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Seasonal Changes in the Testes of Fish *Puntius ticto*, and Their Relation to Heavy Metal Toxicity

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An integrated hypothesis explaining the implications of environmental influence on the reproductive process has not emerged owing to paucity of data. Nevertheless, evidence is at hand to show that favorable physiological disposition for reproduction is brought about with external and internal factors through the mediation of the neuroendocrine mechanisms. It is now clear that the environmental factors impinge on the exteroreceptors and through them affect the central nervous system, the hypothalamus, the pituitary and finally the gonad. Another endogenous factor is the internal rhythm, which is known to regulate at least in part the seasonal reproductive activity (Bullough 1951; Sundararaj and Sehgal 1970). A review of literature reveals that comparatively much work has been done on the structure and seasonal changes of the testes of fishes (Pandey and Mishra 1981; Mukhopadhyay and Sinha 1986). The aim of the present study is to observe seasonal changes in the testes of fish Puntius ticto and their relation to heavy metal toxicity.

MATERIALS AND METHODS

Live specimens of male Puntius ticto were collected from local fresh water resources during the period of September 1984 to December 1985. The observations were based on monthly collections for a period of nearly 2 yr. Most of the specimens were sacrificed immediately on the spot. To observe the effect of heavy metals on seasonal variation in testes, the fishes (of approximately same weight and lenght) were brought to the laboratory and kept in the solution of sublethal concentration (26 mg/L) of cadmium acetate for 96-hr period. The treatment of fishes was done after calculation of LC50 values. The 96-hr LC50 was calculated as per method described by Sehgal and Saxena (1986). To maintain the concentration the chemical mixture was renewed with fresh water every 24 hr.

The length and weight of the body and testes were recorded. The testes were fixed in Aqueous Bouins fluid and processed for histological studies. Paraffin embedding method was used and microtomy was done at 4-6 um thickness.

Haematoxylin and Eosin technique was used for staining the sections. Gonosomatic index (GSI) was calculated for the year on the monthly basis to evaluate the maturity state of the testes in accordance with the formula after Pickford (1953). All diameter measurements were made by ocular micrometer and stage micrometer.

RESULTS AND DISCUSSION

The testes of Puntius ticto are paired elongated structures and remain suspended with mesorchium. Each testes is bean shaped structure, composed of radially arranged lobules bound on the coelomic surface covered by a thin visceral peritoneum. Bettween the basal lamina of adjacent lobules are the interlobular septum. The seminiferous lobules are lined by germinal epithelium. Each lobule in section is associated with the germ cells. Germinal cells in various stages of differentiation are contained within each lobule. Different stages of spermatogenesis, viz., sperm mother cells, spermatogonia, primary and secondary spermatocytes, spermatids and sperms were observed inside the lobules (Figs. 1, 2). The interstitial cells appear to communicate with each other through interlobular connective tissue corridors.

The seasonal testicular changes have been distinguished into five stages on the basis of gonosomatic index, general histomorphology and duration of developmental stages of the spermatogenetic cells. These are as follows:
(i) period of spermatogonial proliferation (November to January, (ii) early stage of maturation (February to April, (iii) advanced stage of maturation or rapid spermatogenesis (Late April to June), (iv) phase of functional maturity or spermiation (July to September), (v) spent testes (Late September to October).

In the period of spermatogonial proliferation the testes were whitish in color. The lobules were small and were filled with few primary germ cells and spermatogonia. Small and narrow spaces appeared in the spermatogonia which was the start of the formation of new lobule boundary. The interstitial cell groups tend to surround the growing and developing lobules. By the end of December lobules were filled with cysts of spermatogonia and primary spermatocytes.

Treatment of sublethal cadmium for 96-hr period in the months of October to December produced some changes in the testes of fish Puntius ticto. The spermatogonia exhibited initiation of vacuole formation. The interstitial cells exhibited abnormal shape with damaged cytoplasm and nucleus. By the end of December the lobules exhibited destroyed spermatogonia and primary spermatecytes. Small spaces between the lobules were noted. The gonosomatic index decreased when compared to untreated fishes (Table 1).

Table 1. Date for one complete reproductive cycle:Seasonal changes in the testes of Puntius ticto (Untreated and cadmium treated group). Mean values.

Months	Average Weight of fish in mg (20 Fish)		Average weight of testes in mg (20 Fish)		Gonosomatic Ind (GSI) (20 Fish)		Diameter of Seminiferous lobules in mm (20 Fish)	
	Untreated Group	Cadmium Treated Group	Untreated Group	Cadmium Treated Group	Untreated Group	Cadmium Treated Group	Untreated Group	Cadmium Treated Group
January	315.2	315.0	13.5	13.0	4.28	4.1	0.04	0.04
February	318.1	317.0	13.9	13.5	4.36	4.2	0.04	0.04
March	320.0	318.2	14.1	13.8	4.4	4.3	0.04	0.04
April	321.0	317.5	28.5	13.9	8.8	4.3*	0.04	0.04
May	322.0	318.7	33.6	19.0	10.4	5.9*	0.08	0.09*
June	324.0	320.8	35.5	19.5	10.9	6.0*	0.12	0.13*
July	324.8	321.5	35.5	19.9	10.9	6.1*	0.19	0.21*
August	324.8	322.5	40.2	20.9	12.3	6.4*	0.21	0.22*
September	318.2	321.4	18.1	17.1	5.6	5.4*	0.15	0.16*
October	316.0	310.3	14.0	12.1	4.4	3.8*	0.10	0.11*
November	314.0	308.7	10.3	9.2	3.2	2.9*	0.91	0.11*
December	310.0	306.6	10.2	8.30	3.2	2.7*	0.71	0.12*

* $P < 0.001$

In the period of early stage of maturation the testes were dirty white in colour. The number of seminiferous lobules increased during this phase. The spermatogonia transformed into the primary spermatocytes. By the end of March all the lobules were packed with irregularly arranged cysts of spermatocytes and spermatids. The connective tissue septa between lobules became prominent and stretched and interstitial cells appeared distinctly (Fig.1).

The fish treated with cadmium acetate for 96-hr period in the months of February to April showed degenerative changes in the testes. The spermatogonia, primary and secondary spermatocytes showed highly vacuolated cytoplasm and damaged nucleus. The connective tissue septa between the lobules ruptured after treatment of heavy metal (Fig.3). The interstitial cells exhibited atrophied nature.

In the advance stage of maturation (Late April to June) maximum activity was seen. The large number of cysts of spermatocytes and spermatids were present in the lobules and its centre was occupied by a small cluster of sprmatids transforming into spermatozoa (Fig.2). The connective tissue starands grew thinner and interstitial cells exhibited their maximum development. The gonosomatic indices and seminiferous lobule diameter were presented in the Table 1.

In cadmium treated fishes the spermatocytes and spermatids exhibited deformed structure in the months of late April to June. The shape of spermatocytes became irregular and the wall of these spermatocytes dissolved (Figs.4,5). The interstitial cells lost their normal structure and became atrophied. The gonosomatic indices decreased and diameter of seminiferous lobules increased when compared to untreated group (Table 1).

In the phase of functional maturity or spermiation the greatly expanded lobules were lined with a layer of resting spermatogonia and were filled completely with spermatozoa. The diameter of seminiferous lobules was presented in the Table 1. The primary germ cells were few in number. The number of interstitial cells was much less and the connective tissue septa were thin. The gonosomatic indices of this group were presented in the Table 1. In this period the testeswere ready to discharge the spermatozoa.

The treatment of cadmium to fish testes at sublethal concentration for 96-hr period in the months of July to September produced deformities in spermatocytes, spermatids and spermatozoa. These spermatogenetic cysts exhibited vacuolozation in the cytoplasm and deformities in the nucleus. The sperm duct also showed damaged structure, its cells became irregular, and nucleus showed atrophied structure. The spermatozoa were destroyed and lobules vacuolated(Fig.6). The GSI and diameter of seminiferous lobules were presented in the Table 1.

With the discharge of sperms, the tension on the expanded lobules was released and they shrunk in the months of September and October. Most of the lobules became empty and contained fibrous debris. Druing this period the interstitial cells exhibited a spent condition and new cells were in the process of formation from the stroma cells.

The vacuolization in the lobules was prominent after cadmium treatment in the month of September and October. A large number of atretic spermatophores were observed. The connective tissue septa ruptured and interestitial cells disappeared.

It is known that Puntius ticto is a seasonal breeder and it spawns in April to August. Prior to the spawning period the testes undergo propagatory stages during the remaining part of the season. The preparation includes various degrees of morphohistological changes in the testes in relation to spermatogenetic activity. However, in the present study the seasonal variation in the testes of Puntius ticto was described and another group of studies was carrier out with the seasonal variation in the testes of Puntius ticto after sublethal exposure (26 mg/L) of cadmium acetate for 96-hr period.

Surprisingly there is no information about the effect of heavy metals on seasonal testicular changes in teleosts. However, Shukla and Pandey (1984) studied the carbamide induced histological alterations during different phases of the testicular cycle of a fresh water perch Colisa fasciatus. They found the abnormal lobular architecture, dissolution of germinal epithelium, prominent vacuolization and necrosis during the preparatory and mature phases. In the present study testes exhibited degenerative changes during all phases of testicular cycle after sublethal exposure of heavy metal. The value of GSI was reduced when compared to untreated fishes (Table 1). In the testes of cadmium treated fishes the early stage of maturation exhibited vacuolization in spermatogonia and spermatocytes. The connective tissue septa between the lobules ruptured and interstitial cells atrophied. In maturation phase the abnormal architecture of lobules were observed and their diameter increased (Table 1). During spermiation the spermatogenetic stages showed abnormalities. The results of preparatory and mature phase were corroborated with the result of Shukla and Pandey (1984) in the testes of Colisa fasciatus after carbamide exposure. It appears from the present study that testicular cycle of Puntius ticto is adversely influenced by the heavy metal metal treatment.

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REFERENCES

Bhatti M N, Al-Dhaham N K (1978) Annual cyclical changes in the testicular activity of a fresh water teleost Barbus luteus (Haekel) from Shett A:Areab Iraq. J Fish Biol 13:321-326

Bullough W S (1951) Vertebrates sexual cycles. Methuen and Co. Ltd., London, 123.

Mukhopadhyay S, Sinha G M (1986) Annual cyclical changes in the testicular activity of a Indian fresh water Major Carp Labeo rohita (Ham)Gegenbaurs Morph Jahrb Leipzing 132:303-321

Pandey K, Misra M (1981) Cyclic changes in the testes and secondary sex characters of fresh water teleost Colisa fasciata. Arch Biol 92:433-450

Pickford G E (1953) A study of the hypophysectomised male killi fish Fundulus heteroclitus (Linn)Bull Bingham Oceanog Collect 14:5-41

Sehgal R, Saxena A B (1986) Studies on toxicities of cadmium and zinc to fresh water teleost Puntius ticto. J Hydrobiol 2: 33-35

Shukla J P, Pandey K (1984) Carbamide induced histological alterations during the various phases of testicular cycle of fresh water perch Colisa fasciatus. Bull Inst Zool Acad Sin Taipei 23:181-186

Sundararaj B I, Sehgal A (1970) Short and long term effects of imposition of total darkness on the annual ovarian cycle of the catfish, Heteropneustes fossilis (Bloch). J Interdiscip cycle Res 1:291-301

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