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Distributional pattern of different cells with special emphasis on the seasonal variations of gonadotrophs in the pituitary gland of *Mystus vittatus* (Bloch, 1794) in relation to testicular activities

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Abstract
Different cell types were identified in the pituitary gland of *Mystus vittatus* on the basis of their characteristic arrangement, distribution and tinctorial properties. The adenohypophysis was subdivided into three distinct zones innervated by the process of neurohypophysis. The acidophilic prolactin (PRL) cells and adrenocorticotropic cells were observed in the rostral pars distalis (RPD). The basophilic gonadotropic cells (GTH) and thyrotropic cells (TSH) were distributed in the middle proximal pars distalis (PPD) and react positively to periodic acid Schiff's (PAS). The acidophilic cell types did not change remarkably their activities and staining intensities throughout the year. The GTH and TSH cells exhibited both quantitative and qualitative variations during the testicular cycle. During growth and maturation period the GTH cells were characterized by intense staining and dense homogenous granules and reached maximum diameters at the end of maturation phase. During spawning period, slight decrease in the staining affinity and GTH diameters were recorded. The seasonal changes in the testis of *M. vittatus* have been described according to its variations in GSI values and frequency percentages of the different male germ cells occurring in the testicular lobules. It was concluded that the GTH cells and testicular activities correlate well during different reproductive phases and possibly the GTH cells provoke changes in the testicular activity of this fish.

Keywords: Adenohypophysis, Cell type variations, Gonadotropic cells, Pituitary gland, Testes, *Mystus vittatus*

1. Introduction
The development of the gonads and the process of reproduction is motivated by hormones secreted by the pituitary gland which are known to mediate between the external environment and the reproductive organs [1]. The study of the histology of the pituitary gland of different teleosts has been published by various authors from time to time [2, 3, 4, 5, 6, 7]. However, the identification and distribution of the cell types in the pituitary gland of different teleosts have attracted attention through histochemical, ultrastructural and immuno-cytochemical points [8, 9, 10, 11]. The secretary cells of the pituitary gland show different pattern of distribution in rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI). One type of gonadotropin secreting cells has been observed in some fish species [11, 12, 13, 14]. However, two GTH cell types have also been described in other teleost species by Mousa and Mousa [15] in *Mugil cephalus*. As far the knowledge is concerned, there are few earlier studies regarding the seasonal changes of the GTH cells in the adenohypophysis of the freshwater teleosts [16, 17].
The aim of the present study is to identify and localize the different cell types with special emphasis to GTH cells in the pituitary gland of male *Mystus vittatus* (Bloch) at all stages of testicular development. This striped dwarf catfish is very much popular as food fish due to high nutrient profile and its availability throughout the year. It is also used as indigenous ornamental fish. Breeding takes place one a year in freshwater bodies during summer monsoon. It takes about nine to ten months to reach the maturity stage. Therefore, the aforesaid study will provide basic information concerning with successful propagation.

2. Materials and Method

Ten adult male of *M. vittatus* (average length 12.5 ± 1.15 cm and mean body weight 22.1 ± 2.12 g) were procured fortnightly throughout the year from a particular stocking pond located in Burdwan town (23.23ºN and 87.866ºE) of West Bengal, India, in order to avoid ecological variations in different water bodies that can affect the development of the pituitary and testes.

2.1. Gonadosomatic Index (GSI)

Twelve numbers of male fishes were collected every month during August to July and the total body weight of the fishes and the total weight of the testes were taken every month to the nearest milligram to calculate the GSI from the following formula:

$$GSI = \frac{weight\ of\ the\ gonads}{weight\ of\ the\ fish} \times 100$$

2.2. Histological methods

To study the seasonal changes of the pituitary cell types and testicular cells, the fishes were sacrificed and pituitary tissues were obtained. The intact brain and pieces of testes of the fish were dissected out and fixed in aqueous Bouin's fixative for 18 hrs. The tissues were then placed in 70% ethanol and subsequently dehydrated with increasing ethanol concentration followed by acetone and cleared in benzene. Tissues were then embedded in paraffin (melting point 56-58 ºC). The testes and mid-sagittal sections of the pituitary gland were cut at 4 µm thickness using a Leica RM2125 RT microtome. Deparaffinized sections were brought to water and stained by adopting various techniques which are as follows:

2.2.1. Periodic Acid Schiff's (PAS) technique of Mc. Manus [18] using Orange G (OG) as the counter stain: (PAS-OG) for demonstration of thyrotrophs, gonadotrophs and somatotrophs.

2.2.2. Alcian Blue (AB) (pH 0.2 and 2.5) combined with PAS-OG by acid permanganate oxidation as adopted by Herlant [19]: (AB-PAS-OG) for demonstration of thyrotrophs, gonadotrophs, prolactin cells and somatotrophs.

2.2.3. Mallory's Triple stain [20]: (MT) for demonstration of gonadotrophs.

2.2.4. Chrome Alum Haematoxylin Phloxine after Gomori [21]: (CAHP) for demonstration of corticotrophs, somatotrophs and thyrotrophs.

2.2.5. Alcian Blue-Orange G-Acid Fuchsin after Slidders [22]: (AB-OFG) for demonstration of somatotrophs, gonadotrophs and thyrotrophs.

2.2.6. Sections of testes were also stained with Iron Alum Haematoxylin (IAH), Delafield's Haematoxylin and Eosin (HE) and Mallory's Triple (MT) stain for identification of different germ cells including interstitial cells.

After staining the sections were dehydrated through ascending series of ethanol, cleared in xylene, mounted permanently with DPX and then examined under a binocular microscope.

From the histological preparation of the testes, the diameters of the various spermatogenetic cells were measured while from the pituitary tissues the diameter of the GTH cells was calculated with the help of reticulo-micrometer and ocular-micrometer. The percentage of occurrence of the GTH cells during different reproductive phases were measured calculating the number of GTH cells per hundred cells at five different sites for a particular fish on a total of 10 different fishes and then averaging their number for a particular phase. The cell diameter of the GTH cells was measured from a total of 20 cells per fish and the measurements were made at four points within each cell at 90º from one another and reported as the mean ± Standard Error of Mean (SEM). The diameter of the various spermatogenetic stages of the fish was measured in a total of 20 cells per fish and the measurements were made at two different axes perpendicular to one another.

3. Results

The pituitary gland of *M. vittatus* is attached ventrally to the floor of the diencephalon of the brain. In the mid-sagittal section and based on the histological characteristics of its cell types, the adenohypophysis is divisible into three component parts viz., anterior rostral pars distalis (RPD), the middle proximal pars distalis (PPD) and the posterior pars intermedia (PI). Although there is no sharp line separation between these zones. Different cell types of the adenohypophysis in *M. vittatus* have been identified on the basis of their staining intensities with various staining methods.

I. Acidophil I cells

Rounded or oblong acidophils occupy the major part of the RPD. Exhibited positive reaction in the cytoplasm with acid fuchsin and orange G but negative to PAS, AB and aniline blue stain and considered as prolactin cells (Fig. 3).

II. Acidophil II cells

They are small in size, spherical or oval in shape, with small eccentric nuclei, located in the border of neurohypophysial branches and also scattered along with other cell types of the RPD. These cells have no affinities to PAS, AB and aniline blue but have strong affinity to erythrosine and acid fuchsin. These acidophil cells are considered as corticotropic cells (Figs. 6 and 15).

III. Acidophil III cells

Rounded or elongated in shape with centric nucleus and acidophilic small cytoplasmic granules stained intensely with orange G in Mallory's triple stain and AB-PAS-OG but negative to PAS, AB and aniline blue. These cells are scattered in the PPD zone comparable with the somatotrophs (STH) (yellow red colour) (Figs.1 and 6).

IV. Cyanophil I cells

These are rounded, oval or oblong in shape with more or less centrally placed nucleus and dense cytoplasmic granules. Cytoplasm intensely stained with PAS, AB and Aniline blue. During maturation phase cytoplasm showed purple blue colour with AB-PAS-OG due to highly PAS (GTH) positive nature. These cells are comparable to gonadotrophs (GTH) and distributed throughout the PPD region. A few GTH cells are also found scattered in the RPD region along with the

acidophils (Figs. 1, 2, 3, 6 and 7).

V. Cyanophil II cells
They are oblong, elongated or irregular in shape with acentric nucleus. The cytoplasm displayed intense stain with PAS, AB due to glycoprotein materials and also exhibited navy blue colour in the cytoplasm with aniline blue of Mallory's triple stain. These cells are comparable to thyrotrophs (Figs.1 and 3) and mainly restricted in the basal portion of RPD and whole part of PPD and also intermingled with cyanophil I cells, *i.e.*, GTH cells.

VI. Chromophobe cells
They are small in size, spherical or oval in shape with small eccentric nuclei and considered as degranulated state of various chromophil cells. These cells are located sparsely in RPD, PPD and PI region (Figs.1 and 11).
The event of spermatogenesis in *M. vittatus* has been divided into five distinct stages, viz., spermatogonia (stage I), primary spermatocytes (stage 2), secondary spermatocytes (stage 3), spermatids (stage 4) and spermatozoa (stage 5). The characteristic features of the aforesaid stages are as follows:

I. Spermatogonia (stage-1)
These are largest ones of all the spermatogenetic cells occurring singly or in the nests attached to the lobule boundary wall (Figs. 4, 17 and 18). These cells are more or less spherical in shape with a rounded nucleus stained with haematoxylin and chromobhobic cytoplasm. The diameters of these cells vary from 10 to 12 µm.

II. Primary spermatocytes (stage 2)
The primary spermatocytes contain relatively lesser amount of chromophobic cytoplasm and strongly basophilic nuclei. The diameters of these cells vary from 8 to 10 µm (Figs. 5 and 18).

III. Secondary spermatocytes (stage 3)
They are more or less spherical in shape with a diameter ranging from 5 to 7 µm. Cytoplasm is hardly seen around the nuclei of these cells. The nuclei are highly condensed and deeply stained with haematoxylin. They last for a short duration and thereafter produce spermatids (Figs. 4 and 17).

IV. Spermatids (stage 4)
These cells remain in dense aggregation within the lumen of the lobules. The nucleus is in highly condensed state and also strongly basophilic in nature. The diameters vary from 2.5 to 3 µm. They undergo a series of transformation resulting in the formation of mature spermatozoa (Figs. 5, 9 and 10).

V. Spermatozoa (stage 5)
The spermatozoa are occurring in the central position of lumen in the lobules. The nucleus is approximately 1.5 to 2 µm in diameter (Figs. 5, 9 and 13).
In between the seminiferous tubules are present the Interstitial cells of Leydig (14 ± 0.35 µm) (Figs. 5, 9 and 17).

3.1. Seasonal changes in the gonadotropic cells in relation to testicular maturation
The seasonal changes in the gonadotropic cells in the pituitary and different germ line cells in the testes have been described on the basis of frequency percentage of GTH cells and various germ cells in the testicular lobules. Accordingly, the reproductive cycle in *M. vittatus* may be grouped into growth, maturation, spawning and post-spawning phases which are as under:

3.1.1. Growth phase (December to February)
The cytomorphological changes in the GTH cells of the pituitary bear a close relationship to the changes in the testis during their growth. The GTH cell is small and it possesses a spherical, azocarminophilic nucleus, situated mainly in the PPD, they are few in number in the RPD. During this phase the average diameter of the GTH cells are calculated to be 9.2 ± 0.3 µm in December to 10.3 ± 0.47 µm in February (Table. 1). An increase in the number and activities of GTH cells during the end of growth phase occurs at the time of differentiation of testicular germ cells (Figs. 1, 2 and 3).
In the present investigation in testes the value of GSI ranges from 0.56 ± 0.06 to 0.859 ± 0.13 (Fig. 19). Spermatogonial cells are the principal cell types encountered during December (Fig. 4). Primary, Secondary spermatocytes and spermatids are noticed in January. However, during the end of February spermatids and spermatozoa are present in considerable numbers (Fig. 5).

3.1.2. Maturation phase (March to May)
During this phase the GTH cells attain their maximum size (Table 1) and occupy almost the entire PPD region and to some extent also in the RPD region. The increment in the volume of the GTH cells, including their cytoplasm is clearly detected (Figs. 5, 6 and 7). The percentage of the GTH cells in the PPD region is about 48%.
During this phase when the testes enter into the maturation phase the GSI aligns between 0.861 ± 0.02 to 1.02 ± 0.19 (Fig. 19). The diameter of the tubules increases considerably. All types of spermatogenic cells appear in this phase. Spermatogonia decrease in number due to its rapid division to produce large numbers of spermatids and spermatozoa. During the end of this phase distended seminiferous lobules are totally packed up with cysts of spermatids and spermatozoa. The interstitial cells in the interlobular spaces become active (Figs. 9 and 10).

3.1.3. Spawning phase (June to August)
In the spawning phase slight increase in the average diameter of GTH cells is noticed. It has a value of 15.1 ± 0.31 µm in June followed by 15.2 ± 0.44 µm in July (Fig. 11). However, decreasing trend has been noticed from August when the diameter of GTH cells is recorded to be 13.15 ± 0.03 µm (Fig. 12). The GTH cells increased in number (Table 1) and staining intensity in PPD region as an indication of cell granulation. In the PPD, the percentages of GTH cells are about 55% during the spawning phase (Fig. 11). During the highest testicular development the percentage of GTH cells attained peak value. The close association of the GTH cells with the blood cells in the PPD zone clearly indicated that the secretory products of GTH are conveyed through the blood vessels (Fig. 11).
In the testes the GSI value is recorded to be 1.11 ± 0.12 and 1.21 ± 0.29 during June and July respectively. However, declining trend of GSI has been observed from August when the GSI is noticed to be 1.09 ± 0.02 (Fig. 19). The testicular lobules attain a maximum width of this stage and the lobule boundary wall becomes very thin. In the late spawning phase the lobules are packed with spermatozoa although few cysts

containing spermatids have also been observed. The interstitial cells are frequently noticed in the interlobular spaces (Figs.13 and 14).

3.1.4. Post-spawning phase (September to October)

The size as well as the number of GTH cells decreased considerably from September onwards and the average size of the GTH cells are calculated to be 11.3 ± 0.3 µm which further decreases to 10.7 ± 0.3 µm in October (Figs.15 and 16). The percentages of GTH cells are found to be 28% (Table 1). In the testes the GSI value declines to 0.396 ± 0.004 (Fig. 19). The diameter of the tubules decreases and the boundary wall gradually becomes thicker. Residual spermatogenic cells are dispersedly present along with cysts of spermatids (Fig. 17). The lobule boundary wall is lined with spermatogonial cells. During the late resting phase or early growth phase primary spermatocytes, secondary spermatocytes, few spermatids and a

very few spermatozoa have been found as nests (Fig. 18). The interstitial cells are prominent in between lobules (Fig. 17). In the present observation the variations in the cell, cytoplasm and nuclear areas of the gonadotrophs follow the same pattern and run parallel to those of the GSI and proliferation of different germ cell in the testis. The GTH cells attained maximum size prior to the spawning activity of the said fish. The size of the GTH cell decreases in the spent fishes due to the release of cellular materials in the blood circulation.

Table 1: Diameters of the GTH cells and its percentage of occurrence in the PPD of male pituitary of *M. vittatus* during different reproductive phases.

Months	Stages of Maturity	GTH cell diameter (µm)			GTH % in PPD
		Maximum	Minimum	Average ± SEM	
Nov	Resting	10	7.5	7.9 ± 0.34	13.5
Dec	Growth	10	8	9.2 ± 0.29	35
Jan		12	7.5	9.7 ± 0.57	
Feb		12	8	10.3 ± 0.46	
Mar	Maturation	14	10	11.55 ± 0.39	48
Apr		14	10	12.35 ± 0.40	
May		16	12	14.0 ± 0.42	
June	Spawning	16	14	15.1 ± 0.31	55
July		16	12	15.2 ± 0.44	
Aug		14	12	13.15 ± 0.29	
Sept	Post- spawning	12	10	11.3 ± 0.29	28
Oct		12	10	10.7 ± 0.29	

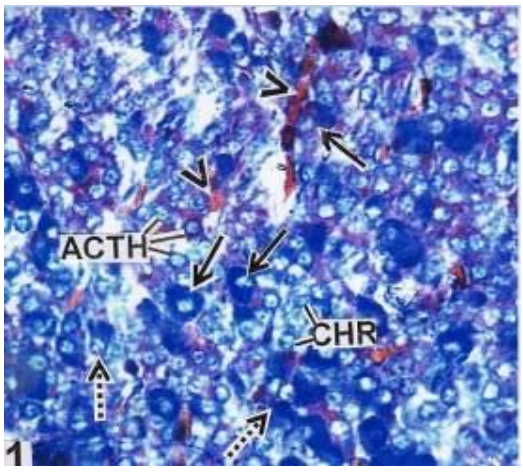

Fig 1: RPD region of pituitary gland during growth phase showing the distribution of GTH cells (solid arrows), TSH cells (broken arrows), ACTH cells and chromophobe cells (CHR). Note blood vessels (arrow heads) in between pituitary cells; (AB-OFG) × 400X.

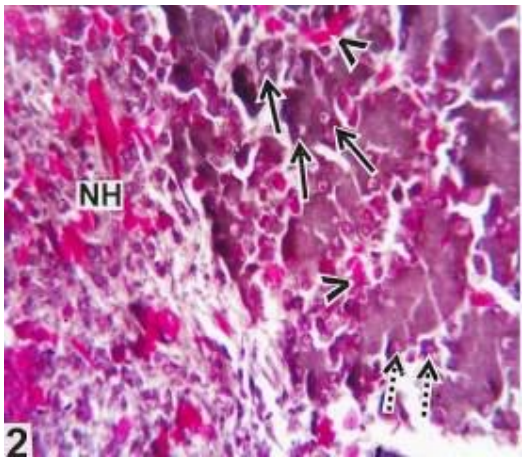

Fig 2: PPD region of pituitary adjacent to neurohypophysis (NH) during growth phase showing GTH cells (solid arrows), TSH cells (broken arrows) and blood vessels (BV) (arrow heads); (CAHP) × 400X.

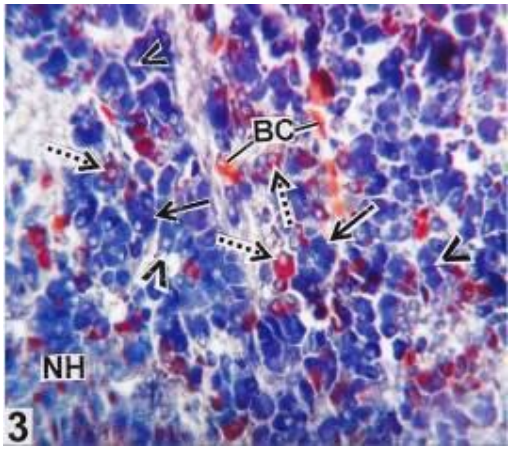

Fig 3: PPD region of pituitary during late growth phase showing dense population of GTH cells (solid arrows) adjacent to blood capillaries (BC). Note the presence of prolactin cells (broken arrows) and TSH cells (arrow heads) in between GTH cells; (MT) × 400X.

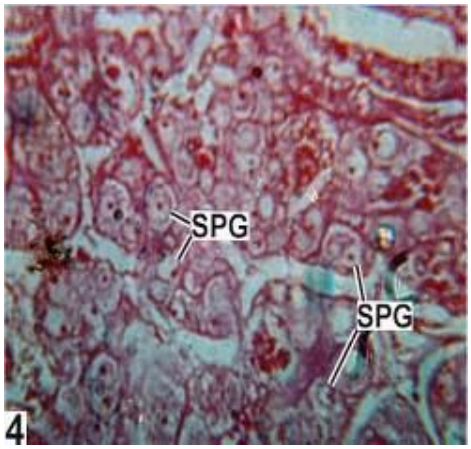

Fig 4: Testicular lobules with spermatogonial cells (SPG) during early growth phase. Note prominent nucleus within SPG; (HE) × 600X.

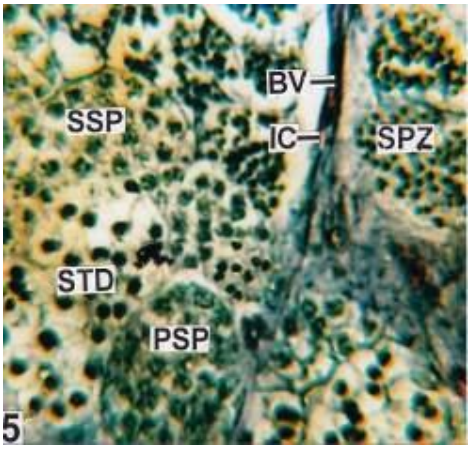

Fig 5: Testicular follicles showing Primary spermatocytes (PSP), secondary spermatocytes (SSP), spermatids (STD) and spermatozoa (SPZ) during growth phase. Note the presence of interstitial cells (IC) adjacent to blood vessel (BV); (IAH) × 600X.

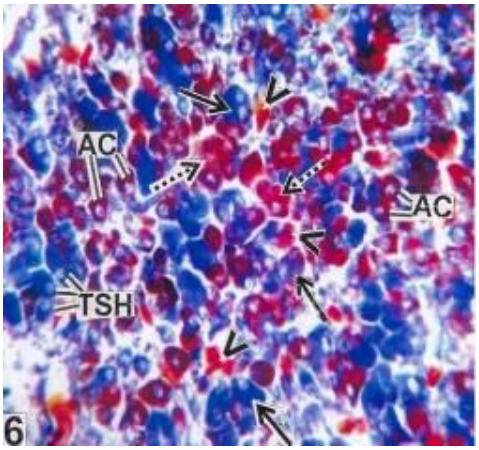

Fig 6: RPD region of pituitary gland during maturation phase showing STH cells (broken arrows), ACTH cells (AC), GTH (solid arrows), TSH cells. Note prominent BV (arrow heads) adjacent to pituitary cells; (MT) × 400X.

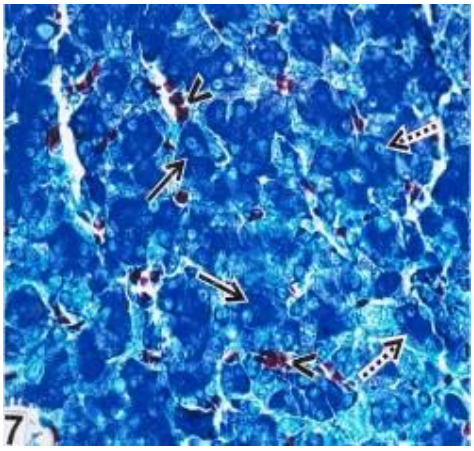

Fig 7: Showing quantitative increment of GTH cells (solid arrows) in the PPD of pituitary showing intense AB stain and acentric nucleus during end of maturation phase. Note the presence of TSH (broken arrows) and BV (arrow heads); (AB-OFG) × 400X.

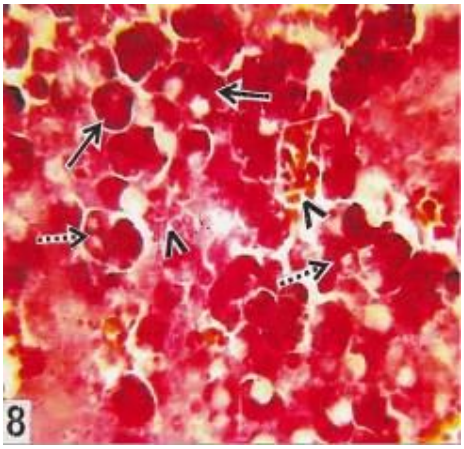

Fig 8: Showing full matured GTH (solid arrows), TSH (broken arrows) in the PPD region of pituitary during end of maturation phase. Note the presence of BV (arrow heads) in between GTH and TSH cells; (PAS-OG) × 400X.

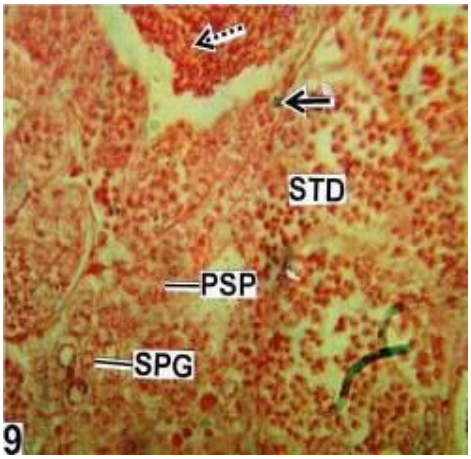

Fig 9: Increment of the diameter of seminiferous tubules. Note the presence of cysts PSP, STD and SPZ (broken arrows). Note decrease number of SPG. Solid arrow indicates interstitial cell; (HE) × 400X.

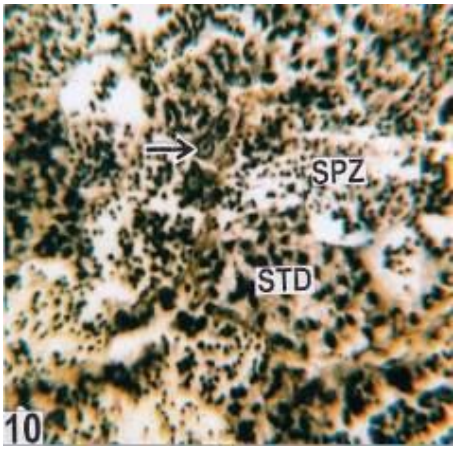

Fig 10: Distribution of STD, SPZ and SPG (arrow head) in the testicular follicle during end of maturation phase; (IAH) × 400X.

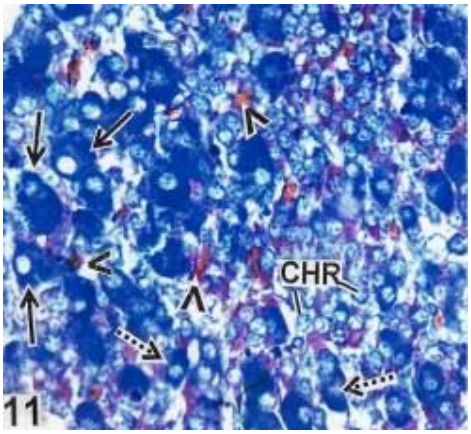

Fig 11: PPD of pituitary gland during spawning phase showing maximum population and dense cytoplasmic stain of GTH (solid arrows), TSH (broken arrows) in between BV (arrow heads). Note scattered CHR cells in between GTH and TSH; (MT) × 400X.

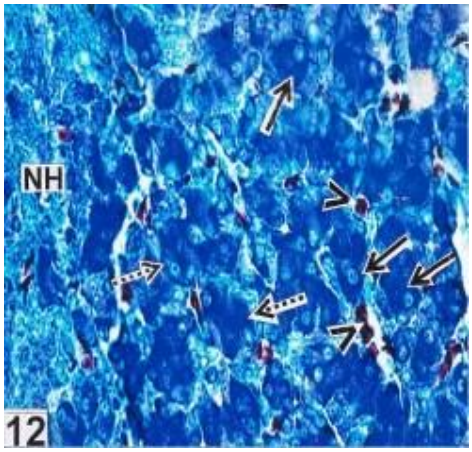

Fig 12: Slight decrease in size and staining intensity of GTH (solid arrows) and TSH (broken arrows) cells in the PPD of pituitary gland during end of spawning phase. Arrow heads indicate BV in between pituitary cells and NH indicates neurohypophysis; (AB-PAS-OG) × 400X.

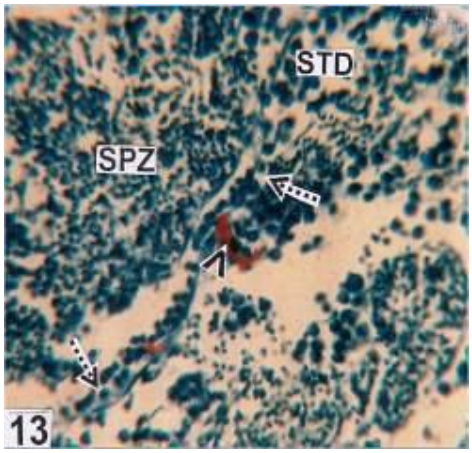

Fig 13: Dense population of SPZ adjascent to cysts of STD in the testicular follicles during spawning period. Note IC (broken arrows) and BV (arrow head); (MT) × 400X.

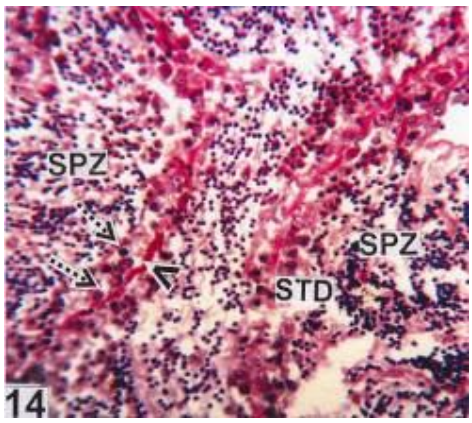

Fig 14: Fully matured SPZ adjascent to cysts of STD in the testicular follicles during spawning phase. Note IC (broken arrows) adjacent to BV (arrow head); (HE) × 400X.

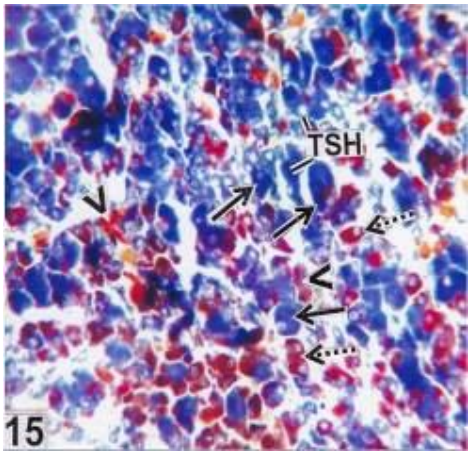

Fig 15: Posterior region of RPD of pituitary gland showing decrease of GTH (solid arrows) and TSH cells during post-spawning phase. Note the presence of ACTH cells (broken arrows) and BV (arrow heads) in between GTH and TSH cells; (MT) × 400X.

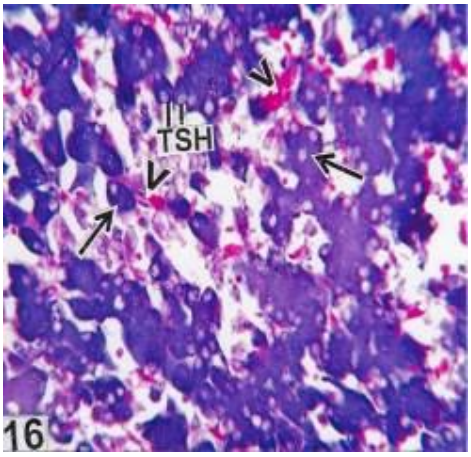

Fig 16: Reduced GTH (solid arrows) and TSH cells in the PPD of pituitary gland during post-spawning period. Note the presence of BV (arrow heads) adjacent to GTH and TSH cells; (CAHP) × 400X.

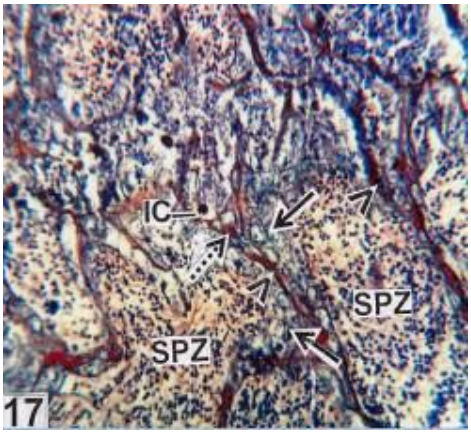

Fig 17: Residual SPZ in the testicular lumen during post-spawning phase. Note the presence of IC adjacent to BV (broken arrow) in the interlobular space. Note the appearance of SPG (solid arrows) along the thickened lobule boundary wall (LBW) (arrow heads). (MT) × 400X.

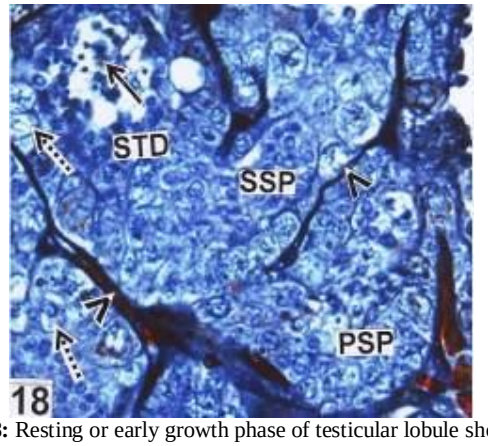

Fig 18: Resting or early growth phase of testicular lobule showing SPG (broken arrows), cysts of PSP, SSP and STD. Note the presence of few SPZ (solid arrow) and thickened LBW (arrow heads). (MT) × 400X.

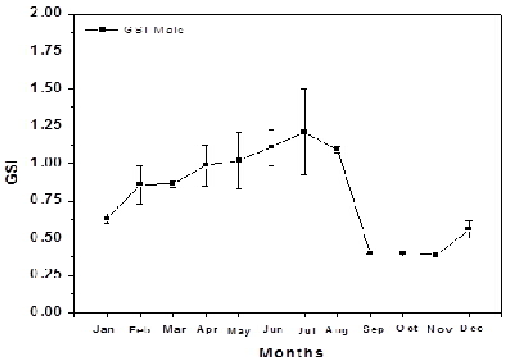

Fig 19: The variation of Gonado Somatic Index (GSI) for male *M. vittatus* during maturation, growth, spawning, post-spawning and resting phases.

4. Discussion

The pituitary gland of *M. vittatus* belongs to the cranio-leptobasic type and is composed of two parts without distinct line of demarcation between adenohypophysis and neurohypophysis. Similar observation has also been reported in the pituitary of Nile tilapia by El-sakhawy *et al.* [23]. The nerve fibres of neurohypophysis give off ramification to the RPD, PPD and PI. The hormone producing cell types is arranged in a mosaic pattern due to the uneven distribution of different cell types within the three regions. Ball and Baker [2] have mentioned that the distribution of cell types in teleost adenohypophysis is extremely regular, so that to a large extent differentiation of cell types in tinctorial techniques becomes easy. Joy and Sathyanesan [24, 25], Jafri and Ensor [26] identified precisely the different cell types situated in the pituitary of a few teleosts. They categorised various cell types in the teleostean pituitaries on the basis of the staining reaction in the cytoplasmic content adopting different staining technology. In the present investigation the prolactin cells provided with acid fuchsin staining granules formed the major component in the RPD. This finding is in agreement with those of Sage and Bern [27], Schreibman *et al.* [28], Joy and Sathyanesan [24] in different teleosts. On the other hand, prolactin cells of RPD are sometimes reported to be chromophobic in some teleosts as advocated by Baker *et al.* [29] and Boddingius [30]. In *M. vittatus* the acidophils of the PPD region *i.e.*, orangeophils have been considered as

somatotrophs are distributed irregularly. Haider [31], Jose and Sathyanesan [4] and Mandal and Sinha [32] reported that prolactin cells are distributed randomly in the PPD of *Heteropneustes fossilis*, *Labeo rohita* and *Catla catla* respectively. The second type of small, spherical or oval acidophils comparable to corticotropic cells which are densely stained with acid fuchsin/erythrosine are scattered in the RPD bordering the neurohypophysis. Ball and Baker [2], Baker *et al*. [29] and Mandal and Sinha [32] reported that adenocorticotropic (ACTH) cells are Lead haematoxylin positive and are located in the RPD bordering the neurohypophysis and occurred in groups. In the present study the cyanoplil I or the gonadotrophs formed the main bulk of cells of the PPD. Few gonadotrophs are also located along the border of the PI. Many authors confirmed cyanophil cells of various types in the different regions of teleostean pituitaries. Jose and Sathyanesan [4] recorded two types of cyanophil cells in the border of RPD and PPD as well as in the PPD proper of *L. rohita*. However, some authors failed to record conclusive evidence regarding the distinction between two types of cyanophils *i.e*., thyrotrophs and gonadotrophs. According to Sage and Bern [27], the thyrotrophs are angular or polyhedral in comparison to gonadotrophs. Jafri and Ensor [26] and Ali [33] showed that the thyrotrophs exhibit navy blue colour while the gonadotrophs impart red colour when AB-PAS-OG-ACF stain is employed in the pituitary of roach. In the present investigation cyanophil I cells or the gonadotrophs are comparatively large, angular or oblong in shape and display purple blue colour in AB-PAS-OG stain and are located mainly in the middle part of PPD. The cyanophil II or the thyrotrophs exhibit navy blue colour with aniline blue and AB-OFG technique and are located in the PPD intermingled with cyanophil I cells. Narayan *et al*. [34] also opined that in *Tilapia mossambica* the thyrotrophs were located in the PPD along with the gonadotrophs.

In many teleosts, a correlation between the gonadotropic cells and the gonadal cycle have been observed showing the hyperplasia, hypertrophy and other signs of increased activity of these cells in association with the maturation of the gonads [35, 36, 37]. In the present investigation in *M. vittatus* the distribution and differentiation of various cell types in adenohypophysis based on tinctorial grounds are well documented. The GTH cells in *M. vittatus* exhibit their prominence having dense stained basophilic cytoplasm with PAS, AB and aniline blue during the maturation of testes. At the end of maturation phase and prior to spawning, this basophil increases in their number. During this period, the GTH cells form the major component of the PPD. This is in conformity with the findings of Krishnan and Diwan [35], Gaber [7] and Al-Absawy [11]. In the present observation pronounced change in cellular diameter as well as accumulation of cytoplasmic contents of GTH cells in the adenohypophysis in *M. vittatus* coincides with the transformation of various germ cells, GSI and beginning of spermiation. The spermatogenetic activity as revealed by the GSI shows an increase in the maturation stages and attains peak values in the late maturation or early spawning. Joy and Sathyanesan [24] have clearly demonstrated that in *Clarias batrachus*, the basophils in the pituitary gradual increase in their number when the gonads start maturing and in those with ripe gonads these cells form the major component of the PPD. During the growth phase the low active condition of the GTH cells is well coincidence with the increase in spermatogonial cells and with the absence of spermatozoa. The GTH cells are more or less inactive and decrease its average number during the post-spawning period. In the present study, another basophilic cell i.e., TSH cells exhibit seasonal changes in their activity during maturation and spawning phases as these cells appear granulated with dense cytoplasmic stain.

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