GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

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HISTOLOGICAL ORGANIZATION AND CHANGES IN THE ARCHITECTURE OF THYROID FOLLICLES AND OVARIAN TISSUES DURING GROWTH, MATURATION AND SPAWNING PHASES IN *OMPOK BIMACULATUS* (Bloch)

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ABSTRACT
The present study dealt with the histological status of thyroid follicles and correlated them with the changes of ovarian activities in female *Ompok bimaculatus.* Thyroid follicles were present around the middle posterior extension of the ventral aorta between the dorsal branchial cartilages and the ventral sternohyoid muscles in close association with blood capillaries. Each follicle possessed a central lumen either fully or partially filled up with agranular eosinophilic colloid materials and the lumen was enriched by a single layer of epithelial cells. The secretory activity of the thyroid follicles fluctuated in harmony with the oogenetic cells during growth, maturation and spawning phases in *O. bimaculatus*. The low follicular activity during growth phase *i.e*. December to February was well in coincidence with the increase of early and late perinucleolar oocytes. During maturation phase *i.e*. March to May the colloids of thyroid follicles were faintly stained having resorption vacuoles as well as peripheral lumen at the outer margin of the colloid. These features were well correlated with the occurrence of the cortical alveolus and yolk vacuole/granule stages in the ovary. The maximum secretory activity as well as atrophied condition of thyroid follicles were noted during spawning phase i.e. June to August and also correlated with the dynamic cytological activities during vitellogenesis. It was concluded that the peaks of thyroid and ovarian activities correlate well and run parallel.

KEYWORDS: Histology, Thyroid, Growth, Maturation, Spawning, *Ompok bimaculatus.*

INTRODUCTION

In teleosts, thyroid hormones have been found to be involved in a variety of physiological processes, such as metamorphosis, growth, metabolism, reproduction and osmoregulation (Leatherland, 1994). The thyroid follicles of fish are loosely scattered in the gill region along the ventral aorta (Wabuke-Bunoti and Firling, 1983; Raine *et al.*, 2011). The hormones are synthesized under the control of a thyroid stimulating hormones (TSH) from the pituitary gland (Eales and Brown, 1993). In the most teleostean fish, the thyroid is unencapsulated and the thyroid follicles are in the form of hollow ball consisting of single layer of epithelial cells enclosing a fluid filled space. In fish, the central control of thyroid hormone is limited to the production and secretion of T_4, which is transformed into biologically active T_3 in peripheral tissues, mainly the liver which is essential for reproductive activities (Leatherland *et al.*, 1990; Eales and Brown, 1993). The seasonal changes in the activity of thyroid follicles in teleosts have been studied and often been correlated with the gonadal maturation (Cyr and Eales, 1996; Chakrabarti, 2014) . Kundu and Chakrabarti (2016) noted that during spawning phase maximum follicular diameter correlated with the dynamic cytological activities during vitellogenesis and highest frequency percentage of mature oocytes in the ovary.

The present study is designed to understand the histological organization of thyroid and histological changes, that is, distribution of thyroid follicles, hyperplasia, homogeneity of the follicles in correlation with the changes in the ovary in *Ompok bimaculatus* (Bloch) during growth, maturation and spawning phases.

MATERIALS & METHODS

Living mature female fishes of *Ompok bimaculatus* (Length varies from 13 cm to 27 cm during various phases) were procured fortnightly from a particular stocking pond of 'Chakdah Fish Farm' located in Chakdah ($23^0 47^{'}$ N - $88^0 51^{'}$E) , Nadia, West Bengal, India in order to avoid ecological variations that can affect ovarian and thyroid development. Data on total body weight and ovarian weight of 10 fishes were taken to calculate the mean gonadosomatic index (GSI) using the formula:

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

Histological methods

After decapitation of fish for obtaining the thyroid tissue lower jaw of fishes were taken out and sternohyoid muscles were removed. The tissues around the ventral aorta and afferent branchial arteries were dissected out and fixed in aqueous Bouin's fluid for 18 hours. After fixation decalcification was made in a mixture of 5 % formic acid and formaldehyde (1:1 volume) for 8 days. Ovaries were cut into pieces and were fixed in Bouin's fluid for 18 hours for histological studies. After fixation the ovaries and decalcified thyroid tissues were placed in 70% ethanol and subsequently dehydrated properly through ascending series of ethanol, followed by acetone and cleared in benzene. Next the tissues were embedded in paraffin (Melting point 56^{o}c–58^{0}c) and serial sections of the tissues were cut at 4 µm thickness.

Deparaffinized sections were brought to distilled water through descending series of alcohols and sections of thyroid and ovary were stained with Delafield's haematoxylin-eosin (HE) and Mallory's triple stain (MT). Some sections of ovary were stained with Iron-alum haematoxylin (IAH). Sections were dehydrated through ascending ethanol series, cleared in xylene, mounted permanently with DPX and then examined under binocular microscope. From the histological preparation of the ovary, the diameters of the various oogenetic cells were measured while from the thyroidal tissues the diameter of the thyroid follicles and the epithelial cell height were calculated with the help of reticulo-micrometer and ocular micrometer.

RESULTS

Thyroid follicles of *O. bimaculatus* is not encapsulated but appears to be highly diffused, it is in the form of groups of follicles (Figs. 1,2) which appears to be scattered around the regions of middle and posterior part of the ventral aorta in between dorsal branchial cartilages and the ventral sternohyoid muscles or connective tissue (Fig. 1). Each follicle possesses a central lumen encircled by unicellular flattened epithelial layers. The lumen of thyroid follicles is either fully or partially filled with granular eosinophilic colloid materials (Figs. 1, 2) and in many stages of reproductive phases they are provided with resorption vacuoles (Fig. 3). On the basis of the amount of colloid, presence or absence of colloidal resorption vacuoles and the height of the epithelial cell layer, altogether four stages representing the stages of activities of thyroid follicles have been recognized in *O. bimaculatus* . The follicles are at the first stage, the non-secretory stage, exhibit the follicular lumen completely filled up with dense colloid having close contact with the epithelial cell layer. The height of the epithelial cell layer is minimum (Figs. 1, 2). The follicles at the second stage, the secretory stage, show the appearance of resorption vacuoles and peripheral lumen around the central colloid has been encountered. The height of the follicular epithelial cell layer is relatively higher in comparison to that of the first stage (Figs. 3, 8, 9). In the follicles of the third stage, the active secretory stage, the number of colloid resorption vacuoles increase considerably and occurs mainly around the entire periphery of the colloid. In this stage, the colloid is liquefied and it appears to be comparatively less in quantity and the height of the epithelial cell layer is at its maximum degree (Figs. 8, 9). Follicles of the fourth stage of collapse or atrophied with scanty colloid. Epithelial layer is irregular without any definite arrangement (Figs. 12, 13).

Oogenesis

The developing oocytes present in the ovary are classified into various stages according to their size, appearance of nucleus, nucleolus and characteristics of cytoplasm. In *O. bimaculatus* the sequence of oocytes maturation has been divided into six developmental stages *viz.* oogonia (stage I), early and late perinucleolus stage (stage II and stage III), yolk vesicle stage (stage IV), yolk granule stage (stage V) and mature follicle (stage VI).

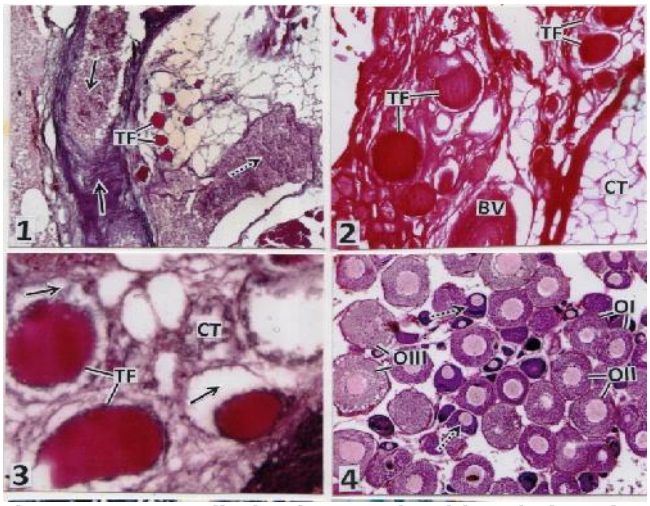

Fig 1: Thyroid follicles (TF) having compact colloid in between dorsal branchial cartilages (solid arrows) and ventral sternohyoid muscle (broken arrow) during growth. (HE) × 100. **Fig 2:** Enlarged view of TF in between connective tissue (CT) during growth phase. Note the thin epithelial layer and luminal colloid in TF. (HE) × 400. **Fig 3:** Secretory stage of TF at the end of growth phase showing the appearance of resorption vacuoles and peripheral lumen (arrows) around the central colloid. (HE) × 1000. **Fig. 4:** Showing oogonia (broken arrow) , oocyte I (OI), oocyte II (OII), oocyte III (OIII) during growth phase. (HE) ×150.

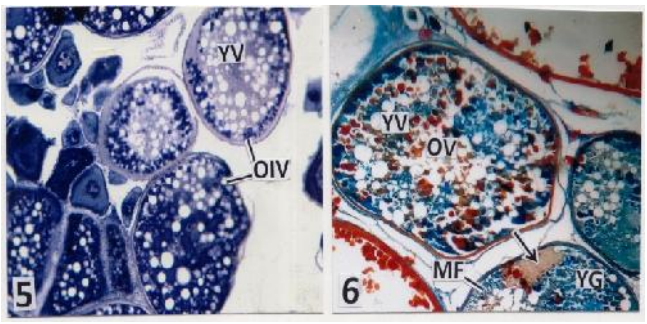

Fig. 5: Showing oocyte IV (OIV) with prominent yolk vesicle (YV) at the middle of growth phase. (IAH) × 400. **Fig 6:** Showing oocyte V (OV) with dense YV and some yolk granules (YG) within the ooplasm and mature follicles (MF) with full of YG at the end of growth phase. Note eccentric position of germinal vesicle (arrow) in MF. (MT)× 600.

Oogonia (stage I) (6.2 µm × 8.0 µm to 7.90 µm × 9.48 µm)

Oogonia are present either singly or in small nests within the ovigerous lamellae. The oogonium consists of an eccentrically placed large nucleus and two to three nucleoli (Fig. 4).

Early perinucleolus oocyte (stage II) (15.80 µm × 17.96 µm to 26.28 µm × 29.54 µm)

The stage consists of a large oval centrally placed nucleus and contained 8 to 10 basophilic nuclei together with fragmented chromatin materials. Cytoplasmic mass is compact and homogenously distributed (Fig. 4).

Late perinucleolus oocyte (stage III) (37.92 µm × 43.10 µm to 53.78 µm × 59.88 µm)

This stage represents the protoplasmic growth period when the cytoplasm as well as the nuclear mass increases in size. Late perinucleolus oocytes are characterized by the appearance of cortical alveoli along the periphery of the cytoplasm (Figs. 4, 10). It consists of oval centrally placed nucleus with an average diameter 10 µm to 12 µm and contain about15-20 basophilic nucleoli together with condensed chromatin materials. This oocyte stage is surrounded by two single layers probably the granulosa and theca (Fig. 4).

Yolk vesicle oocyte (stage IV) (61.47 µm × 69.56 µm to 78.92 µm × 84.65 µm)

The cortical alveoli cover the entire ooplasm at this stage of ova. Most of the vesicles are empty and called yolk vesicles. This stage of oocyte is enveloped with zona radiata, zona granulosa and outermost theca (Fig. 5).

Yolk granule stage (stage V) (113.42 µm × 120.57 µm to 141.49 µm × 152.63 µm)

In this vitellogenic oocyte growth is accompanied by centripetal deposition of yolk globules till the entire cytoplasm becomes filled with yolk and as a result the cell volume and dimeter increase rapidly (Figs. 6, 10). The germinal vesicle moves from the center of the oocyte towards the periphery. The oocyte is enveloped with a thick zonaradiata , middle multinucleated zona granulosa and outer theca (Fig. 11).

Mature follicle (stage VI) (193.45 µm × 219.42 µm to 235.56 µm to 248.32 µm)

In this vitellogenic oocyte, the yolk granules coalesce and remain tightly packed with each other so as to form yolk mass (Figs. 6, 14, 15). The germinal vesicle is eccentric in position and irregular in outline (Fig. 14). The mature follicle ready for spawning exhibits migration of germinal vesicle towards the periphery of the follicle (Fig. 6). The zona radiata becomes thinner and the multinucleated zona granulosa layer is greatly reduced but the theca layer remains unaltered.

Atretic oocytes

Sometimes the developing oocytes fail to attain maturity and are called atretic oocytes. These are characterized by disintegrated nuclei and liquefies yolk granules (Figs. 10,14).

Sequential changes in thyroid follicles and oocyte cells during growth, maturation and spawning phases

In the present investigation the characteristics of thyroid follicles, height of the follicular epithelial cells, GSI, morpho-histological features of various oogenetic cells are found to undergo changes during growth, maturation and spawning phases.

Growth phase (December to February)

As judged by the histological criteria most of the thyroid follicles during this phase appeared to be functionally inactive state *i.e.* non-secretory stage and are surrounded by flat squamous epithelial cell layer (Fig. 1). The diameters of the thyroid follicles have been recorded to be 16.4µm×17.7µm in December. During January the follicular lumen is not completely filled up with the colloid but lumen appears along the periphery of the epithelial cell layer (Fig. 2). In the month of February i.e. end of growth phase the resorption vacuoles along with peripheral lumen around the centrally placed colloid has been encountered which is in the state of liquefaction. The diameter of thyroid follicle in February is recorded to be 22.8µm ×24.6µm to 21.46 µm×30.40µm and the epithelial cell height appears to be 1.6 µm ± 0.08 to 1.94 µm ± 0.26 (Fig. 3). During growth phase the GSI is recorded from 2.08 ± 0.35 to 3.75 ± 0.15. In December and January the GSI value increases gradually from 2.08 ± 0.35 to 2.20 ± 0.06. Gradual increment of GSI is noticed in February and recorded to 3.75 ± 0.15. This phase is characterized by the presence of chromatin nucleolus, early and late perinucleolus stages of oocytes. A few stage III oocytes present having cortical alveoli (Fig. 4). At the end of growth phase cortical alveoli stage increases and considerable number of stage IV oocytes i.e. yolk vesicle stages are formed (Fig. 5). At the onset of March few yolk granule stages i.e. stage V oocytes along with one or two mature follicles are also encountered (Fig. 6).

Maturation phase (March to May)

During March to April, the thyroid follicles are in secretory and active secretory stage. During March the follicles are densely aggregated around the ventral aorta (Fig. 7). The follicular diameter measuring about 31.46 µm ±1.4 × 41.26 µm ± 1.16 to 32.0 µm ± 1.14× 43.84 µm ±1.22. In April an increasement of the vascularization around the follicles is also evident. Some of the thyroid follicles are in close contact with the blood vessels. An increasing trend in the epithelial cell height has been recorded to about 2.63 µm ± 1.12 to 3.0 µm ± 1.5 in April (Fig. 8) .At the end of May the colloids in the thyroid follicles are seen to be faintly stained vacuolated structures at the outer margin of the colloid (Fig. 9). The epithelial cell height recorded to be 3.2 µm ± 1.6 to 3.8µm ± 1.14. The highest oogenetic activity is found to occur during this phase. Majority of the developing oocytes are of stage IV and stage V respectively. At the end of this phase the yolk granules of stage V continued to coalesce (Fig. 10). Promient zona granulosa and zona radiata are present (Fig. 11). Small number of atretic follicles is also found in this stage in between yolk granule oocytes (Fig. 10). During this phase the GSI gradually increase from 5.52 ± 0.35 in March, followed by 8.68 ±1.05 in April and 10.80 ± 2.02 in May respectively.

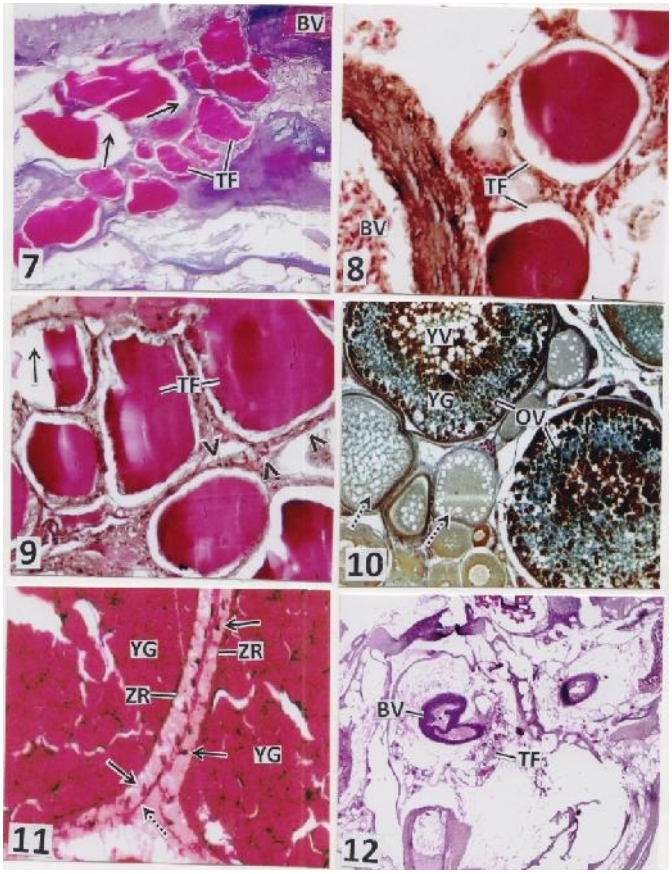

Fig 7: Thyroid follicles (TF) adjacent to blood vessel (BV) during maturation phase showing liquefaction of colloid and appearance of peripheral lumen(arrows). (HE) × 400. **Fig 8:** Mid maturation phase showing active secretory TF adjacent to blood vessel (BV). Note the increasing trend of epithelial cell height of TF. (HE) × 600. **Fig 9:** Active secretory stage of TF showing cuboidal epithelial cells (arrow heads) along the border of TF at the end of maturation phase. Arrow indicates peripheral lumen. (HE) × 600. **Fig 10:** Showing oocyte V (OV) stage with centrally placed yolk vesicles (YV) and dense yolk granules (YG) during maturation phase. Note the presence of oocyte III (broken arrow) and atretic follicle (AF) (solid arrow). (MT) ×600. **Fig 11:** Showing oocyte V (OV) during end of maturation phase with prominent granulosa cells (solid arrows), zona radiata (ZR) and theca layer (broken arrow). (HE) ×1500. **Fig 12:** Showing active secretory follicles and atrophied follicles encircling ventral aorta (BV) during spawning phase. (HE) × 50.

Spawning phase (June to August)

During spawning phase the active secretory follicles and atrophied or stage of collapse with scanty colloid are dominated. The aggregated follicles are closely associated with the blood vessels (Figs. 12, 13). In the month of June, the active thyroid follicles are encircled by cuboidal epithelial cell layer. The height of the epithelial cell layer measuring from 4.2 µm ± 0.06 to 4.8 µm ± 0.05. In July and August the follicles are in their maximum functional state as also reflected by the presence of scanty colloid in the center of the follicles or some follicles are empty (Fig. 13). The follicular diameter has been recorded to be 35.30 µm ± 1.24 × 42.64 µm ± 1.48. Ovary at this stage is full of mature follicles i.e. stage VI oocytes which are larger and irregular in shape, yolk globules condensed and provided with eccentric germinal vesicles (Figs. 14, 15). As the ripe ova are closely packed with each other, inter follicular spaces have rarely been seen (Figs. 14,15). Atretic oocytes

have also been detected (Fig. 14). In June, the GSI value recorded to be 14.58 ± 2.60 while in July GSI value declined to 13.78 ± 1.58 and in August GSI value shows further declining trend (12.50 ± 1.35).

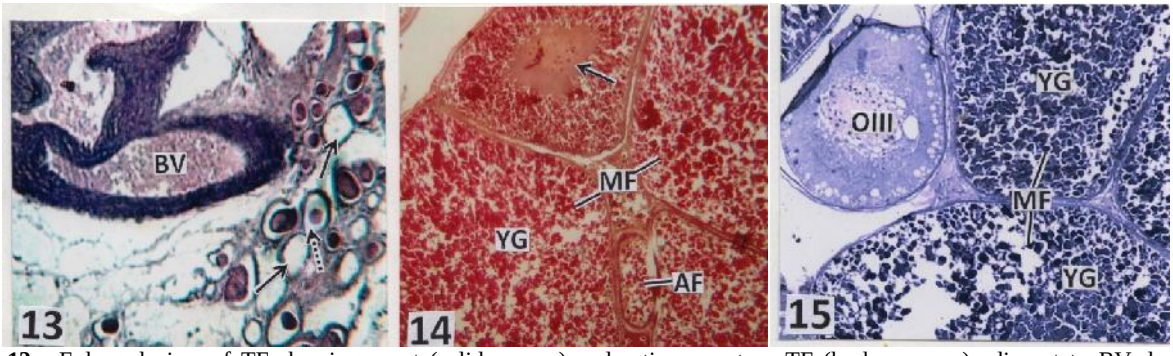

Fig 13: Enlarged view of TF showing spent (solid arrows) and active secretory TF (broken arrow) adjacent to BV during spawning phase. (MT) × 150. **Fig. 14:** Showing large number of mature follicles (MF) with full of yolk granules (YG) during spawning phase. Note the presence of eccentric nucleus (solid arrow) of MF and atretic follicle (AF) in between. (HE) × 600. **Fig. 15:** Showing MF having disintegrated YG during end of spawning phase. Note the appearance of oooocyte III (OIII). (MT) × 600.

DISCUSSION

In the present investigation on the morphohistological structure of thyroid follicles which are found to be of scattered nature in *O. bimaculatus* and loosely distributed in the connective tissue adjacent to the blood vessels corroborates with the findings of Srivastava and Sathyanesan (1971) in *Mystus vittatus*, Joy and Sathyanesan (1981) in *Clarius batrachus* and Schmidt and Braunbeck (2011) in *Danio rerio* . The thyroid follicles in *O. bimaculatus* are mostly round or oval in shape and each follicle possess a central lumen encircled by a single layer of epithelial cells. On the basis of the amount of colloid, presence or absence of colloidal resorption vacuoles and the height of the epithelial cell layer, altogether four stages representing the different stages of activities of thyroid follicles have been recognized in *O. bimaclatus viz*. non-secretory, secretory, active secretory and atrophied . Pandey *et al*. (1976) also considered the epithelial cell height and the follicular diameters to express the different functional state of thyroid follicles in *Heteropneustes fossilis*. Mukherjee (1988) divided thyroid follicles of *Clarias batrachus* in five stages (quiescent, non-secretory, secretory, active secretory and atrophied). Schmidt and Braunbeck (2011) emphasized that a very interesting end point was the observation of changes in the quantify of the colloid as the main site of thyroid hormone synthesis and storage. In the present investigation it is apparent that the degrees of thyroid activities in *O. bimaculatus* have a close relationship with ovarian maturation. It has been found that during the growth phase the low active condition of the thyroid follicles, as revealed by the minimum follicular epithelial cell height is well coincidence with the increase of a number of immature oocytes comprising of the chromatin nucleolus as well as early perinucleolus stages and the absence of mature oocytes. It has been found that during peak spawning phase i.e. June and July the thyroid follicles are in their active secretory state coincides with the increased GSI value and proliferation of vitellogenic oocytes. However, the participation of the thyroid follicles in harmony with the ovarian status in *O. bimaculatus* may or may not be a direct one as the teleostean thyroid is reported to have the capacity to regulate the metabolic rates. Hoar (1957) also suggested that the influence of the thyroid on the gonadal development may be an indirect one because the teleostean thyroid regulates the metabolic state which isnecessary for the maintenance of the gonadal development and hence the implication of thyroid gland in the reproduction of fish is difficult to interpret. Belsare (1974) while studying the cyclical changes of thyroid follicles in *Channa punctatus* advocated that the thyroid activity has got a close relationship with the breeding activity of the fish. A thyroid – gonadal relationship has been established in medaka, *Oryzias latipes* (Nishikawa, 1975).

Epithelial cell height of thyroid follicle represents a classical parameter to detect thyroid activities (Miranda *et al*., 1996; Goleman *et al*., 2002). In the present investigation during maturation phase the increment of epithelial cell height along with resorption vacuoles as well as liquefied colloid in thyroid follicles adjacent to blood vessels are also well coincide with the increasement of yolk vesicle and yolk granule stages. During the spawning phase the most active condition of thyroid in female *O. bimaculatus* as revealed by atrophied follicular cells having maximum epithelial cell height are correlated with the high frequency percentage of mature oocytes. Salmat *et al.* (2012) have stated that the epithelial cells bordering thyroid follicles in *Acanthopagrus latus* are flattened, cuboidal or columnar depending upon their activity. Osborn and Simpson (1978) have suggested that since the elevation in thyroid hormone level during the time of gonadal development in Plaice occurs in both immature and maturating specimen, thyroxin may be regarded as permitting the metabolic changes necessary to save the developing gonad rather than being directly involved in gametogenesis. On the other hand, the TSH cells of pituitary may act directly on the thyroid follicles and are believed to affect the rate of colloid synthesis from the thyroid epithelial cells. The maximum activity of the thyroid epithelial cells has been observed during the peak spawning period (June to August). Therefore, there is a clear phase lag between synthetic activity of the TSH of the pituitary and the colloid synthesis from the epithelial

cell assuming that the height of the thyroid epithelial cells corresponds to the secretory activity of the fish concerned. Thyroid epithelial cells fill the lumen of the thyroid follicles by their colloidal secretions increasing the sizes of the thyroid follicular cells.

ACKNOWLEDGEMENT

The authors are grateful to Dr. Aanandamay Barik, Head of the Department of Zoology, The University of Burdwan, Burdwan for providing necessary laboratory facilities.

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