

CHAPTER - FIVE
SUMMARY AND CONCLUSION

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5.0 Introduction:

In the beginning of 20th century there was wide spread of epidemic malaria, elephantiasis, gastro, cholera, filaria, malaria, dengue and other water born diseases. These diseases are spread through the agency of nsects.

These are due to, an increased organic load and surface run off during the rainy season is resulted into partial eutrophication of river water and the formation of river stagnancies. Such type of habitat become the breeding and spawning place for different types of mosquitoes. This type of situation leads to spread of epidemic diseases. The segments of a and Krishna rivers in Sangli district were selected for this research work. In recent years (2003-07) the repeated floods in Krishna river have created many problems of public heath and the hygiene. There was also considerable increase in the breeding places of mosquitoes such as freshwater ditches, ponds, swamps and marshy wet-lands.

Chemical control of mosquitoes has proved to be hazardous to the environment. Use of insecticides virtually failing, a biological control was the only alternative left to keep the cheak on the insects vector. Destruction of larvae will eventually cause a rapid reduction & control of adult insect population. Since for this research work larvivorous fishes were selected. A no of larvivorous fishes directly feed upon mosquito

larvae, hence there is a very effective control on the population of the mosquitoes.

This research work in larvivorous fishes is mainly concerned with histology and histochemistry of gonads, however their safe maintenance in aquarium & fresh water ponds, or ditches their prolific reproduction and wide range redistribution of them into different localities of Palus Tahsil, is the main theme.

5.1 Materials and Methods :

The present research work is not only related to the reproduction, histology and histochemistry of gonads of the larvivorous fishes but the survey of other larvivorous fishes from the selected area.

Following larvivorous fishes were found in the Krishna and Yerala river and adjoining several streams, irrigation canals and water pools-

Different species of *Puntius*

- *Wallago attu*
- *Rita rita* (Ham)
- *Mystus seenghala*
- *Mastocembelus armatus*
- *Rasbora daniconius*
- *Clarias batrachus*

For the study of histology and histochemistry of gonads in larvivorous fishes, the following fishes were selected- Guppy (*Poecilia*

Plate No. 1 : Caption to Figures

Fig. No. 1 – Note the common freshwater cat fish *Mystus Seengala* X 10

Fig. No. 2- Indian topminnow, *Puntius chola* maintained in the aquaria X 10

Fig. No. 3 –

I - Male *Chanda ranga* with prominent lateral line and lunar shaped visceral pouch X 10

II & III – Female *Puntius Sophore* – Note the prominent caudal black spot and pot belly appearance.

IV, V & VI – Male *Puntius Sophore* – with golden operculum and stream lined body shape X 10

PLATE NO. 1



1



2



3

Plate No. : Caption to Figures

Fig. No. 1 – Note the *Rasbora danicus* collected from freshwater well, note a band of chromatophores on the lateral line from snout to tail also note orange chromatophores on the dorsal surface, during active breeding period X 10.

Fig. No. 2 –

Fig. I to V – Male *puntius sophore*

Fig. VI & VII – Pot bellied female *puntius sophore* obtained from fish catch X 10.

Fig. No. 3 –

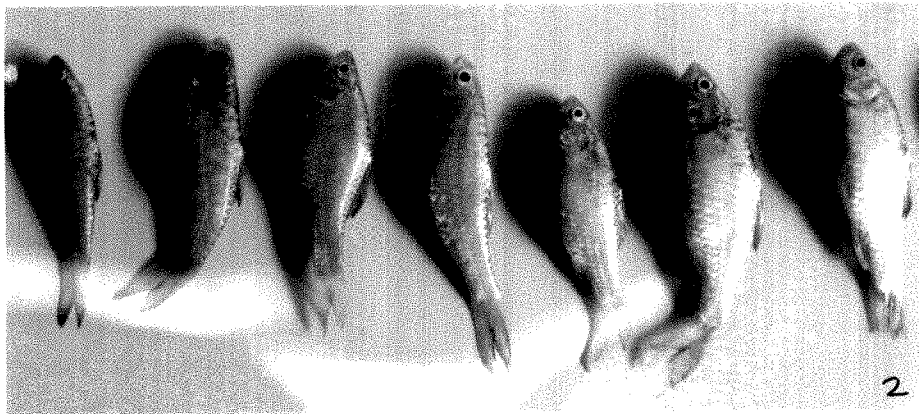
I - Note young larvivorous *Tilapia mosambica* with superior notched wide mouth X 10.

II – Young larvivorous *Channa punctatus*, in dorsal view X 10

III- Freshwater topminnow *P. sophore* mature stage X 10.

IV- Note the banded freshwater topminnow *Puntius sophore* X 10

PLATE NO.



reticulata), *Chanda ranga*, *Puntius sophore*. The live guppies brought to laboratory and kept in aquarium and freshwater garden ponds. They were regularly fed with baby shrimp bran meal and crustacean larvae.

The guppies were proliferated in thousands and millions. With the help of team of college students, the guppies were redistributed in the water reservoirs of different villages in Palus Tahsil.

Chanda ranga and *Puntius sophore* fishes were brought from the fresh catch during their sexual inactive prebreeding, active breeding and the post breeding period. They were also maintained in the laboratory aquarium for acclimatization.

The larvivorous fishes were quickly dissected out for their testes and ovary. The tissue was fixed in cold 2% calcium acetate in 10% formalin (CAF). The fixed tissues were kept at 4⁰C for 24hr. Then the tissues were washed in clean running water for 24hr, dehydrated in ascending grades of alcohol (30%, 50%, 70%, 90% and absolute) embedded in paraffin wax at 58⁰C and sections were cut at 5-7 micron. Following histological and histochemical techniques were employed for the identification of mucosubstances or mucins-

- 1) Routine HE technique
- 2) Alcian Blue pH 1 staining technique.
- 3) Alcian Blue pH 2.5 staining technique.
- 4) PAS (Periodic schiff reagent) staining technique.

5.2 Observations

5.2.1 Guppy (*Poecilia reticulata* peter)

1. Sexual dimorphism:

In guppy, the males are smaller than the females. The full grown males are attractive with allured colouration in their caudal fin and with a lateral prominent black pigment spot.

The males are equipped with sterio type moving gonopodium. The mature male guppy was identified with the presence of hook like structure at the tip of gonopodium.

2. Gonopodium:

Gonopodium is present in male guppy. It was observed at the base of anal fin. At the base of gonopodium a bundles of series of smooth muscels are present. It mainly act as copulatory organ in male guppy.

3. Histology and Histochemistry of testis:

HE staining technique-

T.S. of testis of guppy show intense basophilic staining in primary sperm duct, secondary sperm duct, endothelial capillary, secretory cells of secondary sperm duct. The moderate staining observed in groups of interstitial cells, sertoli cells, spermatogonia also their was faint staining in sperm heads.

AB pH 1 technique and AB 2.5 staining technique-

It was observed the moderate to intense staining .

The staining with AB pH 1 is light blue intensity and the staining for AB pH 2.5 bluish green in colour. The staining was obtained, the AB pH 1 staining was revealed in interstitial connective tissue, the basement membrane of testicular follicles and in few germinal elements of acinus, the staining of AB pH 2.5 was very intense in Leydig cells, and the Sertoli cells of sperm ducts, and the sperms.

PAS staining technique-

The PAS staining was moderate in all testicular acini and their element, however both granular and intense magenta pink staining was revealed in secretory cells of intercalated sperm ducts.

4. Histology and Histochemistry of Ovary-

HE staining technique-The HE staining was revealed in the connective tissue, hilus, germinal epithelium, dividing oocytes and the follicular palcenta.

AB pH1 and 2.5 staining technique -

AB pH 1 staining was selectively very intense in vascular connective tissue of the ovary, the thecal cells of the maturing follicles and the corpus luteum.

The staining was also revealed in the nuclear histoproteins, follicular cells and secretory granules.

PAS Staining Technique –

The sites of follicular placenta, yolk sac, the corpora lutea and the membranous network of ovarian stroma were positive for the PAS staining technique.

5.2.2 Chanda ranga (Ambassis):

1. Histology and Histochemistry of testis :

H E staining technique –

The HE staining was revealed in tunica albuginea, germinal epithelium of the interstitial testicular septa and dividing spermatogonia.

AB pH 1 and AB pH 2.5 staining technique-

Moderate to intense staining obtained in connective tissue capsule in testis, germinal epithelium, spermatogonia and the sertoli cells.

PAS staining technique –

Poor staining in the luminal sperm debris moderate to intense staining was obtained in leydig cells, sertoli cells, the basement membrane of seminiferous tubules etc.

2. Histology and Histochemistry of Ovary–

HE staining technique-

With HE staining eosinophilic staining was obtained in dividing oocytes. In developing follicle brown staining was obtained in cortical cytoplasm, the follicular cells were stained intensely. Two types of yolk bodies were recorded. Negative stain in the oil droplets.

AB pH 1 and AB pH 2.5 staining technique-

The staining was mostly revealed in nuclear histoproteins, perinuclear secretory granules follicular cells and the ovarian stroma.

PAS staining technique –

The PAS staining revealed the granular form around the yolk vesicles and a uniform staining in zona radiata. The PAS staining was also revealed in nuclear histoproteins prior to process of vitellogenesis.

5.2.3 *Puntius sophore* (Ham)

1. Histology and Histochemistry of testis:

HE staining technique-

The staining was revealed in seminiferous lobules and tunica albuginea in general. The intensity of the HE staining was gradually increased from germinal epithelia to spermatides.

AB pH 1 and AB pH 2.5 staining technique-

The moderate staining pattern was observed in groups of interstitial cells, germinal epithelium, spermatogonia and the spermatids.

PAS staining technique –

The testis is revealed for PAS reactivity by the presence of granular, magenta pink colour staining in germinal epithelium, cells of sertoli, interstitial septa.

2. Histology and Histochemistry of Ovary-

HE staining technique –

In ovary, the HE staining was examined during the different phases of ovary such as the quicent stage, maturing stage and post ovulatory stage or spent ovarian stage. The staining was revealed in germinal epithelium of ovary, follicular cells, yolk bodies and the haematoxylene positive staining in the nucleoli prior to vitellogenesis.

5.3 Concluding remarks :

The results are obtained on the testis of continues breeder, Guppy (*poecilia reticulata*), the testis of seasonally breeding glass fish (*Chanda ranga*) and freshwater topminnow (*Puntius sophore*), by employing standard histological and histochemical techniques for the mucins. The cells of sertoli segregated in the sperm ducts forming the accessory reproductive gland like structure equivalent to epididymus in mammal, tested for the homogenous secretion of albumen and other secretary materials. In gonads of larvivorous fishes '3' types of mucins and their functional role in reproduction have been correlated. These are namely neutral, acidic & sulphated are reported.

The gonopodium is a unique copulatory organ in guppy and their working mechanism.

In tests of seasonally breeding teleost prolific production of sperm and ova are recorded, might be due to absence of viviparity.

The ovarian architecture of live bearing guppy is most challenging study pattern, however the regulation of gestation by steroids of corpus luteum and follicular cells is observed. In spite of over production of eggs in oviparous teleost, the two types of follicular atresia recorded namely – hypertrophid follicular atresia and non hypertrophid follicular atresia and its role in the steroid production and the reabsorption of ovarian contents for the fresh cycle.

5.4 Plan and scope for future work :

- 1) Extension work is need on propagation and redistribution of larvivorous fishes in freshwater reservoirs of and zones is required.
- 2) Instead of maintaining guppy in an fresh aquanium they should be propogated in eco-friendly environment of the ponds with cyclic changes of fresh water.
- 3) The role of lysosomal enzymes and lipids in the ovary of guppy is most appreciated, in the future plan of the work.
- 4) There should be search on the other larvivorous fishes for different water reservoirs.
- 5) The *puntius chola* should be propagated in the gentle running streams, ponds and the weirs where they will take the tall of mosquito larvae.

- 6) The muco-polysaccharides of gonads should be invented in gonads of various larvivorous fishes. It will be helpful in the nutrition of fishes and the maintenance of the breeding.
- 7) According to T.S. Eliot, "To make an end is to make beginning the end is where we start from." That is the making end of one problem, to create the thousand problems." Hence study on the larvivorous fishes have vast, infinite scope.