MATERIALS
AND
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The freshwater gobiid fish, *Glossogobius giuris* (HAM) were randomly collected at intervals of 15 days for a period of three years (July 1988 to July 1991) from Sanath Kumari tank, near Bangalore, using cast and gill nets (10mm). These fishes were brought alive to the laboratory and acclimated for 15 days in 50 liters glass aquarium (60"x30"x20") containing aerated tap water prior to using them for experiments. The fishes kept at room temperature of 27±1°C in the laboratory served as controls. Fishes were fed daily with earthworms. The sex of the mature fish was determined by external examination of the shape of urinogenital papillae.

Only large and sexually mature male fishes were used in this study. The body length and weight of each fish were recorded. The male fishes weighing about 40 to 60 gms and length of 150 to 240mm were selected for further experimental studies.

Investigations have been made on the histomorphological aspects of the hypophysis and testis of *G. giuris* in relation to:
1) annual reproductive cycle,
2) exposure of fishes to sublethal quantities of organophosphate pesticide (fenthion):
   a) a light microscopic study, and
   b) an electron microscopic study.

Pituitary and testis of _G. giuris_

To investigate the changes in the pituitary and testis a total of 1250 mature male fish of _G. giuris_ were examined during different months of the year (July 1988 to July 1991). After studying the testis _insitu_, the testis along with the seminal vesicle were carefully removed, weighed and fixed in different fixatives for further histological and histochemical studies. The gravimetric analysis were made and expressed in terms of gonosomatic index (GSI), where,

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GSI = \frac{\text{Weight of the testis}}{\text{Weight of the fish}} \times 100
\]

Histology of the Testis

For histological studies, one of the bilateral testis was divided into anterior, middle and posterior portions and fixed separately in Bouin's Hollande, (3 to 5 days) dehydrated and embedded in paraffin (58-60°C); the other testis was fixed in formal-calcium and treated for localizing lipid. Sections of 5μ thick were cut transversely
and stained in Ehrlich's haematoxylin and eosin as counter stain and in Mallory's triple stain to study the cellular details. The lipoidal content were localized by following Sudan Black 'B' staining methods of McMannus (1946).

Sections from the middle portions of each testis were examined and gross observations made following the method outlined by Murthy (1981) to assess the stage of maturity. The diameter of testis, testicular gland, and seminiferous lobules were measured from every tenth section by calibrated ocular micrometer and its average was calculated.

The stages of maturity were identified under seven distinct stages following Murthy (1981) viz., 1) resting germ cells, 2) primary spermatogonia, 3) secondary spermatogonia, 4) primary spermatocytes, 5) secondary spermatocytes, 6) spermatids and 7) sperms.

These various types of cells can be distinguished based primarily on the progressive decrease in diameter of the nucleus.

Histology of the Seminal Vesicle

The seminal vesicles were fixed in Bouin's Hollande, dehydrated and embedded in paraffin for histological examination. Sections were cut transversely at 5μ thickness and stained in Ehrlich's haematoxylin using eosin as a
counter stain. The area of the six largest locules of the seminal vesicles of each fish was determined using calibrated ocular micrometer. Mean locular area was then determined for each fish for studying seasonal cycle.

Histology of the Pituitary

The brain with hypophysis was carefully separated from the cranium and fixed in Bouin's Hollande (3 to 5 days), Bouin's fluid (24 hours) and Zenker's formal (12 hours). After fixation the tissues were processed and embedded in paraffin (58° to 60°C). Serial sagittal sections of the brain with the pituitary gland were cut at 5μ-6μ and the median sagittal sections with the infundibular stalk was stained in: 1) Cleveland Wolfe's trichrome (Herlant, 1956), 2) Herlant's tetrachrome (Herlant, 1960), 3) Periodic acid-Schiff reagent (Pearse, 1950), 4) Aldehyde fuchsin (Gabe, 1953), 5) Chrome-alum-haematoxylin-phloxin (CAHP) (Gomori's, 1941), 6) Lead haematoxylin technique (Mc Coneill, 1947) and Mallory's triple stain.

The diameter of the cellular and nuclear areas of the pituitary cells viz., prolactin secreting cells in rostral pars distalis (RPD) and gonadotropin secreting cells in proximal pars distalis (PPD) were determined by an ocular micrometer standardised with a stage micrometer.

The average cell and nuclear diameter of pituitary cells was determined by measuring the diameter of 20 nuclei.
from each fish for each month. Data was analysed statistically.

Studies on the effect of organophosphate pesticide (Fenthion) on the pituitary – testicular – system

The impact of pesticides on pituitary – testicular – system of *G. giuris* was studied during different phases of the reproductive cycle viz., 1) resting phase, 2) rapid spermatogenic phase and 3) spawning phase. Adult specimen of *G. giuris* weighing 40 to 60 gms and length of 150 to 240 mm were collected and maintained in the laboratory as described above.

The organophosphate pesticide, Fenthion (0,0-dimethyl-0-(4-methylmercapto-3 methylphenyl)-thiophosphate (40)), was dissolved in ethanol and added separately to the testwater to obtain the desired concentration. The stock solution of fenthion (0.5mg/lit) was prepared separately and the desired concentrations were made by adopting the dilution technique as outlined in APHA (1971). The acclimated fish were divided into four experimental groups of ten fishes each. The first three groups of fishes were placed in sublethal concentration (0.05, 0.25 and 0.5ppm) of fenthion solution, and the last group in freshwater served as control. Ten fishes were kept in each concentration in 20 liters glass aquaria in all experiments. The acclimated fishes were starved for 24 hours prior to their use in the experiment.
and were not fed during the course of the experiment (Dalela et. al. 1978). The water containing pesticide (fenthion) in the glass trough was changed every alternate day.

a) A Light Microscopic Study

The fenthion treated fishes (0.05, 0.25 and 0.5ppm concentrations) were vivisected without anesthesia (at various intervals, 24, 48, 72 and 96 hours). After studying the testis in situ, it was removed carefully, weighed and prepared for histological studies (fixation, staining methods etc. same as in the normal tissue). For comparison of data, all testicular weights were calculated as percentages of body weight (GSI).

The pituitary, intact with brain was fixed in Bouin's fluid and Bouin's Hollande. Serial sagittal sections were cut at 5-6μ and stained in different staining techniques as per the pituitary staining. The sections were examined and the results were tabulated as in figure I - III.

b) An Electron Microscopic Study

Since the cytotoxic effects of the fenthion were seen more conspicuously (based on our light microscopic studies) in pituitary, testis and seminal vesicles treated with 0.5ppm of fenthion solution for 24 hours only. Those tissues were taken and processed for study under the transmission electron microscope.
For these studies, the control as well as the fenthion treated fishes (during spawning phase) were vivisected after 24 hours. The pituitary, testis and seminal vesicles were dissected out under fixatives. These tissues were fixed separately for one hour in 3% glutaraldehyde (GTA) in phosphate buffer at pH 7.4 and at 4°C. GTA-fixed material was post-fixed in 1% osmium tetroxide for four hours at 4°C. Whole of the pituitary, pieces of testis and seminal vesicles were rapidly dehydrated with graded alcohols and embedded in epoxy resin (Sarsa Bharathi and Armugum, 1979 and 1982). The specimen block were kept in incubator at 60°C for 24 hours for proper hardening. Gross trimming and rough sections were made. Sections were cut with LKV ultramicrotome and stained with 1% toluidine blue and examined under compound microscope. The desired area of study was marked and blocks were trimmed in such a way as to include only the desired area. Ultra thin sections were cut at 70-90nm (700-800°A) and placed on copper grids of 3mm size with 400 meshes resting on filter paper. These were stained with uranyl acetate followed by lead citrate for electronmicroscopy (Electron microscope Zesis 952, Philips 420 model and LEM 2000).
Photomicrographs are presented at the end of each section.

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