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# DEVELOPMENT OF THE SECONDARY SEXUAL CHARACTERS ACCOMPANYING CYCLICAL CHANGES IN THE TESTES OF A CATFISH *MYSTUS (M) VITTATUS* (BLOCH)

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**Markandey Misra and Kamleshwar Pandey** (1984) Development of the secondary sexual characters accompanying cyclical changes in the testes of a catfish *Mystus (M) vittatus* (Bloch). *Bull. Inst. Zool., Academia Sinica* 23(1): 57-68. Cyclic changes in the testicular activity of the freshwater catfish *Mystus (M) vittatus* were studied for a period of eighteen months. Accompanied cellular changes in the testes with possible involvement of secondary sexual characters (SSC) have been observed and discussed.

Secondary sexual characters (SSC) in relation to the testicular cycle and steroidogenic structures of fishes has hardly attracted attention of biologists. Kopec (1918, 1928), Wunder (1931) and Niwa (1965a, b) shown that male positive SSC as nuptial colouration is dependent on testicular hormones. Of late, Pandey (1969) has correlated the role of pituitary and gonadal hormones on the manifestation of SSC in *Poecilia reticulata*. Pandey and Misra (1981) have recorded the role of interstitial Leydig cells in the manifestation and maintenance of SSC in *Colisa fasciatus*. The present paper for the first time embodies a comprehensive account of testicular cycle and endocrine structures in relation to SSC in *Mystus (M) vittatus*.

## MATERIAL AND METHODS

Adult male *Mystus (M) vittatus* possess a genital papilla and a spear-shaped thickening at the base of the caudal fins (Swarup and Swaroop, 1975). Male specimens were collected for a year and a half from the local Ramgarh lake, Gorakhpur and were utilized for the present study. The weight (g) of fish and length (cm) and weight (g) of testes were recorded and gonadosomatic index (GSI) was calculated by the following formula:

$$GSI = \frac{\text{Total testes weight}}{\text{Fish body weight} - \text{Total testes weight}} \times 100$$

Pieces from anterior, middle and posterior regions of the testes were fixed in Bouin's (both aqueous and alcoholic), Picromercuroformol (Pandey, 1979) and Baker's formol calcium fixatives. Sections were cut at 5-6 $\mu$m and stained with Heidenhain's haematoxylin,

Heidenhain's Azan, Herlant tetrachrome for routine histological study. Calcium formol fixed tissues were stained with Sudan Black B for histochemical studies. Sections were also subjected to Schultz's test.

## RESULTS

The testes (2.51 ± 0.06 cm — 3.10 ± 0.10 cm average in length) of *Mystus* (*M*) *vittatus* are paired lying posteriorly in the abdominal cavity just ventral to the kidney. The two testes (0.06 ± 0.006 g — 0.18 ± 0.003 g average in weight) are free anteriorly but fused posteriorly with finger-like processes more prominent during breeding season. Both the testes are more or less of equal size, but occasionally one happens to be shorter than the other and are of white to cream hue. The testes consist of numerous distinctly defined lobules held together by a thin covering made up of connective tissues surrounded by an outer delicate layer of peritoneum. The lobules of the testes are of different diameter (96.73 ± 1.72 μm — 173.25 ± 3.20 μm). The lobular wall is a single layer of germinal epithelium while the interlobular space composed of connective tissues, blood capillaries and interstitial Leydig cells (Fig. 1).

**Maturation process of the testis**

*Sperm mother cell*: The cells of germinal epithelium of lobular wall give rise to a spermatogonia or sperm mother cells. Resting sperm mother cells or primary spermatogonia (11.39 ± 0.44 μm average in diameter) and multiplying sperm mother cells or secondary spermatogonia (10.20 ± 0.2 μm average in diameter) are also marked in this fish. They have distinct cell boundaries and conspicuous nucleus with nucleolus. They occur in groups and their chromatin stains deeply (Fig. 3).

*Primary spermatocyte*: The secondary spermatogonia give rise to primary spermatocytes. They are 5.64 ± 0.03 μm average in diameter.

Fig. 1. Showing germinal epithelium and interlobular space with interstitial cells —▶ and blood cells - -▶. ×1000.

Fig. 2. Showing primary spermatocytes —▶ with deeply stained nucleus and secondary spermatocytes - -▶ within the lobules gaining prominance during preparatory phase. ×400.

Their cytoplasm get rarefied and nucleus stains deeply. The synezesis knot has also been marked (Fig. 2).

*Secondary spermatocyte*: They are formed as a result of active division of primary spermatocytes and are smaller in size (4.49 ± 0.07 $\mu$m average in diameter). Secondary spermatocytes have indistinct cell wall and a darkly stained nucleus in the centre (Fig. 2).

*Spermatid*: Secondary spermatocytes are translated into spermatids, which are small, spherical measuring 1.25 ± 0.19 $\mu$m average in diameter. They have indistinct cell wall, 'u' shaped chromatin staining dark with nuclear dyes and are found scattered in the lobules (Fig. 7).

*Spermatozoa*: Apparently spermatozoa appear identical to the spermatids but are smaller (0.87 ± 0.08 $\mu$m average in diameter) in size and are formed by the transformation of the latter. They have strong affinities to the basic dyes (Fig. 6).

**Testicular cycle**

An analysis of the morphological and histological features of the testis throughout the year shown in Table 1, Figs. 8 and 9 makes it possible to divide the testicular cycle of the *Mystus* (*M*) *vittatus* into four well marked phases which can be corroborated with the GSI and prominent manifestation of SSC.

*Preparatory phase* (January-February): The average length and weight of the testes and GSI in this phase have been recorded minimum (*i.e.* 2.54±0.19 cm to 2.57±0.16 cm, 0.07±0.01 g to 0.10±0.01 g and 0.65±0.02 to 0.84±0.03 respectively). The testicular lobules measure 106.49±2.84 $\mu$m to 113.93 $\mu$m average in diameter. The per cent number of sperm mother cells (31.30±0.78%) and primary spermatocytes (29.60 ± 0.57%) in this phase are maximum among other phases of the testicular cycle. The secondary spermatocytes recorded in this phase are 12.20 ± 0.56% to 21.40 ± 0.61%. Some residual spermatozoa still occupy the central area of certain lobules.

The collapsed lobules seem to have been regaining their original shape during this phase (Fig. 2). The testes show a slow but steady renewal of the extra lobular cytoplasmic debris and intralobular spermatogenesis. The interlobular septa of the testes show a gradual yet slow decline in their thickness *i.e.* from 13.40 ± 0.57 $\mu$m to 13.00 ± 0.56 $\mu$m in average.

The interstitial Leydig cells present in the interstices are marked regaining their oval or circular shape (Fig. 3). The average diameter of these cells is 2.25 ± 0.04 $\mu$m during this period. The nucleus occupies a central position and granulation in the cytoplasm appears.

TABLE 1

Monthwise changes in length and weight of testes and GSI of *Mystus* (*M*) *vittatus*

| Months | Length of testes (cm) | Weight of testes (g) | Body weight of fish (g) | Gonadosomatic index (GSI) |
|---|---|---|---|---|
| January | 2.54±0.19 | 0.07±0.01 | 10.74±0.78 | 0.65±0.02 |
| February | 2.57±0.16 | 0.10±0.01 | 11.92±0.61 | 0.84±0.03 |
| March | 2.73±0.09 | 0.13±0.003 | 12.07±0.40 | 1.09±0.05 |
| April | 2.76±0.12 | 0.14±0.003 | 12.43±0.35 | 1.13±0.07 |
| May | 2.88±0.09 | 0.15±0.002 | 12.62±0.24 | 1.20±0.06 |
| June | 3.01±0.02 | 0.16±0.003 | 12.86±0.24 | 1.25±0.06 |
| July | 3.07±0.07 | 0.17±0.003 | 13.00±0.60 | 1.32±0.02 |
| August | 3.10±0.10 | 0.18±0.003 | 13.35±0.23 | 1.36±0.02 |
| September | 3.05±0.13 | 0.17±0.003 | 12.93±0.44 | 1.33±0.02 |
| October | 3.00±0.02 | 0.16±0.006 | 12.58±0.37 | 1.28±0.03 |
| November | 2.63±0.03 | 0.10±0.01 | 11.02±0.71 | 0.91±0.01 |
| December | 2.51±0.06 | 0.06±0.006 | 10.80±0.37 | 0.55±0.01 |

The data are expressed as Mean±SE of ten replicates of *Mystus* (*M*) *vittatus*.

Fig. 3. Showing large sperm mother cells —▶ with deeply stained chromatin and the interstitial cells - -▶ regaining their oval or circular shape. ×1000.

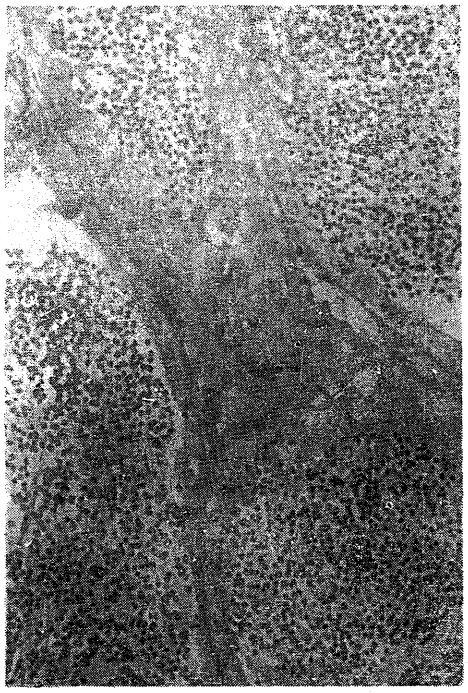

Fig. 4. Showing well expanded lobules —▶ filled with spermatozoa and the well defined interstitial Leydig cells - -▶ in different secretory stages. ×400.

The 3.70 ± 0.22% to 5.90 ± 0.24% of Leydig cells have been recorded the lowest amongst all the phases of the sexual cycle. This phase thus represents a period when the secretion of interstitial Leydig cells is completely utilized for initiation and continuance of spermatogenesis. Due to this reason possibly the SSC are poorly differentiated.

*Maturing phase* (March-May): A gradual increase in the average length (2.73 ± 0.09 cm to 2.88±0.09 cm) and weight (0.13±0.003 g to 0.15±0.002 g) of testes and GSI (1.09±0.05 to 1.20±0.06) have been recorded during this phase. The histological studies reveal that the testes are in active spermatogenic phase. The well expanded lobules measure 135.88±2.19 μm 160.26±4.41 μm average in diameter (Fig. 4). The percent number of sperm mother cells and primary spermatocytes has been recorded decreasing from March to May (11.30±0.35% to 6.30±0.31% and 17.40±0.59% to 9.20±0.46% respectively). However, the per cent number of secondary spermatocytes recorded in March (33.40±0.57%) is the maximum in the entire spermatogenic cycle which gradually decreases and remains 9.80±9.46% in May. Hence, the spermatids/spermatozoa recorded in the lobules exhibit a numerical increase from March to May (30.70±0.66% to 61.40±1.17%).

These data point an overall increase in the testicular parameters leading to the attainment of highest degree of functional maturity. Though gradual yet noticeable reduction in the thickness of interlobular septa (11.10 ±0.45 μm to 8.80±0.40 μm average) has been recorded during this phase. The interstitial Leydig cells resume their final circular shape and the size also tends to increase 2.41 ± 0.03 μm to 3.10 ± 0.03 μm average in diameter. A well defined nucleus occupies the central position in the granular cytoplasm and the number of Leydig cells during this phase is 7.20±0.34% to 13.30±0.45% (Fig. 4).

Rapidly changing cellular features during this phase point that the secretion of Leydig

cells while participating in the spermatogenesis is now also utilized in the differentiation of prominent SSC as well developed genital papilla and a spear-shaped thickening at the base of the caudal fin.

*Spawning phase* (June-October): This phase extends for a fairly long period. The average length of testes increases from June to August when it becomes maximum (3.01 ± 0.02 cm to 3.10 ± 0.10 cm) and then gradually reduces in size at the end of this phase (3.00 ± 0.02 cm). Likewise, the average weight of testes increases from June to August (0.16 ± 0.003 g to 0.18 ± 0.003 g) when it becomes maximum and thereafter falls gradually at the end of this phase (0.16 ± 0.006 g). Consequently GSI shows an increasing trend from June to August (1.25 ± 0.06 to 1.36 ± 0.02) being highest but a gradual decline at the end of this phase (1.28 ± 0.03) has been noticed. The testicular lobules also show a gradual increase in their diameter (156.17 ± 2.84 $\mu$m) at the end of this phase. There is a numerical decline of sperm mother cells from June (4.80 ± 0.27%) to August (1.60 ± 0.13%) which is gradually restored and exceeds (11.50 ± 0.42%). The number of primary spermatocytes (4.70 ± 0.21% to 2.10 ± 0.24 %) and secondary spermatocytes (4.70 ± 0.47% to 1.20 ± 0.11%) decreases from June to September but increases slightly in October (3.80 ± 0.29% and 2.60 ± 0.23% respectively). However, the number of spermatozoa in the lobules show a reverse trend *i.e.* a gradual increase from June (70.80 ± 0.60%) to August (80.70 ± 0.73%) following a decline in the later months (68.70 ± 0.47%).

During this phase the interlobular septa attain maximum reduction (6.90 ± 0.48 $\mu$m to 5.90 ± 0.29 $\mu$m average), however, they acquire a thickness of 9.30 ± 0.58 $\mu$m average in later part of this phase. The interstitial Leydig cells in the interstices while attain their final circular shape and size (3.14 ± 0.02 $\mu$m to 3.17 ± 0.03 $\mu$m average), most of them also show to undergo the process of depletion which is evidenced from the cytolysis and degranulation in the later part of this phase (Fig. 5). Hence, their size recorded in the later part of this phase show reduction (2.11 ± 0.03 $\mu$m average in diameter). Their number during the early part of this phase is though maximum 15.00 ± 0.47% to 15.10 ± 0.39% but this too gradually falls in the later part of this phase (13.40 ± 0.32%). The secretion of the Leydig cells which was primarily utilized in the spermatogenesis has secondarily brought about the differentiation of SSC, thus performed two fold functions. This may be inferred that possibly a lower titre of their section maintains an uniform tempo of SSC similar to that of the previous phase. However, decline in size and number of Leydig cells has already started in the later part of this phase.

*Post-spawning phase* (November-December): Average testicular length, weight and GSI show a fall at the end of this phase being the lowest in the entire testicular cycle (2.51 ± 0.06 cm, 0.06 ± 0.006 g and 0.55 ± 0.01 respectively). The spermiation has resulted in the collapse of the lobules while spermatogenesis appears to be static. Compared with other phases, the average lobular diameter recorded at the end of this phase (96.73 ± 1.72 $\mu$m) is the minimum. The per cent number of germ cells *viz.* sperm mother cells, primary spermatocytes at the end of this phase is 29.60 ± 0.89%, 21.40 ± 0.68% and 10.20 ± 0.43% respectively. Some residual spermatozoa (31.20 ± 0.43%) have been observed in the lumen of the lobules. This shows that the total spermatozoa produced during one cycle are not completely spawned and only 71%-72% of them are shed.

The interlobular septa have been recorded 12.40 ± 0.54 $\mu$m to 14.30 ± 0.54 $\mu$m average in thickness. The interstitial Leydig cells show atrophy and their nuclei get pycnotic (Fig. 7). Hence, the shape appears irregular and their size recorded in this phase is the lowest in the entire Leydig cells cycle (2.11 ± 0.02 $\mu$m average in diameter). Their number is 8.90 ± 0.24% to 7.60 ± 0.39% which is comparatively a much less per cent number from that of the preceding phase.

This phase concludes the spermatogenic

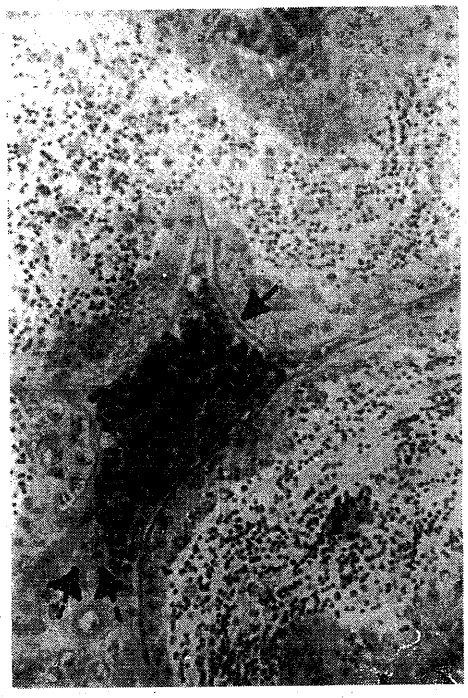

Fig. 5. Showing interstitial Leydig cells —▶ some of them undergoing cytolysis and degranulation with pycnotic nuclei - -▶. ×400.

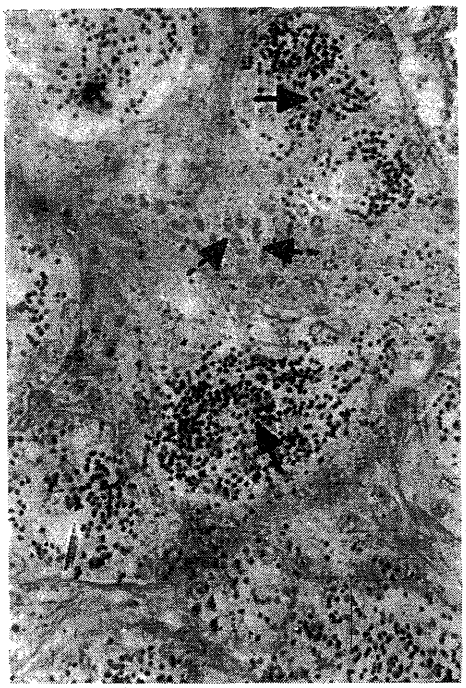

Fig. 6. Showing spermatozoa —▶ in the lobules, some of the interstital cells seen undergoing depletion - -▶. ×400.

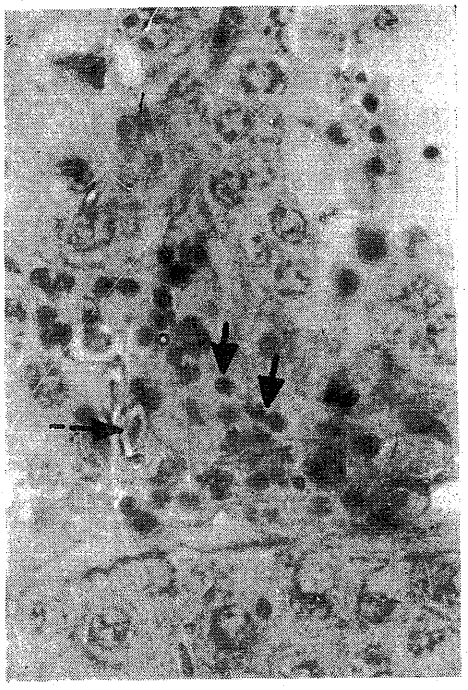

Fig. 7. Showing pycnotic nuclei —▶ of the interstitial cells with a number of blood cells - -▶. ×100.

activity and thereby suggests that the active secretion of Leydig cells has now come to an end. SSC remains almost unchanged in this phase though a dwindling trend is, however, apparent.

## DISCUSSION

Teleostean testes appear to be either lobulated (Sathyanesan, 1960; Dixit and Agrawal, 1974) or convoluted (Gokhale, 1957) or their surface remain smooth (Swarup, 1958; Sanwal and Khanna, 1972 and Pandey and Misra, 1981) during breeding season. In partial agreement with the first category, the finger-like processes of the testes become more lobulated and prominent during breeding season in *Mystus* (*M*) *vittatus* possibly they enhance the functional area in the testis for increased production of spermatozoa. Three categories of fusion of the paired teleostean testes have been recorded, *i. e.* they may be either fused along their entire length (Khanna and Pant, 1966) or completely separate (Dixit and Agrawal, 1974) or fused only in the posterior regions (Sanwal and Khanna, 1972 and Pandey and Misra, 1981). The testes of *Mystus* (*M*) *vittatus* are fused in the posterior region and thus show similarity with the last type.

Histologically, Kerr (1919) in *Polypterus* and Rai (1965) in *Barbus tor* have described that the posterior region of testes is made up of a net work of cavities bounded by sterile lamellae. Sathyanesan (1959) in *Mystus seenghala* have described that the main body of the testes produces sex cells whereas the posterior region serves the function of their storage and conduction so is the case with the *Mystus* (*M*) *vittatus*. As has been pointed above the finger-like process in the anterior margin of testes apparently compensate the functional loss for posterior sterile region. Contrary to it James (1946), Gokhale (1957), Chan and Phillips (1967) and Pandey and Misra (1981) have not described such a sterile part. Swarup (1959) and Srivastava (1978, 1979) have described two peak periods of testicular activity while in agreement to Pandey and Misra (1981) *Mystus* (*M*) *vittatus* displays only one peak period of testicular activity and thus is an annual breeder. Regarding the origin of new crop of sex cells, Barr (1963), Rai (1965), Belsare (1966), Swarup and Srivastava (1978, 1979) and Pandey and Misra (1981) have described their origin from the sperm mother cells present throughout the year in the testes and that *Mystus* (*M*) *vittatus* falls in this category. Foley (1926) and Hayasi (1971) have described the origin from extra vascular cord of germ cells "stroma cells" and "Sertoli cells" respectively. Migratory germ cells are also thought to give rise to the new crop of germ cells by Geiser (1922), Gokhale (1957) and Srivastava and Rathi (1959). These cells in our opinion appear to be derived from the same stock of resting cells. There has been much discussions on the origin, structure, occurrence and function of interstitial cells in teleost fishes. The earlier observations on the interstitial cells in teleostean testes are so contradictory that no definite conclusion pertaining to their functional identity can be drawn from them as evidenced from the work of Hoar (1957) and Lagios (1965).

Leydig cells are distributed singly or in small groups in the interstices between the lobules of several elasmobranchs and teleosts (Robertson, 1958; Chieffi, 1967; Guraya 1967a, b; Bara, 1969; Hyder, 1970; Belsare, 1973; Gresik *et al.*, 1973 and de Vlaming, 1974). The structural features of interstitial Leydig cells in *Mystus* (*M*) *vittatus* show smaller size than the germinal cells but larger than the spermatids and spermatozoa. The cytological features of these cells do not show any similarity with the developing germ cells. They may be round, oval or even fusiform in outline with semigranular cytoplasm and darkly stained prominent nucleus. A numerical and size variations in these cells have been observed corresponding to the changes in the state of spermatogenesis (Figs. 8, 9). The cytological and histochemical features of the Leydig cells of the fish testes correspond to the well established steroid secreting cells of mammalian

gonad (Follenius and Porte, 1960; Stanley *et al.*, 1965; Yaron, 1966; Chieffi, 1967a, b; Bara, 1966, 1969; Oota and Yamamoto, 1966; Follenius, 1968; Simpson *et al.*, 1969; Hyder, 1970 and Nicholls and Graham, 1972). The characteristic features of these cells revealed after histochemical and E. M. studies are abundant lipoproteins, smooth endoplasmic reticulum, mitochondria with a complex internal structure, hydroxysteroid dehydrogenases (HSDH) activity indicative of steroidogenesis and accumulation of cholesterol when secretory activity is very low. The Leydig cells of *Mystus* (*M*) *vittatus* incorporate all the forementioned characteristic features of a typical Leydig cells and show close correlative changes with the testicular cycle of these fishes pointing their functional involvement (Figs. 8, 9). However, sudanophilic lipid droplets have not been observed in the Leydig cells of *Oryzias latipes* (Gresik *et al.*, 1973), guppy (Follenius and Porte, 1960), trout (Oota and Yamamoto, 1966) and cichlid fish (Nicholls and Graham, 1972). But these cells in most of the other fish species undergo conspicuous seasonal changes by showing accumulation and prenuptial depletion of cholesterol containing lipid. In *Fundulus*, small lipid droplets appear only at the peak of sexual activity (Lofts and Bern, 1972).

Based on these observations it is inferred that their mobilization is related to other active secretion of steroid hormone which control the development and maintenance of

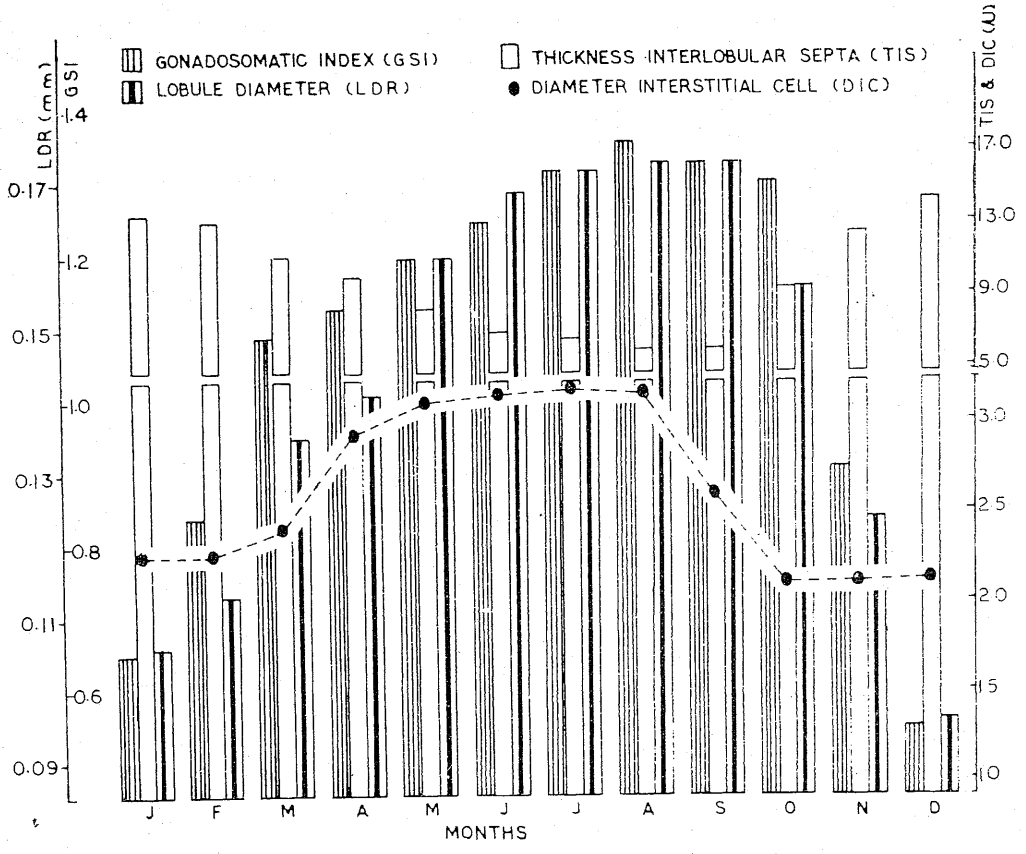


Fig. 8. Histogram showing monthwise changes in gonadosomatic index, lobule diameter, thickness of interlobular septa and diameter of interstitial Leydig cells in the testes of *Mystus* (*M*) *vittatus*. Broken lines connecting closed circles in the histogram specifically indicate monthwise changes in diameter of interstitial Leydig cells.

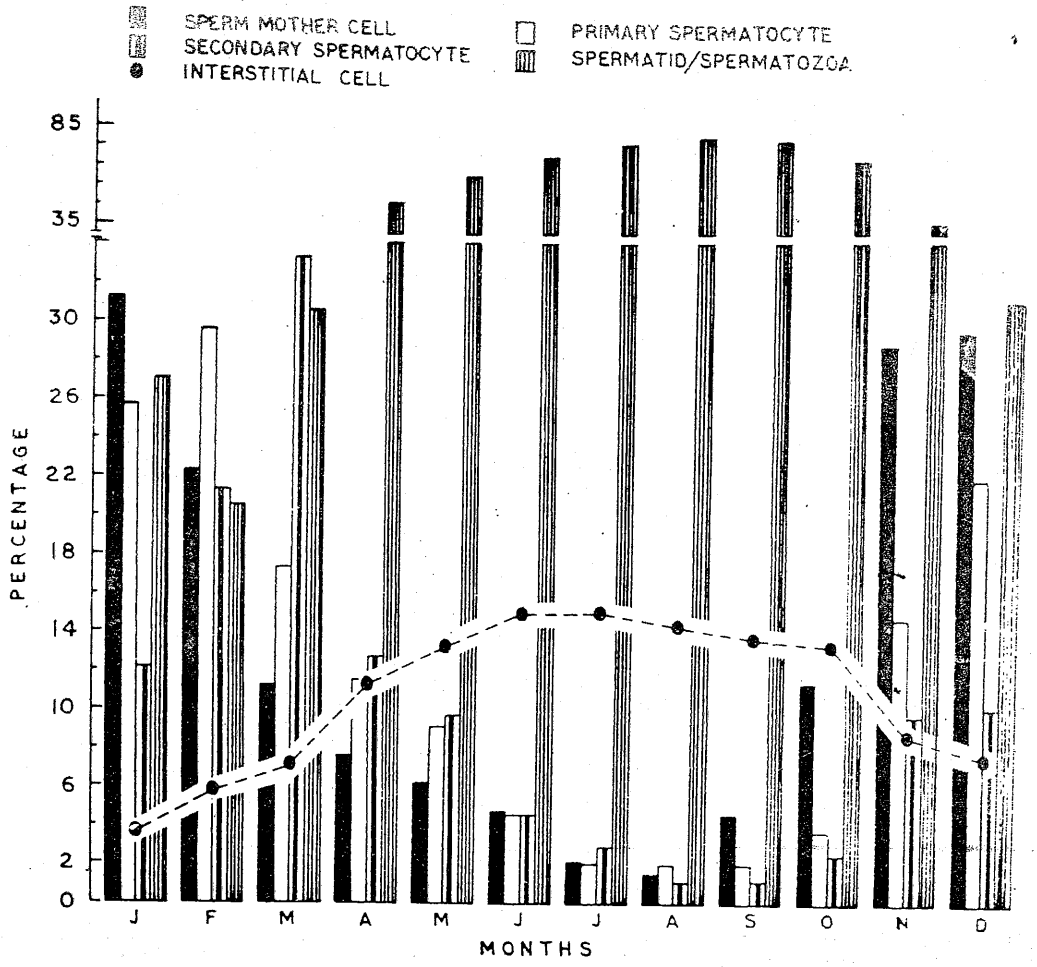


Fig. 9. Histogram showing monthwise changes in per cent number of germinal cells and interstitial Leydig cells in the testes of *Mystus* (*M*) *vittatus*. Broken lines connecting closed circles in the histogram specifically indicate monthwise changes in per cent number of interstitial Leydig cells.

SSC and reproductive behaviour in fishes. The maximum development and activity of interstitial cells have been found to occur just prior to spawning and shortly after when the spermatogenic activity is low in the testes of *Tilapia nigra* (Hyder, 1970) following a rapid development of sexual colouration, nest building activity and establishment of territorial claims, in *Mystus* (*M*) *vittatus* maximum development of Leydig cells has been recorded during spawning phase (June-October) when they acquire their largest size ($3.17 \pm 0.03$ $\mu$m average in diameter) and highest number ($15.1 \pm 0.39\%$) in the month of July. During this period the specimens develop well marked genital papilla and a spear-shaped thickening at the base of the caudal fin at the level of lateral line. While it is recorded minimum during post-spawning period when these characters show a dwindling trend. Adjudged from the cellular changes in the testes, the development and manifestation of SSC closely correspond the state of testicular activity in this fish and that it chiefly depends upon the functional state of interstitial Leydig cells.

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# 條紋黃顙魚睾丸周期性變化與第二性徵之發展

M. MISRA AND K. PANDEY

淡水產條紋黃顙魚睾丸活動周期之變化係根據爲期十八個月之連續研究結果而撰述者。本文且記述有關睾丸細胞之變化與第二性徵形成之連帶關係之觀察結果並加以討論之。