MORPHOLOGICAL & HISTOLOGICAL CHANGES IN THE GONADS OF THE ESTUARINE CICHLID FISH *ETROPLUS SURATENSIS* (BLOCH) DURING GONADAL DEVELOPMENT

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**ABSTRACT**

The morphology and histology of the gonads of *Etroplus suratensis* associated with gonadal development were studied. Seven stages of maturity were arbitrarily recognized according to their macroscopic appearance. Six spermatogonietic stages were recognized microscopically viz. primary germ cells, spermatogonia, primary spermatocytes, spermatids and spermatozoa. In the ovary, nine stages of oocyte maturation were identified and they are chromatin nucleolus, early perinucleolus, yolk vesicle, primary yolk, secondary yolk, tertiary yolk and ripe egg stages. The study also shows that the female fishes are multiple spawners.

**Key-words:** Morphology, histology, gonads, estuarine cichlid, *Etroplus suratensis.*

**INTRODUCTION**

Two species of fish belonging to the family Cichlidae viz. *Etroplus suratensis* and *Etroplus maculatus* are found in Sri Lanka waters. Of these *E. suratensis* (Bloch) is a brackishwater and an important food fish. This fish has recently been introduced into many of the man made reservoirs in Sri Lanka where it now flourishes.

Studies on *E. suratensis* have been a few and the biology of this fish in Sri Lanka is little known. Recently Costa (1983) has studied the morphology, food habits and fecundity of this fish from three diverse habitats in Sri Lanka. The present study concerning the morphological and the histological changes of the gonads during their development was carried out to get an understanding of the reproductive cycle of this popular food fish.

**MATERIALS AND METHODS**

Samples of fish collected from Colombo (Beira) lake were dissected in the laboratory and the gross morphology of the gonads carefully observed. The gonads were then separated into different maturity stages based on the international scale of Hjort (1910).

For histological studies, gonads of known maturity stages were cut into approximately 1 cm pieces and were preserved in 10% formalin, Zenker's fluid and Bouin's fluid. The advanced stages of the female gonads were preserved in Bouin's fluid and in Zenker's fluid. Sections of 6-10 μ were then cut. Because of the yolkly oocytes it was difficult to cut some stages of female...
gonads. To overcome this difficulty, they were soaked in a 9:1 mixture of 60\% alcohol and glycerin, a few minutes prior to the sectioning of the tissue (Baker 1950). Sections were subsequently stained with either Delafield's haematoxylin and eosin or Mallory's triple stain.

The quantitative data for the testis was taken from sections passing through the middle of the testis and for ovaries the measurements were made using the procedures adopted by Pollard (1972).

RESULTS

General morphology of the gonads

The testes are paired and lie immediately below the air bladder. The two halves remain separated for most of their length. They are flattened and elongated and fuse posteriorly to form a common sperm duct. The urinary duct and the common sperm duct join and open into the cloaca by a common pore. In the male the diameter of the anus is larger than that of the urinogenital pore.

The ovaries are also paired organs and lie ventral to the air bladder. They are tubular and join posteriorly to form the common oviduct and open into the cloaca separately from the urinary ducts. In the females the urinogenital opening is larger than the anal opening.

Morphological changes seen in the development of gonads

The size, form and colour of the testis and ovaries changes according to the degree of maturity they attain. Seven arbitrarily chosen maturity stages were recognized here for gonads of both sexes. The macroscopic appearance observed for these maturity stages in the testis and ovaries is given in Tables I and II respectively.

Table I: The maturity stages of testis and their macroscopic appearance in *E. suratensis*.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Macroscopic appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Immature virgin</td>
<td>Minute, thread like, translucent and colourless.</td>
</tr>
<tr>
<td>II Developing virgin</td>
<td>Short, strap like, translucent and white.</td>
</tr>
<tr>
<td>III Developing</td>
<td>Occupy less than half of the body cavity.</td>
</tr>
<tr>
<td></td>
<td>Strap like, opaque and creamy white.</td>
</tr>
<tr>
<td>IV Maturing</td>
<td>Occupy more than half of the body cavity.</td>
</tr>
<tr>
<td></td>
<td>Strap like, opaque and creamy white.</td>
</tr>
<tr>
<td>V Mature</td>
<td>Occupy more than 2/3 of the body cavity.</td>
</tr>
<tr>
<td></td>
<td>Strap like, yellowish white and opaque.</td>
</tr>
<tr>
<td>VI Ripe</td>
<td>Appearance of testis as in V but the milt could be extruded easily by slight pressure on the abdominal wall.</td>
</tr>
<tr>
<td>VII Spent</td>
<td>Testis shrunken and blood shot.</td>
</tr>
</tbody>
</table>
Table II: The maturity stages of ovaries and their macroscopic appearance in E. suratensis.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Macroscopic appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Immature virgin</td>
<td>Ovaries very small and narrow, colourless and translucent. Oocytes invisible to the naked eye.</td>
</tr>
<tr>
<td>II Developing virgin</td>
<td>Ovaries increase in size, creamy white, ovary wall is thick and opaque. Oocytes slightly visible to the naked eye.</td>
</tr>
<tr>
<td>III Developing</td>
<td>Ovaries become more rounded, ovary wall becoming thinner and translucent. Some oocytes visible to the naked eye while some are creamy white and opaque.</td>
</tr>
<tr>
<td>IV Maturing</td>
<td>Occupy half of the body cavity. Ovary wall is thin and translucent. Various sizes of oocytes are visible; larger oocytes are yellow and opaque.</td>
</tr>
<tr>
<td>V Mature</td>
<td>Occupy more than half of the body cavity. Ovary wall is thin and translucent. Large yellow oocytes are present.</td>
</tr>
<tr>
<td>VI Ripe</td>
<td>Same as stage V. But oocytes are brownish yellow and translucent. Slight pressure on the abdominal wall will extrude eggs.</td>
</tr>
<tr>
<td>VII Spent</td>
<td>Flat and blood shot.</td>
</tr>
</tbody>
</table>

General histology of the testis and ovary

Each gonad is enclosed by a very thin and delicate membrane the peritoneum (< 1 μ). In the sections stained with haematoxylin and eosin, the nuclei of the peritoneum can be differentiated from the rest of the tissue, in that they are unevenly spaced and their chromatin is somewhat condensed. Wall of the testis and ovary tunica which is made up of thick fibrous connective tissue lies immediately beneath the peritoneum. This can be differentiated in triple stain preparations.

Testis

In the testis, fibrous connective tissue septa run inwardly from the tunica and divide the testis into a number of lobules. Usually lobules differ in size. Blood capillaries are present both in the wall of the testis and interlobular septa. Within the lobules spermatogenetic cells are present. In E. suratensis six spermatogenetic stages were recognized.

Primary germ cells

These cells are large and more rounded. (diameter 13 μ). The nucleus is large (mean diameter 6.9 μ) and lightly stained. The central region of the nucleus has a single large nucleolus which joins the periphery by thin threads. Cells occur in interstitial areas forming new lobules and in the walls of the existing lobules as clusters. They divide mitotically to form spermatogonia.
Spermatogonia

These cells are smaller than the primary germ cells (diameter—10µ.), having more compact nucleolus than the first stage cells (mean diameter—5.2µ.). The nucleus has a prominent nucleolus. The cells are attached to the lobule wall in groups. They divide mitotically to form primary spermatocytes.

Primary spermatocytes

These cells are small (diameter—7µ.) having little cytoplasm and dense nuclei (mean diameter—4.29µ.) containing dense chromatic material but lack a distinct nucleolus. These cells are often observed in groups.

Secondary spermatocytes

These cells are formed by meiotic division of primary spermatocytes. No cytoplasm can be seen around the nuclei of these cells. The chromatin in the nucleus is very dense and the nucleous very small (mean diameter—2.8µ.). These cells are found in large clusters extending into the lobule lumen. They divide mitotically to form spermatids.

Spermatozids

These cells have no cytoplasm. The nuclei which contain concentrated chromatin are very small (mean diameter—2.13µ.). These cells occur in large clusters. They form the spermatozoa.

Spermatozoa

These cells have pear shaped head (mean width, 1.43µ.) and tail (10µ.). They often occur in the lobule lumen as parachute shaped clumps.

Ovary

The ovary of E. suratensis is connected to the dorsal body wall by a thick mesovarium. A large blood vessel passes between the tunica and the mesovarium. Oocytes of various sizes are embeded in a loose connective tissue stroma of the ovary and these show no definite arrangement. The stroma and the tunica contain many blood capillaries. In the ovary of E. suratensis nine stages of oocyte development have been identified.

Chromatic nucleolus stage

In this stage oocytes occur in nests. They are very small (diameter-20µ.). and cells have distinct nuclei, surrounded by a layer of cytoplasm.

Early perinucleolus stage

The oocytes range in diameter from 20–100µ., and are variously shaped depending upon the stresses imposed on them by expanding oocytes around them. The nucleus (diameter 15–45µ.) is still larger relative to the cytoplasm, and contains 6–16 basophilic nucleoli per section. The cytoplasm gradually becomes more extensive. A thin layer (follicle) envelopes the small and large oocytes.
Late perinucleolus stage

Most of these oocytes at this stage are regular in shape, ranging in diameter from 70–170 μ. The nucleus (35–60 μ) which contains basophilic nucleoli (12–28 per section) becomes eosinophilic. The cytoplasm of the oocytes tend to lose their affinity for haematoxylin. In some oocytes a non cellular membrane (1 μ in thickness = Zona radiata) is present between the developing oocytes and the follicle layer.

Yolk vesicle stage

Oocytes range in diameter from 160–200 μ. The nucleus (35–75 μ) still has basophilic nucleoli 15–28 per section. Minute yolk vesicles (1–2 μ diameter) are present in the peripheral region of the cytoplasm. The zona radiata gradually increases in thickness (1–2 μ), staining bright pink with haematoxylin and eosin and blue with Mallory's triple stain. The follicles become thickened at this stage and forms a cellular layer with deeply staining nuclei (the granulosa). A thin layer of connective tissue (the theca) surrounds the granulosa.

Primary yolk stage

Oocytes at this stage range in diameter from 210 to 430 μ and are characterized by the presence of minute yolk granules in the cytoplasm. The yolk vesicles vary in size (4–6 μ diameter). The zona radiata and granulosa progressively increase in thickness from 3–6 μ and 1–3 μ respectively.

Secondary yolk stage

These oocytes range in size from 360–560 μ. The nucleus starts to disintegrate. Yolk vesicles increase in size (10–16 μ). Yolk granules and yolk vesicles occupy about half of the cytoplasm. The zona radiata remains unchanged while the granulosa increases in thickness (3–5 μ).

Tertiary yolk stage

The oocytes increase in size (180–700 μ). Some oocytes show disintegrated nucleus and some have none at all. Yolk granules coalesce to form large granules (16–30 μ in diameter). Yolk granules and yolk vesicles occupy most of the cytoplasm. The thickness of the zona radiata tends to decrease (3–5 μ). The cells of the granulosa increase in diameter.

Mature yolk stage

These oocytes range in diameter from 710–820 μ. Cells have no nuclei. Yolk vesicles and yolk granules occupy almost entire cytoplasm of the oocytes. The yolk granules have coalesced to form larger packed granules. The peripheral yolk tends to fuse, forming a continuous border within the zona radiata. Zona granulosa increases in thickness (upto 16 μ).
Ripe egg stage:

These oval shaped eggs reach a diameter of 1000μ at the shortest axis and 1900μ at the longest axis as seen in the formalin fixed material. They are translucent brownish yellow in colour and separated from the connective tissue of the theca.

In addition to the above oocyte development stages, atresia of oocytes which fail to attain maturity was also observed.

Histological changes in the testis and ovary during the gonadal development

No changes were noted in the peritoneum and its thickness always remained constant in the testes as well as in the ovaries. The tunica was seen to decrease in thickness. Blood capillaries become dilated with development.

Testis

Lobules of the testis increase in size and their walls become thinner with development. Changes were seen regarding the occurrence of the different spermatogenetic stages in the lobules as well as their relative abundance with development.

Maturity stage I: Immature virgin

Though this stage was observed macroscopically, the fine thread like structure of the testis at this stage made its sectioning very difficult.

Maturity stage II: Developing virgin

Thickness of the testis wall is 6μ and lobules range in size from 15 to 62μ. Five spermatogenetic stages were observed; viz. primary germ cells, spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids. Some lobules are filled with primary germ cells and spermatogonia while the rest show them attached in groups to the wall of the lobule which contains a lumen. Primary spermatocytes are present in groups near the primary germ cells and spermatogonia. Secondary spermatocytes extend to the lobule lumen, spermatids lie only in the centre of the lobule lumen in clusters. Primary germ cells and spermatogonia occur in large numbers but only a few spermatids were observed. All lobules are spermatogenetically active. It is possible that spermatogenetic stages are undergoing divisions.

Maturity stage III: Developing

The lobule of the testis range in diameter from 32–110μ. Six spermatogenetic stages were observed in this stage. Lobules filled with primary germ cells and spermatogonia were not seen as in the previous stage. All lobules contain a lumen having secondary spermatocytes, spermatids and spermatozoa. Primary germ cells and spermatogonia were found adjacent to the lobule wall only. All six stages of spermatogenesis can be seen in some lobules. Secondary spermatocytes and spermatids increase considerably and spermatozoa are rarely found. All lobules are spermatogenetically active.
Maturity stage IV: Maturing

Walls of the testis and the lobules were seen to decrease in thickness (3–5μ). All six spermatogenetic stages were observed. Some of the lobules contain little or no primary germ cells and spermatogonia. Few lobules containing only spermatids and spermatogonia were also observed. Therefore, it could be that while some lobules show decreasing spermatogenetic activity others show advanced spermatogenesis at this stage.

Maturity stage V: Mature

Thickness of the wall of the testis and the lobules were about 2μ. Lobules of the testis increase in size (100–280μ). All lobules of the testis in this stage are packed with spermatids and spermatozoa. Spermatogenesis is less advanced and number of spermatozoa seems to be higher than for the other types.

Maturity stage VI: Ripe

Lobules reach from 120–290μ in size. All spermatogenetic stages were observed. Primary germ cells and spermatogonia occur adjacent to the lobule wall only. In some lobules spermatozoa are depleted, but the rest remain filled with spermatozoa. Primary spermatocytes are rarely seen. At this stage spermatogenesis continues but restricted to a few lobules.

Maturity stage VII: Spent

Walls of the testis and lobules shrunk. Therefore all lobules are small in size. Primary germ cells and spermatogonia (small numbers) were observed.

Ovary

Considerable changes were seen in the occurrence of the oocytes maturation stages and in the atretic oocytes.

Stage I: Immature virgin

Four stages of oocyte development were observed; chromatin nucleolus, early peri nucleolus, late peri nucleolus and yolk vesicle stages.

Stage II: Developing virgin

In addition to the previous stages primary yolk stage was also present.

Stage III: Developing

Six oocyte development stages were observed. Additionally secondary yolk stage was present. Atretic oocytes were also seen.

Stage IV: Maturing

In addition to the previous stage, tertiary yolk stage was observed. Atretic oocytes were also seen.
Stage V: Mature

Eight stages of oocyte development including the mature yolk stage were observed. Atretic oocytes were also present.

Stage VI: Ripe

Atretic oocytes were observed.

Stage VII: Spent

Atretic oocytes and chromatin nucleolus, early peri nucleolus, late peri nucleolus and primary yolk stage were observed.

Fig. 1 shows the percentage frequency distribution of different oocyte diameters present in various maturity stages. This shows that oocytes upto 200μ occur in large numbers in all maturity stages. In stage II the differentiation of maturing oocytes begin and the complete separation of oocytes (ripe eggs) from the maturing batch occurs in stage VI.

Fig. 1. Frequency distribution of oocyte diameters in ovaries at each maturity stage (Grouped in 50μ sizes).
DISCUSSION

Though there are observed differences in the gross morphology, the general order of morphological changes of the gonads of *E. suratensis* in the development of gonads appears to be the same as described for most other teleost fish (Gokhale, 1957; Pollard, 1972 and Davis, 1977).

The general histological structure of the testis of *E. suratensis* is the same as in other teleost species. The formation of germ cells is similar to that which has been observed in many fish. In some teleost species an extra spermatogenetic stage namely, elongate migratory germ cells has been recorded (Gokhale, 1957). However, such a stage was not evident in this study and therefore is similar to the observations made for *Clupea harengus* (Bowers and Holdiday, 1961) and for *Galaxias maculatus* (Pollard, 1972).

During the development of testis several marked histological changes occur. In the maturing stage of the testes of *E. suratensis*, while some lobules show advanced spermatogenesis, a few lobules show decreasing spermatogenetic activity. This feature has also been noted in *Tandanus tandanus* by Davis (1977), who stated that these lobules later function as sperm storage lobules. This may also be the case with *E. suratensis*.

The general histological structure of the ovary of *E. suratensis* is also not much different from the other teleost species described so far.

The sequence of oocyte maturation was divided into nine stages after Yamamoto (1956). Apart from some minor differences the general pattern of development and histology was similar to those observed in other teleost fishes. The yolk vesicle in the oocytes of the ovary of *E. suratensis* followed a centripetal sequence of development and occupied the full cytoplasm of the oocytes. A similar pattern has been observed in *Ophicephalus punctatus* (Belsare, 1962). It was observed in *E. suratensis* that with oocytes maturation, the egg gets surrounded by three membranes (theca, zona granulosa and zona radiata). Similar observations have been made by Yamamoto (1956), Gokhale (1957), Hurly and Fisher (1966) and Davis (1977). Hurly and Fisher (1966) also observed minute pore canals in the zona radiata of the oocytes of *Salvelinus fontinalis* and the granulosa has been recognized as having a nutritive value during yolk formation; the nutrition transfer from the granulosa to the cytoplasm of the oocytes taking place via these pore canals of the zona radiata.

Certain histological differences were observed between the maturity stages which correspond to macroscopic external changes that take place in the ovary. In stage I only immature oocytes (chromatic nucleus, early perinucleolus, late perinucleolus and yolk vesicle) were observed. In stage II presence of primary yolk stage shows that the deposition of yolk in the ovary of *E. suratensis* occurs at this stage. Stages III to IV can be considered as the maturing phases of the oocytes. Stage V is the mature egg stage and ripe eggs are present in stage VI. Atretic oocytes are present in stage VII.
Prabhu (1956) had suggested that intra ovarian egg studies of fish often helps to obtain details about the nature of the spawning. In *E. suratensis* the percentage frequency distribution of oocyte diameters (Fig. 1) shows that stage V of the ovary has three batches of oocytes-immature, maturing and mature. It is possible to conclude that *E. suratensis* is a multiple spawner.

REFERENCES


