Seasonal Changes in the Cytomorphology of the Thyroid Follicles and its Corelation with the Testicular Cycle in the Asian Striped Catfish *Mystus vittatus* (Bloch, 1794)

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Available online at: www.isca.in, www.isca.me

Received 11th May 2014, revised 6th June 2014, accepted 19th September 2014

Abstract

The seasonal changes in the cellular morphology of the thyroid gland were studied in male Mystus vittatus. It was found to be composed of discrete follicular structures dispersed mainly in the sub pharyngeal region around the ventral aorta. The follicles were found to be composed of single layered epithelial cells with agranular eosinophilic colloids in their central lumen. The secretary activity of the follicular cells was found to be in accord with the seasonal phasic changes of the male reproductive cycle. The maximum secretary activity of the thyroid was studied during the spawning phase while the minimum activity of the follicles was traced during the post-spawning and resting phases. So, it was established that the thyroidal secretional activity corresponds to the seasonal testicular cycle of Mystus vittatus and hence, the breeding behaviour of male is controlled by the thyroid gland.

Keywords: Thyroid, follicle, colloid, testis, *Mystus vittatus*.

Introduction

The thyroid gland in teleosts has been recognised as an endocrine structure essential for the control of metabolism, osmoregulation, growth, development and metamorphosis¹⁻³. Considerable work has been done focussing the role of the thyroid gland related to functions like migration⁴, metabolism, osmoregulation and differentiations⁵⁻¹⁰. The gross morphology of the teleostean thyroid are found to vary from scattered follicles to encapsulated structures located in the sub-pharyngeal region in some cases^{11,12}. The seasonal changes in the thyroid gland activity of teleosts have been studied and often been correlated with the gonadal maturation^{7,13-15}. Since, high metabolic alterations takes place during the testicular development in teleosts¹⁶, it is evident that there will be a variation in the histology of the male thyroid.

In our present work an attempt has been made to investigate more precisely the correlative changes between different functional states of the thyroid follicles with the changes in the testes during different reproductive phases in *Mystus vittatus* (Bloch). This small indigenous fish species has been selected due to its nutritional value in terms of proteins, micronutrients, vitamins and minerals ¹⁷ and high market price.

Material and Methods

Specimen collection and calculation of Gonado Somatic Index (GSI): Adult male *M. vittatus* were procured fortnightly throughout the year from a particular stocking pond in order to avoid ecological variations in different ponds that can affect the

development of gonad and thyroid. About 162 male fishes with body weight ranging between 10g to 18g and body length ranging between 8cm-14cm were used for our experimental purpose. Every month data on total body weight and testicular weight of 12 fishes were taken to calculate the mean GSI using the following formula:

$$GSI = \frac{\text{Gonadal weight}}{(\text{Total body weight-Gonadal weight})} X \ 100$$

Histological methods and Statistical measurements: To study the seasonal changes of the thyroid and testis, the fishes were at first sacrificed and for obtaining the thyroid tissue, lower jaw of the fishes were taken out and the stenohyoid muscles were removed. The tissues around the ventral aorta and afferent branchial arteries were dissected out and fixed in aqueous Bouin's fixative for 18 hours. The thyroid was decalcified in a mixture of 5% formic acid and formaldehyde (1:1 volume) for seven days¹⁵. The testis were fragmented (for better penetration of the fixative) and fixed in aqueous Bouin's fixative for 18 hours. The thyroid and testis were then dehydrated through upgraded series of ethanol, followed by acetone and cleared in benzene. Tissues were embedded in paraffin (melting point 56°C-58°C) and serial sections were cut at 4μm thickness. The sections of the testis were stained using iron-alum haematoxylin (IA), Mallory's triple stain (MT) and the routine Delafield's Haematoxylin and Eosin (H&E) while that of the thyroid were stained using Delafield's Haematoxylin and Eosin (H&E) only. All the tissues were cleared in xylene and mounted permanently in DPX and then observed under a binocular microscope.

From the histological preparation of the testis, the diameters of the various testicular cells were measured while from the thyroidal tissues the diameter of the thyroidal follicles and the epithelial cell heights were calculated with the help of reticulo-micrometer and ocular micrometer. The cell height of the thyroid epithelium and the follicular diameter were measured from a total of 20 follicles per fish and the measurements were made at four points within each follicle at 90^{0} from one another and reported as the mean \pm Standard Error of Mean (SEM). The diameters of the various testicular stages of the fish were measured in a total of 20 cells per fish and the measurements were calculated from two different axes perpendicular to one another.

Results and Discussion

Gonadosomatic Index (GSI): The thread like elongated testis of *Mystus vittatus* is located in the dorsal part of the coelom. In the spawning season, the testis appears to be translucent white with prominent denticulations. The weight, colour and morphology of the testes vary in different seasons of the year. The seasonal variation of testes can be quantified using the parameter Gonadosomatic index (GSI) as defined in earlier section. Figure 1 depicts the seasonal change of GSI for adult male *Mystus vittatus* throughout the year.

Our present study showed that the GSI increased slightly from December onwards till February i.e. throughout the growth phase. This can be attributed to the maximum proliferative activity of the spermatogonia and spermatocytes during the growth phase.

From March onwards the GSI was found to increase rapidly throughout the maturation phase and continued up to July i.e., the mid of the spawning phase and then gradually started declining during the late spawning period. These findings are in accordance with that of Hossain *et al.*¹⁸ and might be due to testicular proliferation of large number of spermatocytes and spermatids during the maturation phase and spermatids and spermatozoa during the spawning phase. However, the GSI value was observed to decline from the end of spawning phase, i.e., from August onwards perhaps due to the gradual discharge of cohorts of spermatozoa.

In November, few spermatogonial cells were observed to reappear on the wall of the testes, which failed to show any proliferative activity and hence found to be under resting condition with minimum GSI value. These findings keep uniformity with the view of other workers¹⁸.

Histology: Thyroid: The activities of the thyroid gland could be studied taking into account the seasonal variations of follicular cell diameter shown in figure 2 and the epithelial cell height shown in figure 3. The epithelial cells surround the lumen containing the colloidal materials.

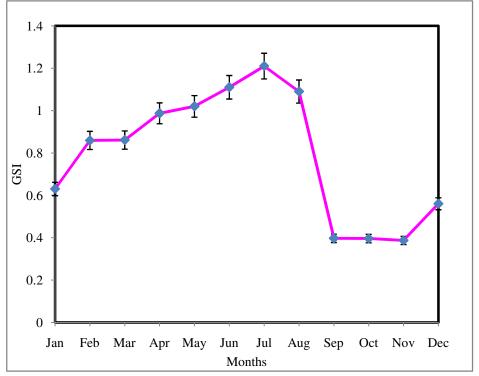
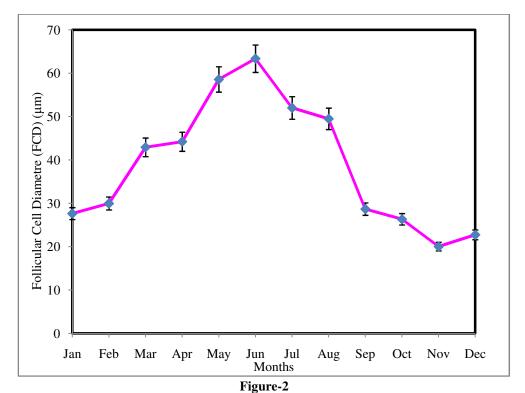


Figure-1
Seasonal changes in the Gonadosomatic index of male *Mystus vittatus* under controlled condition



The Follicular cell diameter (FCD) for male *Mystus vittatus* during different reproductive seasons (the vertical lines at the measurement points are indicating the SEM)

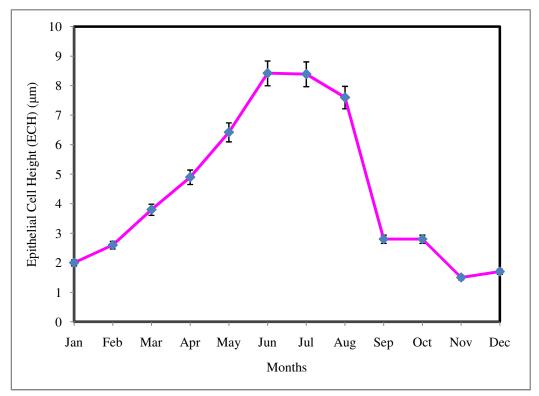


Figure-3
The Epithelial cell height (ECH) for male *Mystus vittatus* during different reproductive seasons (the vertical lines at the measurement points are indicating the SEM)

It was observed from our study that the thyroid follicles in *M. vittatus* did not occur as individual encapsulated structures but were found to be arranged in groups as shown in figures 4 and 5, scattered around the regions of middle and posterior part of the ventral aorta in between dorsal branchial cartilages and ventral stenohyoid muscles as shown in figure 5 or connective tissue as already reported in case of female *M. vittatus*¹⁵. The thyroid follicles were found to be round or oval in shape and were frequently dispersed around the blood vessels as shown in

figures 4, 8, 10 and 12. They were found to be composed of two distinct components, the central lumen filled partly or fully with agranular, eosinophilic and almost homogeneous colloids as depicted in the figures 4, 5, 8 and 10. The follicles were dispensed with resorption vacuoles as shown in the figures 8, 10, 13 and 15, during the active reproductive phases and surrounding them were the single layer of nucleated epithelial cells as depicted in the figures 4, 10 and 12, whose height varied greatly throughout the year.

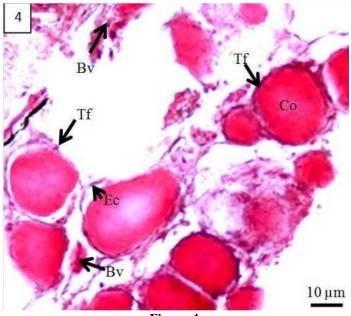


Figure-4

Histological photograph of the non-secretory thyroid follicles (Tf) of growth phase of *Mystus vittatus* filled with colloid (Co) and bordered with squamous epithelial cells (Ec). Note the presence of blood vessels (Bv) adjascent to Tf (H&E)

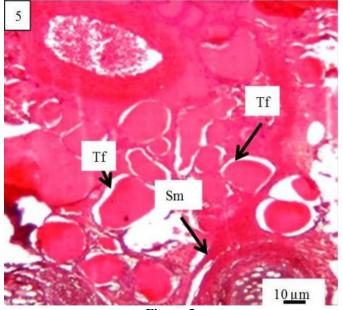


Figure-5

Histological photograph showing partially secretory stage of Tf in *Mystus vittatus* lying adjascent to ventral stenohyoid muscles (Sm) during growth phase. Note narrow colloid free space along the margin of Tf. (H&E)

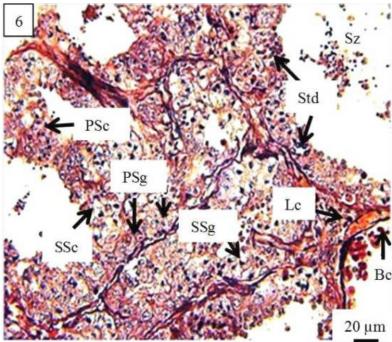


Figure-6

Histological photograph showing the testicular cells of different stages during growth phase of *Mystus vittatus* with Primary spermatogonia (PSg), Secondary spermatogonia (SSg), Primary spermatocyte (PSc), Secondary spermatocyte (SSc), Spermatids (Std) and Spermatozoa (Sz). Note the presence of Leydig cells (Lc) in between spaces of the testicular lobules and the nests of blood cells (Bc) adjascent to it. (H&E)

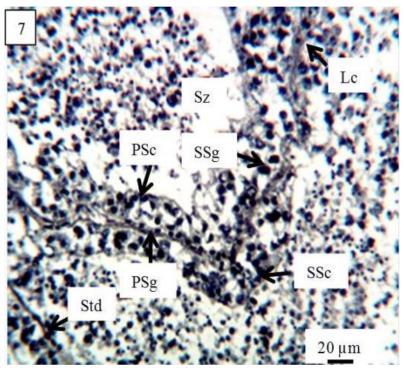


Figure-7

Histological photograph showing late growth phase with Primary spermatogonia (PSg), Secondary spermatogonia (SSg), Primary spermatocyte (PSc), Secondary spermatocyte (SSc), Spermatids (Std) and Spermatozoa (Sz) and Leydig cells (Lc) in Mystus vittatus (IA)

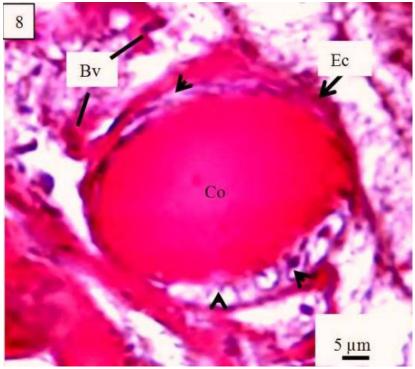


Figure-8

Histological photograph of the secretary stage of thyroid follicles (Tf) in *Mystus vittatus* during maturation phase showing cuboidal epithelial cell layer with empty space within the follicle (arrow heads) and blood vessels in between the follicles. (H&E)

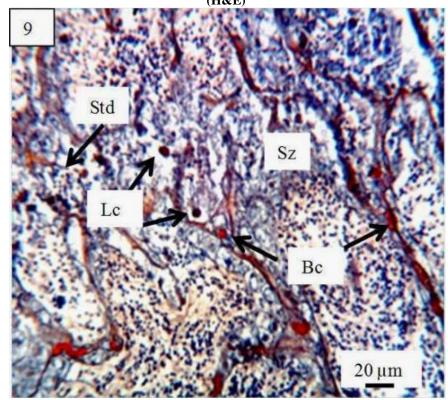


Figure-9

Histological photograph of testicular stage during late maturation phase showing Spermatids (Std) and Spermatozoa (Sz) in *Mystus vittatus*. Note prominent Leydig cells (Lc) with adjascent Blood cells (Bc). (MT)

Spermatogenesis: The sequential process of the formation of sperm is known as spermatogenesis and it occurs within the testis. The organization of the germ cells in the testes of the teleosts is of unrestricted type^{19,20}. It means that the spermatogonia are localized in all extentions of the inner seminiferous tubule wall, and not restricted to a particular portion. Histologically, the sequence of spermatocyte maturation in *Mystus vittatus* had been divided into six distinct developmental stages viz., primary spermatogonia (stage-I), secondary spermatogonia (stage-II), primary spermatocytes (stage-III), secondary spermatocytes (stage-IV), spermatids (stage-V) and spermatozoa (stage-VI).

In between the seminiferous tubules, the presence of the tubule boundary cells or intersticial cells of Leydig was observed in the present study. Some workers had established that the fish spermatozoa show more morphologic diversity than those of other vertebrates, and their ultrastructure provides parameters for phylogenetic analysis^{21,22}. Our histological observations showed the following testicular cell types in male *Mystus vittatus*.

Primary spermatogonia- stage-I (13.2 \pm 0.05 μ m): These cells appeared to be round or oval in shape and were the largest in size among the testicular cells with large diffused chromophilic and basophilic eccentric nucleus and almost unstained cytoplasm. These cells mostly occurred in nests and were found to be attached to the lobule boundary wall in the inner margin of the seminiferous tubule as shown in the figures 6, 7, 17 and 19.

Secondary spermatogonia- stage-II ($10.04 \pm 0.02 \,\mu m$): These cells were the product of mitotic division of the primary spermatogonia. These were found to present in groups with relatively smaller and rounded appearance with a centrally placed nucleus and lesser cytoplasmic content as shown in figures 6 and 7.

Primary spermatocytes-stage-III (7.8 \pm 0.31 μ m): These cells were found to contain lesser amount of chromophobic cytoplasm and possessed strongly basophilic nuclei which stained deep with Haematoxylin. They were formed from stage II by mitotic division as shown in figures 6 and 7.

Secondary spermatocytes-stage-IV ($5 \pm 0.61 \, \mu m$): These cells were smaller in sizes and were produced as a result of reduction division from the primary spermatocytes. In this stage, the cytoplasm was hardly visible around the nucleus under the light microscope. The nucleus appeared to be deeply stained and hence highly condensed. These cells appeared in large nests situated towards the lumen of the testicular lobules as depicted in figures 6, 7.

Spermatids-stage-V(2.2 \pm 0.02 \mum): These cells appeared to be round, oval or crescent shaped having no visible cytoplasm. These cells were found as compact aggregation adjacent to the lumen of the lobules as shown in figures 6, 7 and 9.

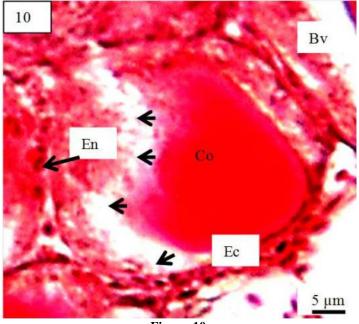


Figure-10

Histological photograph of active Tf bordered with prominent thick Epithelial cells (Ec) and Epithelial nuclei (En) showing liquefaction of colloid during late maturation phase in *Mystus vittatus*. Note active state of blood vessels (Bv) in between Tf. Solid arrows indicate active secretary stage of Tf. (H&E)

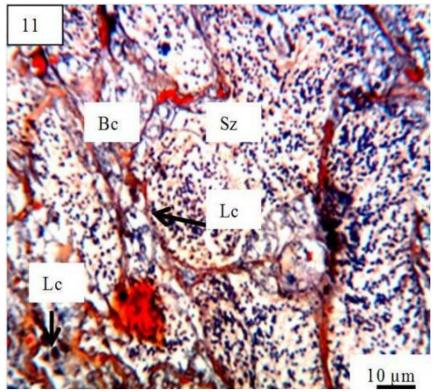


Figure-11

Histological photograph showing early spawning phase provided with prominent Leydig cells (Lc), blood cells (Bc) and lumen almost packed with mature spermatozoa (Sz) bearing long tails in *Mystus vittatus*. (MT)

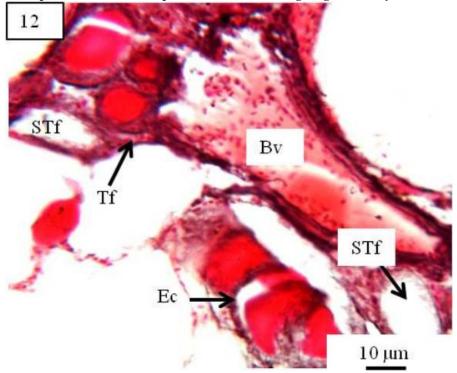


Figure-12

Histological photograph of Thyroid follicle (Tf) showing central colloid (Co) within lumen during spawning phase in *Mystus vittatus*. Note thick and prominent epithelial cells and few spent thyroid follicles (STf) Bv indicates blood vessels adjascent to Tf (H&E)

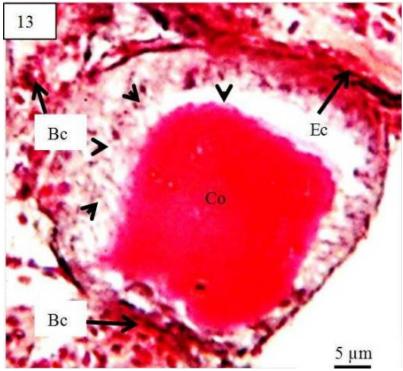


Figure-13

Histological photograph of Thyroid follicle (Tf) during late spawning phase showing resorption vacuoles at the periphery (arrow heads) and central colloid in *Mystus vittatus*. Note epithelial cells bordering the Tf and the Blood cells (Bc) surrounding it (H&E)

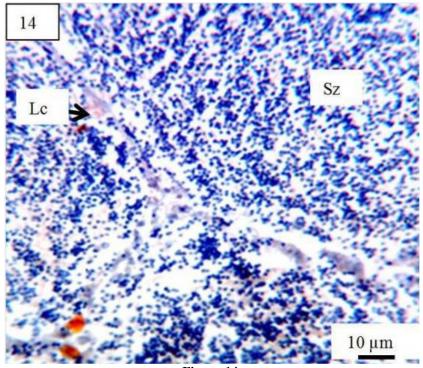


Figure-14

Histological photograph of Testis fully packed with mature spermatozoa (Sz), during the late spawning phase in *Mystus vittatus*. Note the Leydig cell (Lc) adjascent to the blood cells. (MT)

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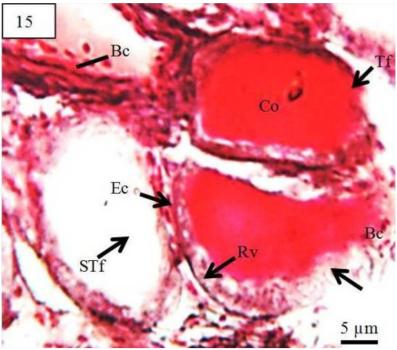


Figure-15

Histological photograph Showing active secretory stage of thyroid follicles (Tf) during spawning phase with resorption vacuoles (Rv) in *Mystus vittatus*. Note the ruptured follicle (Rf) with invading blood cells (Bc) increased epithelial cell height and less amount of colloid (Co) in the centre of Tf. STf indicates sunken thyroid follicle. (H&E)

Spermatozoa-stage-VI (1.5 \pm 0.04 μ m): These cells were produced by the second meiotic division of the secondary spermatocytes. Under the light microscope, these appeared as deeply stained with a spherical nucleus and a faint long tail. The spermatozoa were found to be occurring in clusters in the central position of the lumen of the lobule as shown in figures 6, 7, 9, 11, 14 and 19.

Leydig cells (14 \pm 0.35 \mum): These cells were found in the inter-lobular boundary walls in variable numbers during the reproductive cycle and reports are available suggesting the changes in its morphology during the entire seasonal cycle^{23,24}. These were found to be oval or round in shape and associated with the blood vessels as shown in figures 6, 9, 11 and 14.

Sequential changes in the thyroid follicles and testis during different reproductive phases: As revealed by the histological observations, the characteristics of the thyroid follicles were found to change in accord with the frequency of the occurrence of various testicular cells during the different reproductive phases of the fish under study. The characteristics of the thyroid follicles have been studied by considering the height of the follicular epithelial cells and follicular cell diameter along with the colloidal compactness of the thyroidal lumen.

Growth phase (December to February): During this phase the thyroid follicles were found to be in non-secretory stage as revealed by the surrounding flat squamous epithelial cell layer.

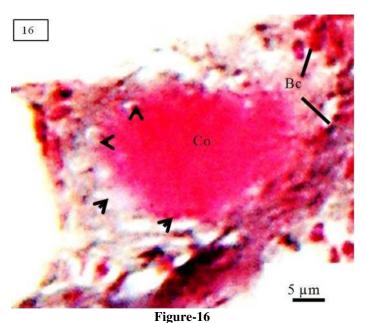
Figure 2 shows that the average diameter of the thyroid follicles in *M. vittatus* ranged from 22.73 \pm 0.88 μm in December to 29.96 \pm 0.62 μm in February. Figure 3 shows that the height of the epithelial cell layer ranged from 1.7 \pm 0.2 μm to 2.0 \pm 0.2 μm . The oval or rounded follicles were filled up with dense eosinophilic colloids which in most cases were in contact with the epithelial cell layer as shown in figures 4 and 5.

The GSI was recorded from 0.560 ± 0.06 to 0.859 ± 0.13 as shown in figure 1. The primary and secondary spermatogonia that stain deep with the above mentioned stains were observed to be located towards the periphery of the testicular lumen. Large numbers of primary and secondary spermatocytes, most of which had large round nuclei with a distinctive chromatin pattern were seen to lie above the spermatogonial cells. Few spermatogonia occurred at the sites close to the lumen. Cohorts of spermatozoa were observed at the centre of some of the lumen as shown in figure 6 indicating that the fishes were not the first time spawners. During the late growth phase, the cellular density increased towards the lumen indicating rapid cellular proliferation as shown in figures 6 and 7.

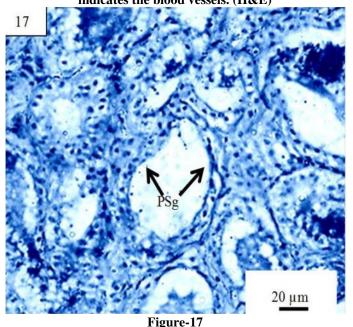
Maturation phase (March-May): During this phase, the thyroid follicles were found to be in secretory and active secretory stages. An increasing trend in the epithelial cell height as depicted in figure 3, has been recorded from about 1.2 ± 0.5 μ m in March to 4.7 ± 1 μ m in May. The oval or elongated follicles measured about 26 ± 1.4 μ m in March to 40 ± 1.4 μ m in

May as shown in figure 2. With the onset of March and during the months of April and early May, the follicular lumen were observed to be completely filled up with deeply stained colloid having vacuolated structures at the outer margin of the colloids as could be observed from figure 8. This stage could be said to be the stage of maximum colloid storage. The degree of vascularization was found to be at its peak and the blood cells were closely associated with the follicular layer as shown in figure 8.

Figure 1 depicted that the GSI measured from 0.861 ± 0.02 in March to 1.020 ± 0.19 in May. The spermatogonial cells continued to occur and in March large numbers of spermatocytes were found adjascent to the spermatogonial cells, while the spermatids and spermatozoa dominated during the late maturation phase and large Leydig cells became visible adjascent to the blood cells in the inter-lobular spaces as shown in figure 9.



Histological photograph of Thyroid follicle (Tf) discharging its content at the onset of post-spawning phase in *Mystus vittatus*. Note the distorted epithelial cell (Ec) lining and the rapidly increasing resorption vacuoles (arrow heads). Be indicates the blood vessels. (H&E)



Histological photograph of Testis during post-spawning phase showing thickened lobule boundary walls and primary spermatogonial cells (PSg) in *Mystus vittatus*. (MT)

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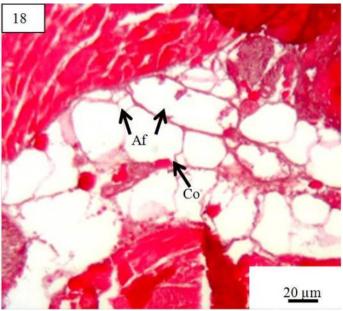


Figure-18

Histological photograph of Thyroid follicles of *Mystus vittatus* in their quiescent state with many Atrophied follicles (Af) and few follicles bearing very little colloid (Co) in them.

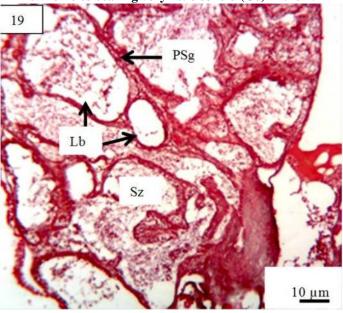


Figure-19

Histological photograph of Testis in their resting phase showing thicker lobule boundary (Lb) walls and emerging Primary spermatogonial cells (PSg) in *Mystus vittatus*. Note that the testicular lumen contains spent spermatozoa (Sz)

Spawning phase (June to August): This phase was found to be dominated by the follicles of the active secretary and atrophid stages. During the months of June, July and August i.e., spawning phase, it could be observed from the figures 10, 13 and 15 that most of the follicles in male were surrounded by either cuboidal or columnar epithelial cell layers. The height of the epithelial cell layer was observed to be $7 \pm 1.1~\mu m$ during June to $9 \pm 1.2~\mu m$ during August as shown in figure 3.

The follicles were at their active functional states as shown in the figures 10, 13 and 15 by a large number of vacuoles around the entire margin of colloid. The follicular diameter measured about $55\pm1.2~\mu m$ in June to $68\pm1.1~\mu m$ in August as shown in the figure 2. Some of the follicles as could be observed from the figures 12 and 15 have been found to be empty with high epithelial cell layer.

In the testis during the early spawning phase quite a number of Leydig cells were visible with blood vessels near their vicinity. The spermatozoa formed bulk of the cellular population with very few earlier stage cells as shown in figure 11. In the late spawning phase the lobule boundary wall were observed to become very thin. Spermatogonial cells along with the spermatogonia almost disappeared. The testicular lumens slightly increased in diameter and were found to be fully packed with mature spermatozoa bearing very long tails. The spermatozoan heads as could be seen from figure 14 appeared to be deeply stained suggesting highly condensed chromatin material inside it.

Post spawning phase (September to October): During this phase the atrophid follicles with irregular epithelial lining ruptured at places were found to be frequent as shown in figure 16. The epithelial cells gradually began to decrease in height denoting fall in their activity for colloid synthesis. Most of the follicles appeared to be empty and distorted. It was observed from our study that the testes gradually decreased in volume and size. The lobule boundary cells i.e., the spermatogonia reappeared. Few of the lobules were still found to be filled with spermatozoa which were recognized as residual spermatozoa. But majority of the lobules were observed to be nearly empty. Spermatogonial cells rapidly increased in number as shown in the figure 17 and the tunica albugenia increased in thickness.

Resting phase (November): During this phase faint thyroid follicular outline with almost empty lumen were noticed. The epithelial height declined to $1.5 \pm 0.02 \, \mu m$ in November as shown in figure 3. In some of the follicles negligible amount of colloid was not very uncommon as observed in the figure 18.

Externally the testes appeared to be thread-like. Large number of spermatogonial cells were observed in figure 19 attached to the inner wall of the testicular lobules that could be distinctly identified. This phase is considered to be most inactive due to slower rate of cell proliferation hence, such name.

Discussion: The present study showed that the GSI remained almost stationary at the beginning of the growth phase and then increased slightly perhaps due to the rapid proliferation of the early stages of the testicular cells. From the beginning of maturation phase the GSI increased slowly but steadily up to the mid-spawning phase due to maximum proliferation of the spermatids and spermatozoa as also observed by the expansion of the testicular lobules. However, in comparison to the female GSI ¹⁵, the value for male fish is almost negligible. Again, from the end of spawning phase the GSI decreased sharply denoting release of the mature spermatozoon in large number from the testicular lobules.

In our present histological analysis of the thyroid follicles significant monthly changes in follicular cell height as shown in figure 2 and epithelial cell height as shown in figure 3 had been noticed round the year which again had been found to occur in

pace with the testicular cycle in *M. vittatus*. The diffused groups of thyroid follicles which were found to be scattered in *M. vittatus* corroborates with the findings of Srivastava and Satyanesan ²⁵ and Joy and Satyaneshan ²⁶ in *Clarias batrachus* and Abbas *et al.* in *Epinephelus aeneus* ²⁷. From our study, the functional state of thyroid follicles in male *M. vittatus* during different reproductive phases may be grouped in five distinct stages: non-secretary, secretory, active secretary, spent or atrophied and quiescent on the basis of the amount of colloid material present, epithelial cell height and the presence or absence of colloidal resorption vacuoles. Mukherjee ²⁸ also divided the thyroid follicles of *Clarias batrachus* into five stages (non-secretary, secretary, active secretary, atrophied and quiescent).

In the present study it has been found that during the spawning phase the thyroid follicles were in their active secretory state while the quiescent phase of the thyroid follicles coincides with the resting or spent phase of the testis. Belsare opined²⁹ that in Channa punctatus, the thyroid activity had a close relationship with the breeding activity rather than the temperature of the habitat. In M. vittatus, the tall columnar epithelial cells with basophilic and vacuolated colloid were observed during the late maturation and spawning phase indicating an active thyroid gland. In the post-spawning and resting phases, the epithelial cells appeared to be flattened indicating their atrophied and quiescent states respectively. Volkoff et al.³⁰ found that in the Atlantic stringray, Dasyatis sabina, the follicular cells varied in size and shape, according to the activity of the gland. Salamat et al. reported that³¹ the surrounding epithelial cells of the thyroid follicles in Acanthopagrus latus were flattened, cuboidal or columnar depending upon their activity. They further noticed that during the warm season tall, columnar epithelial cells with basophilic colloid containing vacuole-like spaces, characteristics of an active thyroid gland were seen.

During the maturation phase (from March to May), the colloid storing activity reached its maxima and the follicles remained densely packed with the colloid. With the advent of spawning or discharge phase the colloid boundary cells appeared darker and thicker hence the epithelial cells remain most active during this time. The colloid appeared to be vacuolated towards the margin and less in quantity and this period was considered to be the active secretory phase of the thyroid. Huge metabolic activities were assumed to occur during this period. Figure 15 shows such a follicle in which the epithelial cells have ruptured and have been invaded by the blood cells. It has been suggested by Bargmann, ³² that this was one way in which the stored hormone was released into the blood stream.

In the post spawning period, the testicular activities became very slow and at the same time the thyroid tissue exhibited relatively smaller, atrophied and distorted follicles with very little colloid in them. The epithelial layer also showed signs of irregularity. With the release of mature spermatozoa the testis receded in size and at the same time the follicles became atrophied or empty as could be seen from figure 18, without noticeable colloidal materials. Hence, the thyroid in M. vittatus played an active as well as vital role in controlling the seasonal cycle of spermatogenesis. Ortiz et al. reported³³ that in Solea senegalensis, the thyroid represented the colloid filled follicles surrounded by a cuboidal epithelium during summer, suggesting a high activity state of this organ. Nishikawan³⁴ advocated that the thyroid follicles have been found to be in most active condition during spawning phase and the thyroid activity becomes low as soon as the fish enters the post-spawning phase. A number of blood vessels were found to be lying near the thyroid follicles indicating the pathway of their contents through the blood stream. Osborn and Simpson reported³⁵ the elevation of thyroid hormone level during the time of gonadal development in plaice and rainbow trout occurs in both immature and maturing specimens, and thyroxine may be regarded as permitting the metabolic changes necessary to save the developing gonad rather than as being directly involved in gametogenesis.

Conclusion

From this present study, it may be concluded that the thyroid in *M. vittatus* plays a vital as well as active role in controlling the seasonal cycle of spermatogenesis. Therefore a systematic control upon the thyroid gland will prove to be beneficial for controlling spermatogenesis of the fish under study. This will in turn be profitable in the field of aquaculture in south-east Asia, where this fish is economically very important.

Acknowledgements

The author is grateful to the University Grants Commission, Eastern Regional office, Kolkata, West Bengal for financing this research work.

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