25 ml. and .25 ml. respectively. (Table I p. 9, Fig. 20B p. 62).

Preparatory phase: (June)

Morphologically the testes in June are like those of the spawning period and their average volume is .20 ml. (Table I p. 9, Fig. 20B p. 62).

Seasonal variation in gonad volume:

The variation in the volume of the gonads in these fishes throughout the annual cycle was recorded by the liquid displacement method (Pullouich, 1939). The volume of the liquid (water) displaced by gonads of thirty fish of each month was measured and then an average monthly volume both for ovary and testis was deduced (Table I p. 9). This was done from the fixed material keeping their time of fixation as constant.
These estimations are approximate but still they help to give an idea of their seasonal variation on a comparative basis.

From a study of the morphological changes which the gonads of *Ambassia renza*, *Puntius ticto* and *Rohtee rotio* undergo, it is concluded that in the post-spawning phase of *Ambassia* and *Rohtee* the ovaries and testes are small immature with a pale colour, whereas in the pre-spawning, spawning and preparatory phases the gonads become mature and well developed and acquire a deep colour. In *Puntius*, however, they are never in a spent condition during the year as the spawning period lasts very long.

The volume of the gonads shows great seasonal variation depending upon their degree of maturity they have attained. From the data on average volume, given in Table I, it is indicated that in *Ambassia* and *Rohtee* there is an appreciable increase in the volume of the gonads during the pre-spawning, spawning and preparatory periods. The volume of the gonads in the post-spawning period on the other hand decreases sharply showing their spent condition. In *Puntius*, however, the post-spawning and pre-spawning periods are not clearly recognizable as its spawning period extends for a greater part of the year. The volume is never too less during the year so as to show the spent condition of the gonad. In *Puntius* it is striking to note that the volume of the gonads increases with the fall in temperature, their maximum average volume is present in December (Table I p. 9, Fig. 99, p. 59).
Histological changes in the gonads:

Ovaries:

In Ambassia range a section of the ovary of the post-spawning phase (October-November) shows distinct ovigerous lamellae protruding towards the inner side of the lumen of the ovary, which in this phase is very distinct (Fig. 21) p. 70). The ovigerous lamellae contain numerous oogonia and primary oocytes. The outer covering of the ovary is thick with a few blood vessels. The number of corpora atretica is large. The post-ovulatory follicles persist in the month of October but in November they are completely absent.

In the pre-spawning phase (December-January) the ovigerous lamellae consist mostly of primary and secondary oocytes apart from the mature ova. The lumen of the ovary becomes obliterated. The outer covering of the ovary is not very thick. Corpora atretica are present during these months. They are more in December and less in January. Post-ovulatory follicles are entirely absent in these months. (Figs. 21b p. 70)

In the spawning phase (February-March and July-September) majority of the ova have advanced towards maturity. In February, many small developing oocytes are seen along with the mature ova. Corpora atretica are very few and post-ovulatory follicles are absent. In March, a large number of mature eggs are seen which are closely packed together with small inter-follicular spaces between them. Primary and secondary oocytes are still noticed. There is an increase in the number of corpora atretica. Post-ovulatory follicles are
Fig. 21 - Sections of the ovary of *Ambassis* (Azan)

A. - Ovary of November fish showing a large number of primary oocytes and oogonia.

B. - Ovary of January fish showing primary and secondary oocytes and corpora lutea.

Abbreviations:

PR.C.L. - Corpora lutea

S.O. - Secondary oocyte
Fig. 21
Fig. 22. - Sections of the ovaries of Ambassia (Azem).

A. - Ovary of March fish showing a large number of mature ova.

B. - Ovary of April fish showing primary and secondary oocytes along with the mature ova and post-ovulatory follicles.

Abbreviations:

PO.F. - Post-ovulatory follicle.
Fig. 23 - Sections of the ovaries of *Ambassia* (Azen).

A. - Ovary of fish of June (late preparatory period) showing a large number of closely packed mature ova and a few primary oocytes.

B. - Ovary of fish of July (spawning period) showing a large number of mature ova along with a few primary and secondary oocytes.
present. In July and August also a very large number of mature ova are present apart from the developing oocytes. Corpora strata are present in large numbers. Post-ovulatory follicles are absent in July but in August they are present. In September, the ovaries contain a few matured ova which are loosely distributed. Primary and secondary oocytes are also present. Corpora strata are present in large numbers. Post-ovulatory follicles are also present in good number. (Fig. 22A, 28B, p. 72-74)

In the preparatory phase (April-June) the ovaries contain a large number of mature ova closely packed together with the inter follicular space reduced to the minimum. Primary and secondary oocytes are also present. Corpora strata are present in all these three months. Post-ovulatory follicles are present only in April, they are, however, completely absent in May and June (Fig. 23A, 23B, 72-74).

In Puntius ticto, in the preparatory phases (November-December and May-June) the ovary shows a large number of oogonia, primary and secondary oocytes along with mature ova. Corpora strata are also present in large numbers. Post-ovulatory follicles are present more in November than in December. Similarly in the second preparatory phase the post-ovulatory follicles are present very much more in May than in June. (Fig. 25A, 25B, p. 77-79).

In the spawning phase (July-October and January-April) the section of the ovary shows fully matured ova closely packed. In between the mature ova are also present a
Fig. 24 - Sections of the ovary of Puntius (Azan).

A. - Ovary of October fish showing primary and secondary oocytes along with mature ova and post-ovulatory follicle.

B. - Ovary of December fish showing a large number of mature ova along with primary and secondary oocytes.

Abbreviations:

M.O. - Mature ovum

PO.F. - Post-ovulatory follicle.
Fig. 25  - Sections of the ovary of Puntius (Azan).

A. - Ovary of May fish showing a large number of oogonia, and primary oocyte along with a few secondary oocytes.

B. - Ovary of June fish showing secondary oocytes and mature ova along with primary oocytes.

Abbreviations:
OC. - Oogonia
Fig. 26 - Sections of the ovary of Puntius (Azan).

A. - Ovary of July fish showing a large number of mature ova.

B. - Ovary of August fish showing primary and secondary oocytes, mature ova corpore stratica and a large number of post-ovulatory follicles.

Abbreviations:

PO.F. - Post-ovulatory follicle

PR.C.L. - Corpora stratica
considerable number of primary and secondary oocytes and
corpora atretica. Oogonia are comparatively less in number
during the spawning phases (Fig. 24 A, p. 74 B). Post-ovulatoy
follicles are present in varying numbers (Table V, p. 19).

In Rochlea obtia, in the post-spawning phase (September-
November.), the ovaries show the presence of a large number
of oogonia, primary and secondary oocytes. The ovigerous
lamellae are distinct and the outer layer of the ovary is
thick. The ovaries of September contain a few mature ova
also. Corpora atretica are present in large numbers. Post-
ovulatory follicles persist in September but in October and
November they are entirely absent (Fig. 5, p. 24).

In the pre-spawning phase (December-February) the
ovaries contain primary and secondary oocytes along with a
considerable number of oogonia. In the later pre-spawning
phase (February) secondary oocytes, and mature ova are more
in number. Corpora atretica are present in a varying number.
Post-ovulatory follicles are entirely absent (Fig. 7, p. 84).

In the spawning phase (March-May and July-August) the
ovaries consist of a large number of closely packed mature
ova. Intermixed the large mature ova small maturing oocytes
(Primary and secondary) are also present. The number of cor-
pora atretica is comparatively larger than the preceding phase.
Post-ovulatory follicles are absent in March and July, but
they are present in April, May and August. (Figs. 28, 30, p. 84).

In the preparatory phase (June) the ovaries show the
presence of a large number of primary and secondary oocytes
Fig. 27 - Section of the ovary of Robteq (February fish) showing primary and secondary oocytes along with mature ova (Haematoxylin eosin).

Fig. 28 - Section of the ovary of Robteq (April fish) showing a large number of mature eggs, primary and secondary oocytes along with corpora atretica (Haematoxylin eosin).

Abbreviations:
M.O. - Mature ovum
PR.C.L. - Corpora atretica
S.O. - Secondary oocyte
Fig. 29 - Section of the ovary of Rohtee (June fish) showing a large number of secondary oocytes along with mature ova and primary oocytes (Hematoxylin eosin)

Fig. 30 - Section of the ovary of Rohtee (July fish) showing a large number of closely packed mature ova, a few primary oocytes and corpora atretica (Hematoxylin eosin)

Abbreviations:
M.O. - Mature ovum
N. - Nucleus
P.O. - Primary oocyte
PR.C.L. - Corpora atretica
S.O. - Secondary oocyte
FIG. 29

FIG. 30
besides a number of mature ova. Corpora atretica are present in a fairly good number. Post-ovulatory follicles are also present. (Figs. 29 p. 86).

Testes:

In Ambassia renza the testes in October and November (post-spawning period) consist of lobules having spermatogonial cells undergoing early stages of spermatogenesis. A large number of germ cells are also present. Lobules are filled with spermatozoas but the latter are less in number as compared to the spawning period. Interstitial tissue between the lobules is thick (Fig. 31A, B p. 89).

The testes in the prespawning phase consist mainly of spermatocytes and spermatids but germ cells are also present. There is an increase in the number of spermatogonial cells. Interstitial tissue is thin. A large number of lobules filled with spermatozoas are visible.

In the spawning phase (February-March and July-September) the testes consist of lobules densely filled with spermatozoas which are seen as darkly stained round bodies. Spermatogonia, spermatocytes and spermatids are also present. The number of germ cells is less. Interstitial tissue between the lobules is thin. (Fig. 32A, B p. 89).

In the preparatory phase (April-June) the lobules of the testes show different stages of spermatogenesis. Lobules filled with spermatozoas are present in large numbers. Lobules containing spermatogonia, spermatocytes and spermatids are also present. The number of germ cells is less. Interstitial
Fig. 31 - A. Section of the testis of *Ambassis ranga*
showing primary germ cells (Haematoxylin eosin)

B. Section of the testis of *Ambassis ranga*
showing spermatogenesis and primary and
secondary spermatocytes (Haematoxylin eosin)

Fig. 32 - Sections of the testes of *Ambassis ranga*.

A. - Testis of March fish showing lobules filled
with spermatozoa and lobules with different
stages of spermatogenesis (Azan).

B. - Testis of July fish showing a large number of
lobules filled with spermatozoa (Haematoxylin
eosin)

**Abbreviations:**

I.T. - Interstitial tissue

P.G.C. - Primary germ cell

P.SPC. - Primary spermatocyte

S.SPC. - Secondary spermatocyte

SP.C. - Spermatocoele

SP. - Spermatozoo
Fig. 33 - Sections of the testes of *Ambassis ranza*  
(Heamatoxylin eosin)

A. - Testis of June fish showing the lobules densely filled with spermatozoa.

B. - Testis of April fish showing a few lobules containing spermatozoa and also empty lobules.

C. - Testis of August fish showing lobules with little spermatozoa.

Abbreviations:

I.T. - Interstitial tissue
SP. - Spermatozoa
tissue between the lobules is thin (Fig. 33A, p. 91).

In *Puntius ticto*, the histological structure of the testes does not vary much except for the spawning months. The number of germ cells is never very large during the year.

In the spawning periods the testes consist of lobules containing mostly spermatocytes. Spermatogonia, spermatocytes, and spermatids are also present. (Figs. 34B, C, p. 94-95).

Even in the preparatory periods, which intervene the long periods of spawning, the lobules consist of spermatogonia, spermatocytes, spermatids and spermatocytes in abundance. Thin interstitial tissue is present between the lobules throughout the year (Figs. 35A-D, p. 96).

In *Rohitee nctic*, the testes of the post- spawning months consist of lobules containing different stages of spermatogenesis (Figs. 37A, p. 98).

In the pre-spawning and preparatory phases the lobules of the testes are clearly defined and filled with spermatocytes. Large number of spermatogonia are present. Spermatocytes and spermatids are also present (Figs. 38B, C, p. 98-100).

The testes of the spawning months consist mainly of lobules densely packed with spermatocytes. Germ cells and spermatogonia along with the spermatocytes and spermatids are also present (Figs. 38B, A, p. 100).

The study of the histological changes in the gonads of these fishes reveals that in *Ambassa* and *Rohitee*, during the post- spawning months, the ovaries and testes consist mainly of early stages of oogenesis and spermatogenesis respectively.
Fig. 34 - Sections of the testes of *Puntius tetra* (Haematoxylin eosin)

A. - Shows primary and secondary spermatocytes.
B. - Testis of February fish showing lobules containing spermatozoa and various stages of spermatogenesis.
C. - Testis of April fish showing lobules filled with spermatozoa.

Abbreviations:

P.SPC. - Primary spermatocytes
SP.C. - Spermatosonia
S.SPC. - Secondary spermatocytes
SP. - Spermatozoa
Fig. 35 - Sections of the testis of *Puntius ticta*

(Heematoxylin eosin).

A. - Testis of June fish showing lobules densely filled with spermatozoa.

B. - Testis of July fish showing lobules containing different stages of spermatogenesis and also lobules containing spermatozoa.

C. - Shows testis of October fish with lobules containing spermatozoa and also different stages of spermatogenesis.

D. - Shows testis of December fish showing lobules densely filled with spermatozoa.

Abbreviations:

I.T. - Interstitial tissue
P.G.C. - Primary germ cell
S. - Spermatids
SP. - Spermatozoa
SP.G. - Spermatoronia
Fig. 36 - Section of the testis of *Echthea cotia* showing different stages of spermatogenesis (Haematoxylin eosin)

Fig. 37
A. Section of the testis of November fish showing lobules with different stages of spermatogenesis (Haematoxylin eosin)

B. Section of the testis of January fish showing lobules containing spermatocytes and other stages of spermatogenesis.

Abbreviations:
P. C.C. - Primary germ cell
P. SPC. - Primary spermatocyte
S. - Spermatids
SP.C. - Spermatogonia
S. SPC. - Secondary spermatocyte
SP. - Spermatozoa
Fig. 38 - Sections of the testis of *Poichta coticus*.

A. Shows testis of March fish showing a large number of lobules filled with spermatozoas
   (*Haematoxylin eosin*)

B. Shows testis of July fish showing the lobules densely filled with spermatozoas (*Haematoxylin eosin*)

C. Shows testis of June fish showing some lobules containing spermatozoas and others containing various stages of spermatogenesis (*Azan*).

**Abbreviations:**

*SP.C.* - Spermatoconia

*SP.* - Spermatозоы
Spermatozoa are present throughout the year but they are abundant in the pre-spawning, spawning and preparatory phases. In the ovaries mature ova are formed during the pre-spawning and preparatory phases but they are largest in the spawning period. Corpora stractica are present throughout the year. The post-ovulatory follicles (ruptured follicles) are present only during certain periods. In Puntius ticto, however, the ovaries and testes are never found in a spent condition. Different stages of ova are present in the ovary throughout the year in good numbers. Corpora stractica are present throughout the year as in Ambassia and Rohitee. Unlike Ambassia and Rohitee post-ovulatory follicles are also present throughout the year. In the testes lobules, filled with spermatozoa, are present throughout the year and the histological structure is only slightly different in different seasons.

Quantitative assessment:

The study of the reproductive cycle in Ambassia and Puntius has been tackled statistically on the basis of changes in the ovary regarding the size of the ova and is based after a similar study in Phoxinus phoxinus (Bullock, 1939). Diameter of the different developing stages of the ova was measured by means of an okulometer disc calibrated with the stage micrometer slide. In case of Ambassia and Puntius ovaries (in section) of eight and ten individuals respectively were taken into account for this study. More than 4,000 eggs were thus measured in each month. Graphs were plotted for every month with the diameter of the
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Note: Table II

Division = 17.5'
Fig. 39 - A. & B. Graphs showing the relative number of eggs of different diameters in the ovaries of *Ambassia ranea* at various times of the year.
FIG. 39
Fig. 40 - Graph showing the relative number of eggs of different diameters in the ovaries of *Ambassia renna* at various times of the year.
FIG. 40
Table III - Showing the relative numbers of eggs of different diameters in the ovaries of *Puntius ticto* at various times of the year.
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Fig. 41  - A. & B. Graphs showing the relative number of eggs of different diameters in the ovaries of Puntius ticin at various times of the year.
developing oocytes serving as the abscissa and the relative number of eggs serving as the ordinate. In a somewhat similar way the testes cells from comparable areas in each month were counted. The results are summarized in Table II, III, IV and V and the graphs are shown in Figs. 39A, 40A, 41A, 42A, 43A, 44A, 102-110, 143-147.

From the data, obtained by this quantitative study of the ovaries in Ambassia ranca, it can be inferred that in the post spawning phase (October-November) the oocytes of a lesser diameter are very large in number while those of a larger diameter are comparatively less in number (Fig. 40 p. 106). In the pre-spawning period (December-January) oocytes with larger diameter increase in number. The number of smaller oocytes is also very large (Fig. 40, 39A p. 106). In the spawning period (February-March) the number of larger oocytes increases enormously but a large number of smaller oocytes are also present (Fig. 39A p. 104). But in July-August and September the number of smaller oocytes is reduced appreciably and the number of larger oocytes is increased considerably (Fig. 39B p. 104). In the preparatory period (April-June) both smaller and bigger oocytes are present in equal numbers (Fig. 39B p. 104).

In case of Puntius tinca eggs with large diameter are present throughout the year, but their number is more during the spawning period (January-April and July-October). On the other hand number of oocytes with smaller diameter is less (Fig. 41A, 42A p. 110). In the preparatory period (November-December and May-June) the number of oocytes with smaller
Table IV and V - Showing the relative number of cell types in the testes of *Ambassis range* and *Puntius ticto* at various times of the year.
Fig. 42 - A. & B. Graphs showing the relative number of cell types in the testes of *Ambassa* range at various times of the year.
Fig. 43 - A. & B. Graphs showing the relative number of cell types in the testes of *Puntius ticto* at various times of the year.
diameter is comparatively more than those of larger diameter (Fig. 41A p. 116).

In the testes of *Ambassia ranze* spermatocytes are immense during the spawning, pre-spawning and preparatory phases but they are comparatively less in the post-spawning period (Table IV p. 113, Fig. 42A p. 115).

In *Puntius ticto* spermatocytes are immensely present throughout the year but their number further increases during the spawning period (Table V p. 113, Fig. 43A p. 117).

In *Ambassia* spawning activity seems to be almost equally spread over in the two spawning phases. However, in *Rohitee* the spawning activity is mainly restricted to the month of July. In *Puntius*, on the other hand, spawning activity continues for several months.

A quantitative study of the corpora stricte and post-ovulatory follicles present in the ovaries of *Ambassia, Puntius* and *Rohitee* was made in order to assess the spawning periods more precisely in these fishes. For this study eight females of *Ambassia* and *Rohitee* and ten females of *Puntius* were taken into consideration. Corpora stricte and post-ovulatory follicles were counted from twenty sections of ovaries of each one of these fishes in each month. The results are summarized in Table VI.

The data obtained in this study clearly shows that the presence of post-ovulatory follicles (ruptured follicles) can serve as a criterion on which greater reliance can be placed for indicating the period of spawning of the fish
<table>
<thead>
<tr>
<th>MONTH</th>
<th>Number of corpora atretica</th>
<th>Number of post-ovulatory follicles</th>
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<tr>
<td></td>
<td>Ambassia</td>
<td>Puntius</td>
</tr>
<tr>
<td>October</td>
<td>258</td>
<td>71</td>
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<td>November</td>
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<td>45</td>
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<td>August</td>
<td>59</td>
<td>57</td>
</tr>
<tr>
<td>September</td>
<td>111</td>
<td>55</td>
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</tbody>
</table>

**EXPLANATION:**

Table showing the number of corpora atretica and post-ovulatory follicles in *Ambassia, Puntius* and *Rohtee* during the different months of the year.
concerned. It is based on the assumption that the presence of post-ovulatory follicles in the ovary would be preceded by the spawning of the fish.

The first appearance of the post-ovulatory follicle would mean that spawning took place in the preceding month but the subsequent appearance of post-ovulatory follicles in the next following months would also suggest the persistence of some of the preceding post-ovulatory follicles in their process of gradual absorption.

In *Ambassia renge* the post-ovulatory follicles are seen in March which means that spawning has taken place in the preceding month. Similarly, these follicles are present in August, thereby suggesting that spawning took place in July. These follicles persist till the end of the spawning and beginning of the post-spawning periods. In *Rohtees* these follicles are present in April, because spawning begins in March, the follicles, however, persist till June. The post-ovulatory follicles are again seen in August because the second spawning period of the fish begins in July. They are entirely absent in the later period of the post-spawning phase. In *Puntius* the post-ovulatory follicles are present throughout the year showing that the fish breeds for most of the period in its annual cycle.

**Discussion:**

The morphology of the gonads and gonoducts in *Ambassia renge*, *Puntius ticto* and *Rohtee cotic* shows a typical teleostean disposition.
In the formation of the primary oocyte Bullough, (1939) has described a synizesis stage in which the chromatin is clumped to one side of the nucleus. Such a stage could not be seen in *Ambassia, Puntius* and *Rohtee*. Without getting clumped, the chromatin breaks up into small bits which arrange themselves beneath the nuclear membrane. The presence of a yolk nucleus has been described by some workers (Wheeler, 1924; Choudhry, 1952; Sathyanesan, 1962 and Khanna and Pant, 1967). Such a nucleus is absent in *Ambassia, Puntius* and *Rohtee*.

The formation of fish eggs with special reference to the vitellogenesis and the histochemistry of the yolk granules has been studied by Yamamoto (1956, 1957 and 1963). He classified fish eggs on the basis of having a "continuous mass of yolk" or a "non-continuous mass of yolk". The former has minute yolk globules as found in *Fundulus* (Marza et al., 1967) and *Glyptosternum pectinopterum* (Khanna and Pant, 1967) while the latter contains large yolk globules as reported by Yamamoto, (1958) in *Clupea, Belsare*, (1962) in *Ophiocephalus* and *Barr*, (1963) in *Pleuronectes*.

In *Ambassia ranza*, *Puntius ticto* and *Rohtee cotio* the eggs belong to the first category as they have minute yolk globules.

There is a great controversy regarding the formation of the new crop of eggs. Wheeler, (1924) and Yamamoto, (1956) have stated that the new crop of oocytes are formed from the follicle cells. In his study of *Cottus baikdii* Henn (1927) observed that the oocytes are derived exclusively from the
pre-existing oogonia. A similar conclusion was reached by Craig-Bennett, (1934) who observed many oogonial divisions in the stickle-back shortly after spawning. In an exhaustive study of a number of species belonging to the Cobiiformes and Blenniformes, Eggert (1931) observed that the oogonia divide mitotically to give rise to oocytes shortly after spawning. Mendoza, (1943); and Bera, (1960) are of the opinion that the new oocytes develop from the germinal epithelium covering the lamellae. Belsare, (1962) believes that in Ophioccephalus punctatus the new crop of oocytes develop from the residual oogonia as is seen in the spent ovary of Musil cephalus (Stenger, 1959) and Plecostomus altivelis (Horine, 1961). In Phoxinus phoxinus Bhargava, (1966) has shown that the oogonia and primary oocytes are also formed from the thickened granulose of the post-ovulatory follicle.

In Ambassia, Puntilus and Rohtee the oogonia are always found inside the ovigerous lamellae which indicate that they develop from the germinal epithelium covering the lamellae.

The stromatic follicles and their probable significance in the fish ovary have been discussed by some authors. Jaski, (1939) states that some of the mature ova give rise to the corpus luteum in Lebiasites reticulatus. In Rhodeus amarus, Bretschneider and Duyvène de Wit (1947) have proved that the mature ova during the reproductive period from the corpora lutea. Hoar, (1955 and 1957) states that these structures in the fish ovary have been termed corpora lutea because they develop from the follicular epithelium and theca as in higher
vertebrates. He, however, gave two terms pre- and post-
eovulatory corpora lutea, the former resulting from the oosiosis
of unspawned ripe ova and the immature oocytes of the spent
fish and the latter resulting from the follicles after the
discharge of the ripe ova. In the whiting and the Norway
Pout (Gokhale, 1957) the resorption of the oocytes results in
the formation of glandular structures which may be a form of
corpus luteum but there is no evidence to show an endocrine
function. Tromp-Bloem, (1959) has clearly shown the absence
of corpora lutea in the ovaries of Gasterosteus aculeatus
during the reproductive period.

Recently, Bell, (1960 and 1965); Bhargava, (1966) and
Rejalakshmi, (1966) have used the term corpora arctica or
stretis of the oocytes for the unspawned ripe oocytes or the
immature oocytes undergoing oosiosis.

In the present work on Ambassis ranga, Puntius ticto and
Rohtee cotio the term corpora arctica has been used for the
unspawned ripe ova or immature oocytes undergoing oosiosis.
The follicles undergoing resorption after the discharge of the
ripe ova have been termed as the post-ovulatory follicles,
a term which seems more meaningful and justified than the
term ruptured follicle.

The study of corpora arctica in the ovaries of these
three fishes reveals that they are present throughout the
year. In Ambassis, however, they are less in the early
spawning phase. Sometimes hypertrophied granulosal cells
of the arctica follicles (corpora arctica) are regarded as
the possible source of the ovarian hormone (Hoar, 1955). In
the present study there cannot be given any histological
evidence in favour of the endocrine function of these cells.
It has been suggested that the so called pre-ovulatory
corpore luteum, which are universally present in fish ovaries,
form the main mass of endocrine tissue of the teleost ovary
(Duyvène (Bretscheider and de Wit, 1947; Hoar, 1955; Ball, 1960).
However, the chemical nature and site of production of the
hormone or hormones remain unknown (Pickford and Atz, 1957).

Hypertrophy of the follicular epithelium to form "luteal"
tissue after ovulation has been shown to occur in some fishes.
Craig-Bennett (1931) has reported the occurrence of a short
lived corpus luteum in the stickle back. Post-ovulatory
hypertrophy of the follicle of Fundulus heteroclitus
(Matthews, 1938) results in the formation of a structure which
closely resembles a corpus luteum. Recently, however, in
Gobius giurus (Rajalakshmi, 1966) it has been reported that
the post-ovulatory follicle undergoes resorption without the
formation of corpus luteum.

The ruptured follicles have been compared to the
mammalian corpora lutea by Craig-Bennett, (1931) whereas Mendoza,
(1943); Gokhale, (1957); Tromp-Bloem, (1959); Stenger, (1959) and
Bars, (1960) do not regard it comparable to the corpus luteum
of the mammalian ovary.

The present study of the post-ovulatory follicle in
Ambassis ranca, Puntius titteo and Rohttee cotic shows that
these follicles undergo resorption without the formation of
corpus luteum as in Cobius giurus (Rajakshmi, 1966). These follicles, are therefore, not comparable to the mammalian corpora lutea in any way.

Nickle and Butenberg, (1936) and Prabhu, (1956) have studied the spawning periods in some fishes. They have found a close connection between spawning tendencies and the development of the intra ovarian eggs. The individual spawns once a year when the mature ova are sharply differentiated from the general stock. If in addition to the mature ova two distinct groups of maturing ova are present the fish spawns twice in a season. When there will be no sharp separation between the general egg stock and the maturing eggs and many intermediate stages will be present in addition, the fish spawns throughout the year. This has been found true for the three fishes studied. Ambassia renza and Rohitee cotic breed twice in a season like Cobicocephalus punctatus (Belsare, 1962) because two groups of maturing ova in addition to the mature ova are seen in the ovaries. Puntius ticto breeds all the year round as there are present different developing stages of the ova almost throughout the year.

Histological structures such as corpora stroticas and ruptured follicles are described in the study of the seasonal changes in the ovary of quite a number of fish. The ruptured follicles or post-ovulatory corpora lutea (post-ovulatory follicle) have never been considered quantitatively to determine the spawning period of the fish.
In the present work, the appearance of the post-ovulatory follicles in the ovary is found to be of a cyclic nature. A quantitative study of these follicles clearly indicates that their presence in the ovary could reasonably serve as a criterion to determine the spawning activity of the fish.

In all the three fishes studied corpora lutea are found throughout the year. Their presence does not very much help in elucidating the reproductive cycle of the fish. However, the post-ovulatory follicles are present in certain periods of the year and as such much reliance has been put on them in following the reproductive cycle (Table VII p. 119). Other methods such as measurement of egg diameter on a statistical basis and changes in volume of the gonads in different seasons, after a similar study in Phoxinus by Bullough (1939), have further helped in clearly elucidating the reproductive cycle of these fish (Table II p. 102, 103, Fig. 18-20, p. 55, 56, 92, 104, 106, 110, 115, 117).

The early literature on the occurrence of interstitial tissue in the teleost testis is contradictory. Craig-Bennett (1931) found Leydig cells easily recognizable in the testes of Casterosteus aculeatus and these showed cyclical behaviour which could be correlated closely with the sexual activity of the fish. Potter and Hoar, (1954) have reported the presence of typical interstitial cells in the testes of Oncorhynchus keta. The work of Marshall and Lofts (1956, 1957) have shown that some teleosts (Esox lucius, Salvelinus willifordii, Leboc sp.) lack a true interstitium. According to Robertson, (1958) interstitial cells are present in the testis of the rainbow-trout Salmo gairdnerii.
In the present work interstitial tissue is present in the testes of *Ambassia*, *Puntius* and *Rohtee*. In *Ambassia* and *Rohtee* the interstitial tissue becomes thin during the spawning and preparatory periods. In *Puntius*, however, it is always thin.

In the case of testes in *Ambassia*, *Puntius* and *Rohtee* the spermatogonia, spermatocytes, spermatids and spermatozoa are formed from the primary germ cells of the follicles only.

In the reproductive cycle of *Fundulus heteroclitus* (Matthews, 1938) spermatids are evident during the fall and early winter. In *Phoxinus* (Bullough, 1939) there is a replenishment of spermatogonia and primary spermatocytes but further stages in spermatogenesis are delayed until the spring. In *Perea* (Turner, 1918), *Gambusia* (Geiser, 1924) and *Gasterosteus* (Craig-Bennett, 1931) spermatogenesis is complete and a potential maturity occurs in the male several months prior to the breeding season. Weisel, (1943) studied the testes of the Sockeye Salmon. He found that in the spawning season the testes exhibit vacuolization and pycnosis in the epithelial cells lining the ducts, in the spermatogonia and the follicle cells.

In *Heteromeustes fossilis* (Chosh and Kar, 1952) the males do not exhibit any seasonal changes in the testicular activity. Sundararama, (1960) has, however, reported seasonal changes in the testes of the same fish.

In the present work the seasonal changes in the testes indicate that while the spermatozoa are seen throughout the year but they increase in number during the spawning periods.
Ecologic and neurologic factors are believed to be important in the control of the reproductive cycle and there is much evidence to suggest that the effect of these factors are mediated through the endocrine glands (Ghosh and Kar, 1952). The work of Matthews (1939) on Fundulus shows that the gonadotrophic hormones of the anterior pituitary influence the ovarian function in fishes.

The possible endocrinological mechanism responsible for the gonadal periodicities in Ambassia, Puntius and Rohitee is discussed on page 242.