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Research Article

HISTOLOGICAL CHANGES IN THE ACTIVITY OF THE THYROID FOLLICLES AND ITS IMPACT ON OVARIAN TISSUES IN *MYSTUS VITTATUS* (BLOCH, 1794) DURING GROWTH, MATURATION AND SPAWNING PHASES

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ABSTRACT
The thyroid gland of *Mystus vittatus* was unencapsulated, composed of follicles scattered mainly around the ventral aorta in the sub pharyngeal region. Each follicle provided with agranular eosinophilic colloid materials in the central lumen was encircled by a single layer of epithelial cells. The follicular cells varied according to the secretary activity in harmony with the reproductive cycle. The low active condition of the thyroid follicles during the growth phase i.e., December to February was well in coincidence with the increase of early and late perinucleolar oocytes. The most active condition of the thyroid follicles were noted during spawning phase i.e., June to August as revealed by the maximum follicular cell height and also correlated with the highest frequency percentage of oocyte V and oocyte VI stages. Thereafter, the thyroid activity gradually proceeded at a low level up to December and revealed an abrupt increase in April i.e., active maturation phase. It was concluded that the peaks of thyroidal and ovarian activities correlate well and run parallel.

Keywords: *Thyroid, Ovary, Mystus vittatus*

INTRODUCTION
The gross morphological structures of the teleostean thyroid are extremely variable from scattered follicles to encapsulated structures occurring in the sub-pharyngeal region in few cases (Chavin, 1972; Gorbman *et al.,* 1983). In teleosts, seasonal changes in the activity of the thyroid gland have been described in several species and often been correlated with the gonadal maturation (Singh, 1968; Leatherland 1982). It is now a well established fact that the seasonal changes in thyroid gland of vertebrates including the fishes are influenced by several environmental factors (Dodd and Matty, 1964; Osborn and Simpson, 1978). On the contrary, Belsare (1971) remarked that the cyclical changes of thyroid activity in *Channa punctatus* have got a close relationship with the breeding activity of the fish instead of the temperature of the habitat. Majority of the teleost fishes are seasonal breeders and histological studies provide an opportunity to understand the cellular kinetics of the gonads, recruitments, development and resorption of gonadal cells and finally in staging the maturity stages of the gonads (Lee *et al.,* 2008). It has however, been reported by many investigators that since large amount of gonadal steroid hormones are secreted during gonadal maturation in teleosts, some of the apparent thyroid activity during these times might be related to be thyrotrophic effects of the steroid hormones themselves (Higgs *et al.,*1976; Singh and Raizada, 1979).
The aim of the present work is to examine more precisely the correlative changes between different functional states of thyroid follicles with the changes in the ovary during different reproductive phases in *Mystus vittatus* (Bloch). This small indigenous fish species has a nutritional value in terms of proteins, micronutrients, vitamins and minerals (Ross *et al.,* 2003).

MATERIALS AND METHODS
Specimen Collection and Measurements
Adult female *M. vittatus* (Bloch) of length 10 to 12 cm and weight 30 to 50 g were procured fortnightly throughout the year from a particular stocking pond located in Burdwan in order to avoid ecological

variations in different ponds that can affect ovarian and thyroidal development. About 176 female fishes were sacrificed during our experimental period. Every month data on total body weight and ovarian weight of 15 fishes were taken to calculate the mean Gonadosomatic index (GSI) using the following formula:

$$GSI = \frac{\text{Total ovary weight}}{\text{Body weight-Weight of the ovaries}} \times 100$$

Histological Methods

To study the seasonal changes of thyroid and ovary, the fishes were sacrifised and for obtaining the thyroid tissue, lower jaw of the fishes were taken out and 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. Decalcification was made in a mixture of 5% formic acid and formaldehyde (1:1 volume) for seven days. The ovaries were cut into pieces and were fixed in aqueous Bouin's fixative for 18 hours. The thyroid and ovaries were then placed in 70% ethanol and subsequently dehydrated properly through graded ethanol, followed by acetone and cleared in benzene. Tissues were embedded in paraffin (melting point-56° C-58° C) and serial sections of the tissues were cut at 4µm thickness.

De-paraffinized sections were brought to water through down-graded alcohols and the sections of thyroid were stained with Delafield's haematoxylin-eosin and that of ovary with iron-alum haematoxylin, Delafield's haematoxylin-eosin and Mallory's triple stain. Sections were dehydrated through ethanol series, cleared in xylene, mounted permanently with DPX and then examined under a binocular microscope. From the histological preparation of the ovaries, the diameters of the various oogenetic cells were measured while from the thyroidal tissues the diameter of the thyroidal follicles and the epithelial cell height 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 ± Standard Error of Mean (SEM). The diameter of the various oocytic stages of the fish were measured in a total of 20 cells per fish and the measurements were made at two different axes perpendicular to one another.

RESULTS AND DISCUSSION

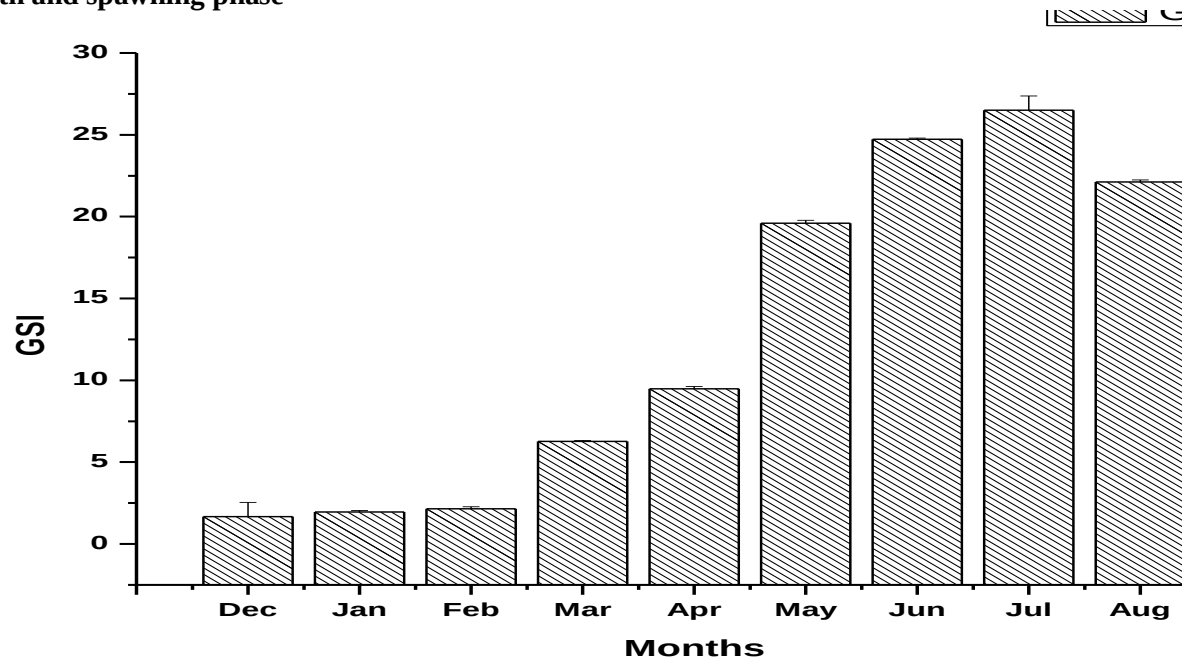
Gonadosomatic Index (GSI)

In our present study it has been observed that the values of GSI in *Mystus vittatus* vary greatly during different reproductive phases. The highest GSI value (26.51 ± 0.87) has been noticed during July when the ovaries remained packed with the fully mature follicles. In December, January and February the ovary enters the growth phase and are found with mean GSI values of 1.67 ± 0.87, 1.96 ± 0.08 and 2.15 ± 0.13 respectively. With the onset of maturation phase i.e., during the months of March and April the GSI values are recorded to be 6.26 ± 0.07 and 9.48 ± 0.14 respectively. In May the GSI value increases sharply showing the value of 19.60 ± 0.17. In June when the spawning season begins the GSI value rises to 24.72 ± 0.08 finally reaching its peak in July. In August the GSI value is recorded to be 22.12 ± 0.12 (Table 1).

In the present study it has been observed that GSI increased marginally but remained almost stationary during the growth phase. This might be due to gonadal proliferation of early and late perinucleolus oocytes in the ovary. Thakur (1978) reported that after post-spawning period GSI in the female fish remained stationary for a prolonged period indicating the dormant phase of ovary. However, from the end of maturation phase GSI increased rapidly and continued up to spawning phase due to maximum proliferation of vitellogenic oocytes and gradual accumulation of yolk granules in the oocytes. This result suggested that adequate food availability helped the fish in recruitment of vitellogenic oocytes and in maintaining the maturation process in the ovary (Garg and Jain, 1984; Mukherjee *et al.*, 1989). However,

GSI value declined from the end of spawning phase i.e., from August onwards due to gradual discharge or reabsorption of yolky oocytes.

Table 1: The variation of Gonadosomatic Index (GSI) for female *Mystus vittatus* during maturation, growth and spawning phase



Histology

Thyroid: Thyroid follicles of *M. vittatus* is not encapsulated but appears to be highly diffused, it is in the form of groups of follicles (Figure 1) which appears to be scattered around the regions of middle and posterior part of the ventral aorta in between dorsal branchial cartilages and ventral stenohyoid muscles (Figure 2) or connective tissue. The thyroid follicles are mostly round or oval in shape and frequently aggregated around the blood vessels (Figure 6). Each follicle posses a central lumen encircled by a single layer of epithelial cells. The lumina of thyroid follicles are either fully or partially filled with agranular eosinophilic colloid materials (Figures 1 and 2) and during active reproductive phases they are provided with resorption vacuoles (Figures 9 and10).

Oogenesis: The ovary in most teleost fishes is a hollow sac-like organ into which extend numerous ovigerous folds called ovigerous lamellae lined with germinal epithelium. The development of new crop of oogonia actually takes place in these lamellae. In *M.vittatus,* the sequence of oocyte maturation has been conveniently divided into six distinct developmental stages viz., oogonia (stage-I), early perinucleolus stage or chromatin nucleolus stage (stage-II), late perinucleolus stage (stage-III), yolk vesicle stage (stage-IV), yolk granule stage(stage-V) and mature follicle (stage-VI).

Oogonia (stage-I) (10 ± 0.05 µm): Oogonia are present either singly or sometimes appear in small nests within the ovigerous lamellae. The oogonium consists of an eccentrically placed large nucleus and basophilic cytoplasm (Figure 3). An oogonium has to pass through a number of maturation stages in order to become a mature ovum.

Early perinucleolus (stage-II) (22 ± 0.64 µm): This stage consists of a large oval centrally placed nucleus which contains about 8-10 basophilic nuclei together with fragmented chromatin. The cytoplasm is basophilic in nature (Figure 3).

Late perinucleolus oocyte (stage-III) (92 ± 1.18 µm): This stage may also be called as the protoplasmic growth stage when the cytoplasm as well as the nuclear mass increases in size. It consists of a large oval

centrally placed nucleus with an average diameter of 20 µm ± 0.61 and contains about 12-20 basophilic nucleoli together with condensed chromatin materials. The cytoplasm is basophilic in nature. This stage is characterized by the appearance of cortical alveoli along the peripheral region of the ooplasm. A thin follicular layer enclosing the zona radiata is also noticed in this stage (Figure 4).

Yolk vesicle oocyte (stage-IV) (118 ± 1.96 µm): The cortical alveoli of stage-III oocyte finally cover the entire ooplasm of stage IV oocyte. Most of the vesicles are empty. The nucleus is large spherical and centrally placed having condensed chromatin materials. The nucleoli of variable sizes and number (15 to 25) are arranged around the periphery of the nucleus. The oocyte is enveloped with a thick zona radiata, the middle multinucleated zona granulosa layer and outermost theca made up of connective tissue (Figure 7).

Yolk granules oocyte (stage-V) (424 ± 1.08 µm): In this vitellogenic oocyte, formation of yolk globules takes place and as a result the cell volume and diameter increases very rapidly. The nucleus is large and shows irregular outline. The staining nature of the cytoplasm is totally changed from basophilic to acidophilic nature due to incorporation of yolk materials. The oocyte is enveloped with a thick zona radiata and the middle multinucleated zona granulosa with distinct nucleus (Figure 13).

Mature follicle or mature ova (stage-VII) (558 ± 1.12 µm): In these vitellogenic oocytes, the yolk granules coalesced and remained tightly packed with each other so as to form yolk mass. The germinal vesicle is eccentric in position and irregular in outline (Figures 8, 12 and 13). The zona radiata and syncytial zona granulosa layer greatly increase but theca layer remain unaltered (Figure 13).

Atretic oocytes: The developing oocytes that fail to attain maturity and undergo resorption are called atretic oocytes. These shows signs of shrinkage are characterized by irregular shaped, disintegrated nuclei and liquefied yolk granules. The granulosa cells proliferate in enormous number and invade inside the follicle where they engulf the yolk materials by phagocytosis. In the present study, the atretic oocytes have been found during the maturation and spawning phases (Figure 12).

Discharged follicle: The post-ovulatory corpus luteum develops from the follicular cells immediately after the discharge of mature ovum, when the theca and follicular cells are left behind. The granulosa layer shows definite changes in structure. Its cells become irregular in shape, then elongate radially and thereafter increase in size.

Sequential Changes in the Thyroid Follicles and Ovary During Different Reproductive Phases

The activities of the thyroid follicles and the frequency of various oogenetic cells, as revealed by the histological studies are found to undergo changes in harmony with growth, maturation and spawning phases in the fish under study. The activities of the thyroid follicles have been studied by considering the height of the follicular epithelial cells along with the follicular and colloidal diameters.

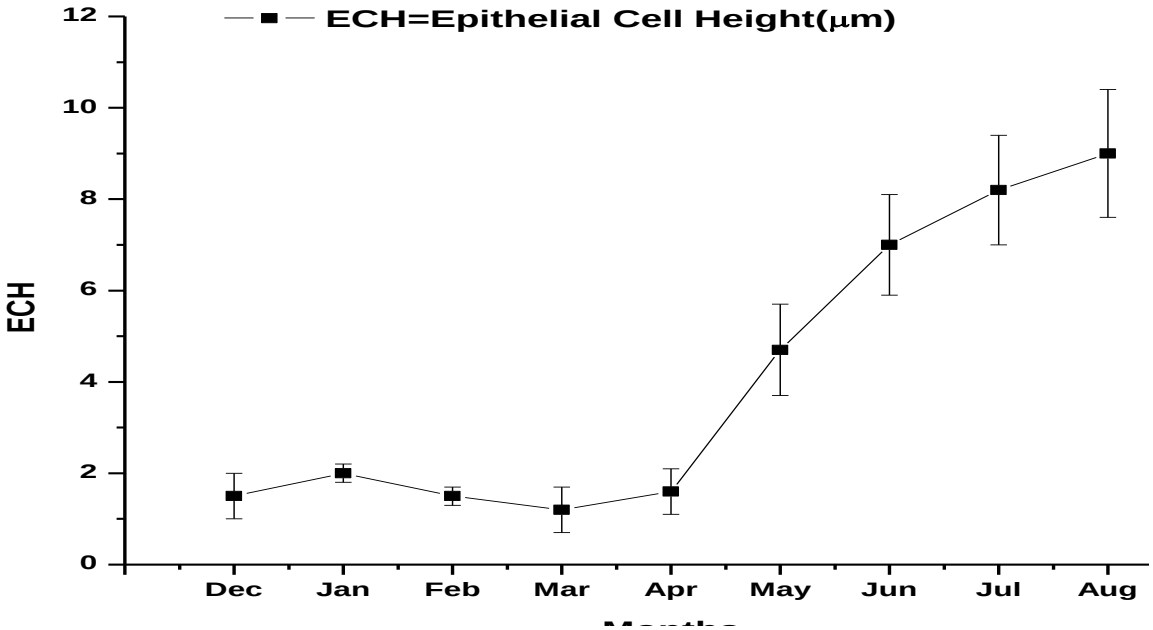
Growth phase (December to February): During this phase the thyroid follicles are in non-secretory stage and are surrounded by flat squamous epithelial cell layer. The average diameter of thyroid follicles in *M. vittatus* ranges from 16 ± 1.7 µm in December to 22 ± 2 µm in February (Figures 1 and 2). The height of the epithelial cell layer varies from 1.5 ± 0.2 to 2.0 ±0.2 µm (Table 2). Follicles are round or oval in shape and follicular diameter measures about 16 ±1.2 µm in December to 42 ±2.0 µm in February (Table 3). They are filled up with dense eosinophilic colloid and in most cases the colloid is in contact with the epithelial cell layer (Figures 1 and 2).

During growth phase the GSI is recorded from 1.67 ± 0.87 to 2.15 ± 0.13. This stage is dominated by chromatin nucleolus; early and late perinucleolus stages of oocytes. The oogonia are few in number and lie within the lamellar epithelium. The percentages of late perinucleolus oocytes are gradually increasing during the end of this phase (Figures 3 and 4).

Maturation phase (March-May): During this phase, the thyroid follicles are in secretory and active secretory stage. An increasing trend in the epithelial cell height has been recorded to about 1.2µm±0.5 in March and 4.7 ±1 µm in May (Table.2). The follicles are oval or elongated in shape and follicular diameter measures about 26 ±1.4 µm in March to 40 ±1.4 µm in May (Table 3). With the onset of March and during the months of April and early May, the follicular lumen are completely filled up with deeply stained colloid but having vacuolated structures at the outer margin of colloids.(Figures 5and 6). This

stage can be said to be the stage of maximum colloid storage. The degree of vascularization is found to be at its peak and the blood cells are closely associated with the follicular layer (Figure 5). At the end of this phase the height of the epithelial cell layer increase considerably measuring about 4.7 ±1 µm and having prominent nuclei (Figures 5 and 6).

Table 2: The Epithelial cell heights (ECH) for female *Mystus vittatus* during Growth, Maturation and Spawning seasons (the vertical lines at the measurement points are indicating the SEM)

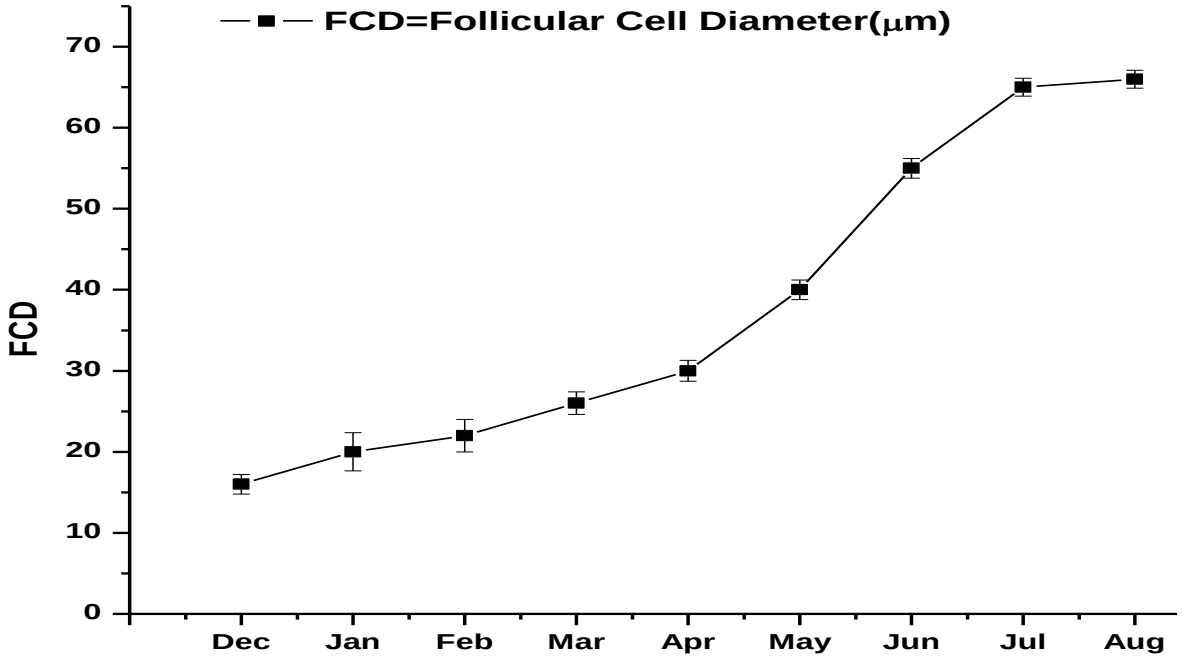


The highest oogenetic activity is found to occur during this phase. Different stages of vitellogenic oocytes are characteristically present. However, majority of the developing oocytes are of stage IV and V respectively (Figures 7 and 8). From March onwards the yolk granules continued to coalesce and migrate centripetally. Subsequently with the advancement of maturity, the immature oocytes are decreased in number. Towards the end of May, the number of developing follicles attains maximum abundance while the number of immature oocytes declines to a minimum. A few atretic follicles are also found in this phase. The zona granulosa and zona radiata have an average thickness of 3.5 ± 0.12 µm and 2.55 ± 0.08 µm respectively (Figure 7).

Spawning phase (June to August): This phase is dominated by the follicles of active secretary and atrophid stage. During the months of June, July and August i.e., spawning phase most of the follicles in female are surrounded by either cuboidal or columnar epithelial cell layers (Figures 9 and11). The height of the epithelial cell layer is maximum measuring 7 ± 1.1 µm during June to 9 ± 1.2 µm during August. The follicles are at their active functional states as reflected by a large number of vacuoles around the entire margin of colloid (Figures 9 and10). The follicular diameter measures about 55 ±1.2 µm in June to 68 ± 1.1 µm in August (Table 3). Some of the follicles have been found empty with high epithelial cell layer (Figure 11).

The ovaries of this stage are full of ripe ova, most of them belonging to stages V and VI. In July, the oocytes of stage VI become large and irregular in shape. The yolk globules begin to break up and the mature follicles are provided with eccentric germinal vesicle (Figures 12 and13). The zona radiata and zona granulosa appear prominent and thicker than before with an average thickness of 8 ± 0.4 µm and 12 ± 0.12 µm respectively (Figure 12 and 13). Some of the mature follicles collapse inward and zona granulosa and zona radiata break in several places. Atretic oocytes (Figure 12) and few discharged follicles have also been detected.

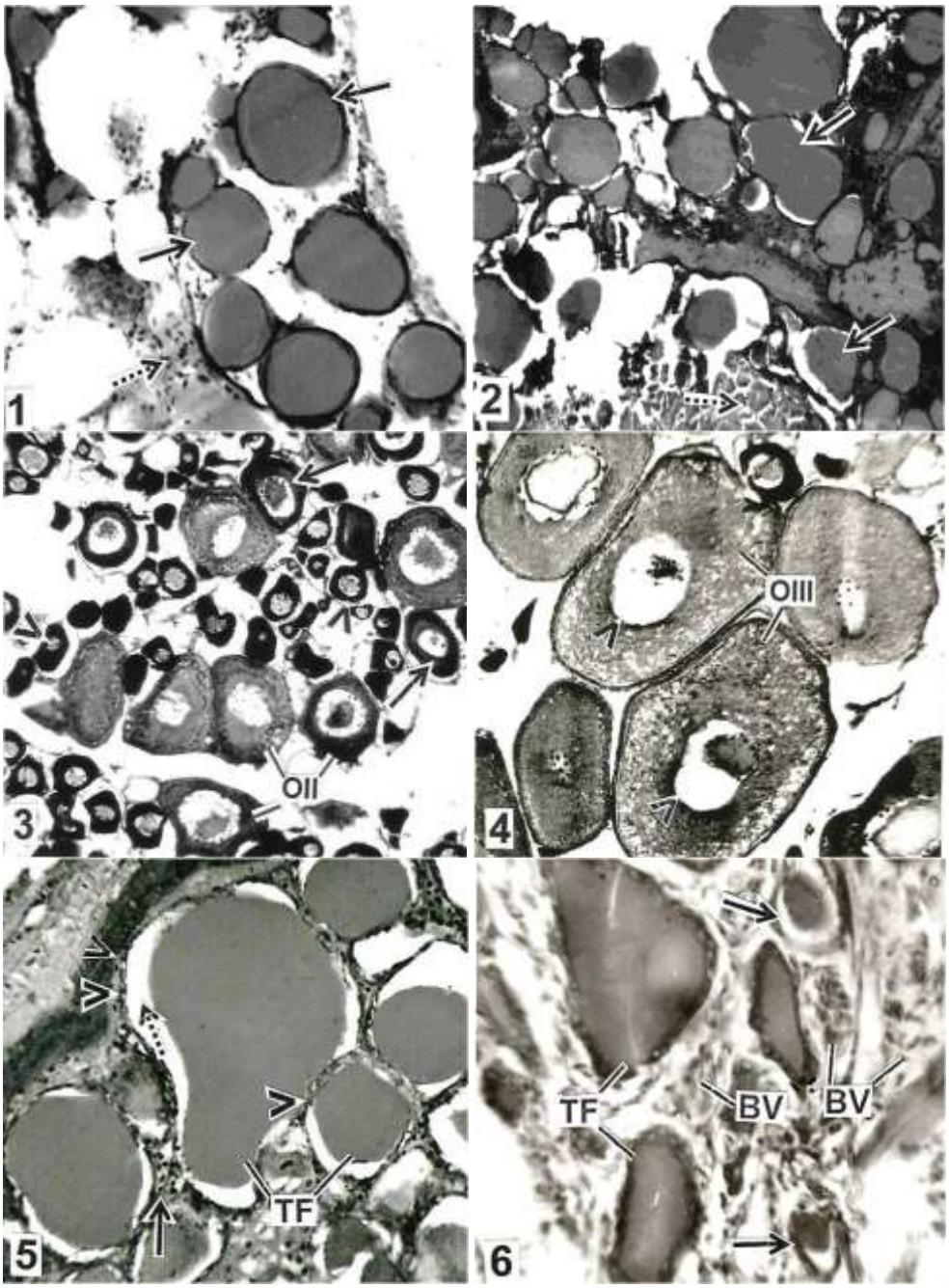
Table3: **The Follicular cell diameters (FCD) for female *Mystus vittatus* during Growth, Maturation and Spawning seasons (the vertical lines at the measurement points are indicating the SEM).**

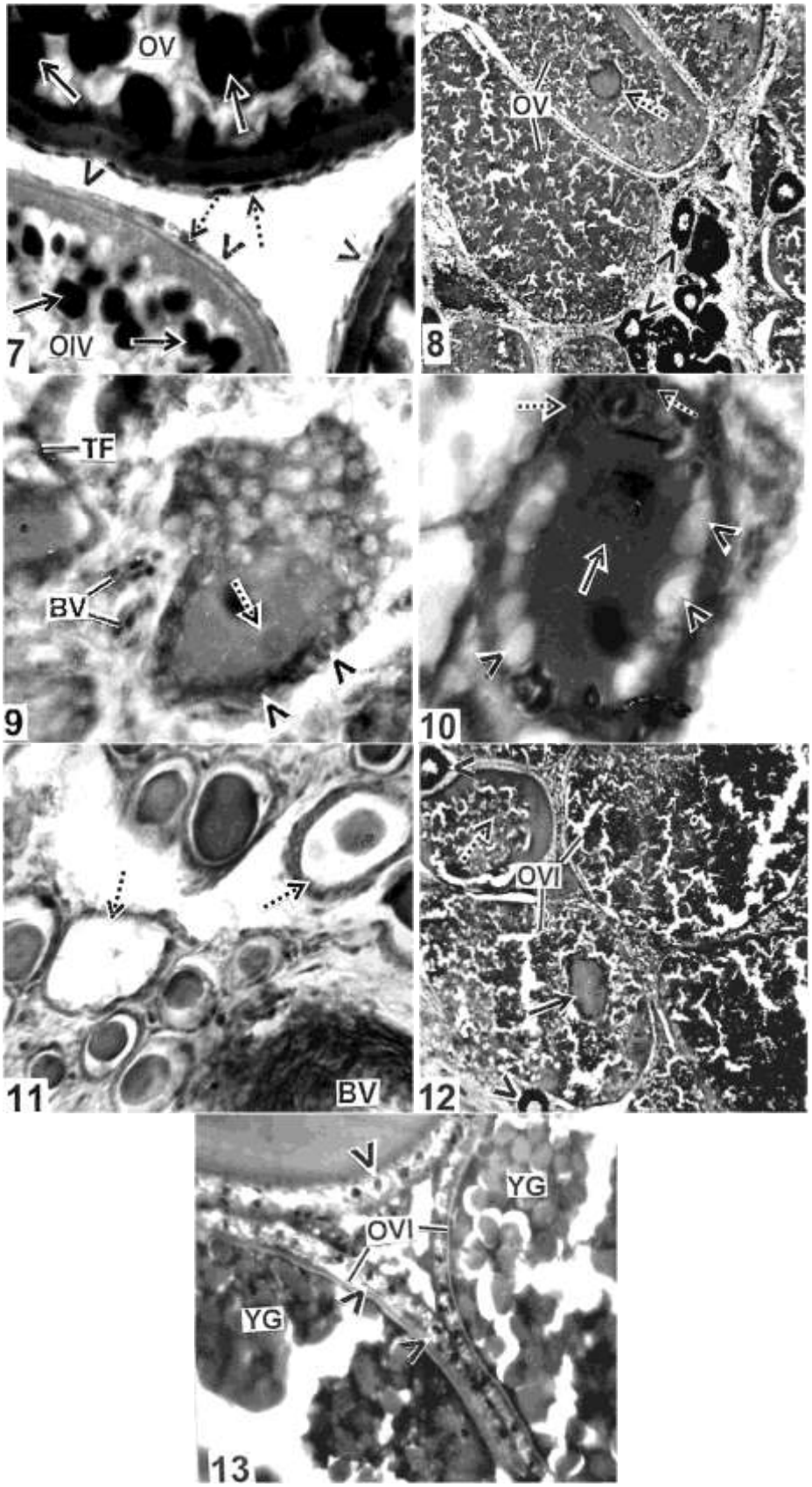


In the present investigation the diffused and groups of thyroid follicles which are found to be scattered in *Mystus vittatus* corroborates with the findings of Srivastava and Satyanesan (1971) and Joy and Satyaneshan (1981) in *Clarias batrachus*. The present histological studies reveal that the degree of functional state of thyroid follicles in *Mystus vittatus* during different reproductive phases may be grouped in four distinct stages: non-secretary, secretory, active secretary and spent or atrophied on the basis of amount of colloid material present, epithelial cell height and the presence or absence of colloidal resorption vacuoles. Mukherjee (1988) also divided thyroid follicles of *Clarias batrachus* into five stages (quiescent, non-secretary, secretary, active secretary and atrophied). In the present study, it is apparent that the degree of thyroid activities in *Mystus vittatus* seems to have a close relation with gonadal maturation. Maximum activity thyroid follicles have been encountered during spawning phase and a low activity of the same during growth phase. This finding is in conformity with the findings of Belsare (1971) while studying the cyclical changes of thyroid gland in *Channa punctatus*. He also opined that the thyroid activity has got a close relationship with the breeding activity of the fish rather than the temperature of the habitat. Salamat *et al.*, (2012) reported that the surrounding epithelial cells of the thyroid follicles in *Acanthopagrus latus* are 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. Ortiz *et al.*, (2006) reiterated that thyroid represented colloid-filled follicles surrounded by a cuboidal epithelium during summer, suggesting a high activity state of this organ.

In the present investigation, it has been found that during the growth phase the low active condition of the thyroid of female *Mystus vittatus*, has been noticed as revealed by the minimum follicular epithelia cell height. This phase is the potential storage phase for the thyroid. These features are well coincidence with the increase in the percentages of early and late perinucleolus oocytes in the ovary. On the other hand, during the spawning phase the most active condition of the thyroid in female as revealed by columnar epithelial cells and resorption vacuoles in the colloid in harmony with the high frequency of fully matured follicles.

Thus it can be conjectured that the degree of activity of thyroid follicles in *Mystus vittatus* coincide with the ovarian status. A thyroid-gonadal relationship has been established in medaka *Oryzias latipes* by Nishikawan (1975). He 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. However, the participation of the thyroid follicles in harmony with the gonadal status in fish under study may or may not be a direct one as the teleostean thyroid is reported to have a capacity to regulate the metabolic rates. Osborn and Simpson (1978) suggested that since the elevation of thyroid hormone level during the time of gonadal development in plaice and rainbow trout occurs in both immature and maturing specimens, thyroxine may be regarded as permitting the metabolic changes necessary to save the developing gonad rather than as being directly involved in gametogenesis.





Figures 1 to 13: Microphotographs of sections of thyroid follicles and ovary of *Mystus vittatus* showing architecture of cells during growth, maturation and spawning phases (Delafield's haematoxylin-eosin; HandE., Iron alum haematoxylin: IA., Mallory's triple: MT stain).
Figure 1: Non-secretory thyroid follicles (TF) (solid arrows) of growth phase filled with colloid and bordered with squamous epithelial cells. Note the presence of blood cells adjacent to TF (broken arrow) (HandE) × 400.
Figure 2: Showing partially secretory stage of TF (solid arrows) adjacent to ventral stenohyoid muscles (broken arrow) during growth phase. Note narrow colloid free space along the margin of TF (HandE) × 400.
Figure 3: Immature oocytes of different stages during growth phase. Oogonia (arrow heads) with centrally placed nucleus and a rim of basophilic cytoplasm, Oocyte I (arrow) and Oocyte II (OII) (IA) X 400
Figure 4: Showing late perinucleolus stage (OIII) with prominent nucleus (arrow heads) with nucleoli, condensed chromatin material and cortical alveoli (IA) × 400.
Figure 5: Secretary stage of thyroid follicles (TF) during maturation phase showing cuboidal epithelial cell layer with prominent nucleus (arrow heads) and empty space within the follicle (broken arrow). Solid arrow indicates blood cells in between the follicles (HandE) × 400.
Figure 6: Active TF bordered with prominent thick epithelial cells showing liquefaction of colloid during late maturation phase. Note active state of blood vessels (BV) in between TF. Solid arrows indicate active secretary stage of TF (MT) × 400.
Figure 7: Oocyte IV (OIV) and Oocyte V (OV) stage during maturation phase. Note prominent granulosa cells (broken arrows) and theca (arrow heads). Solid arrow indicate yolk globules within mature oocyte (HandE) × 600.
Figure 8: Showing Oocyte OV (OV) stage provided with almost coalesced yolk granules during late maturation phase. Note the migration og germinal vesicle towards the periphery of mature oocyte (broken arrow). Arrow heads indicate oogonia (MT) × 400.
Figure 9: Thyroid follicle (TF) showing congregation of resorption vacuoles within lumen leaving small amount of colloid (broken arrow) during spawning phase. Note thick and prominent epithelial cells (broken arrow). BV indicates blood vessels adjascent to active TF (MT) × 1000.
Figure 10: Thyroid follicle (TF) during spawning phase showing resorption vacuoles at the periphery (arrow heads) and central colloid (solid arrow). Note epithelial cells bordering the TF (HandE) × 1000.
Figure 11: Showing spent stages of thyroid follicles, TF during spawning phase. Note increased epithelial cell height (broken arrows) and least amount of colloid in the centre of TF. BV indicates blood vessel (HandE) × 400.
Figure 12: Mature oocyte (OVI) with eccentric germinal vesicle (solid arrow) during spawning phase. Note some immature oocytes (arrow head) and atretic oocyte (broken arrow) (HandE) × 400.
Figure 13: Mature oocytes (OVI) with compact yolk granules (YG). Note outer theca and prominent active granulosa cells with prominent nucleus (arrow heads) in the zona granulosa layer (HandE) × 600.

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