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Seasonal changes in the architecture of hepatocytes in relation to ovarian activities during growth, maturation, spawning and post-spawning phases in *Mystus vittatus* (Bloch, 1790)

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ABSTRACT
The present investigation dealt with the observations made on the histological architecture of hepatocytes and correlated them with the seasonal changes of ovarian activities in *Mystus vittatus* (Bloch, 1790). Histologically, each hepatocyte of female *M. vittatus* was provided with distinct nucleus and granular basophilic cytoplasm. Various female germ line cells were also recognized on the basis of size and histoarchitecture of the cells. However, on the basis of relative abundance and size of the different oocytes the event of oogenesis has been found to occur in four distinct phases, growth, maturation, spawning and post-spawning. It was found that during growth and maturation phases the amount of cytoplasmic granules of hepatocytes was increased as well as the nuclei became hypertrophied. During spawning phase the cytoplasmic granules were sparse in the hypertrophied hepatocytes. However, no significant alterations were noticed in the hepatocytes during post-spawning phase. Cytological variations in the hepatic cells were correlated with the different reproductive phases in female *M. vittatus*.

Keywords: Seasonal changes, Hepatocyte architecture, Ovarian activities, Growth, Maturation, Spawning, Post spawning, *Mystus vittatus*.

1. Introduction
The teleostean fishes being the cheapest source of animal protein have drawn the attention of many fishery biologists relative to their cultural and management practices. However, the knowledge of the reproductive cycle and its functional mechanism are of prime importance concerning the successful management of fisheries which is pertinent to the gross production. It is known that the ovarian cycle in majority of freshwater teleosts which are seasonal breeders undergo remarkable changes during various periods of the season [1, 2, 3, 4, 5, 6, 7].
However, during the reproductive processes, the demand of energy may be higher than the energy available from the food during that period [8] and the fish meet with this energy demand by utilizing the stored nutrients accumulated during the excess food supply of the time of low energy demand [9]. The gradual increase in total weight and associated morpho-histological changes in ovary of the fish from the pre-spawning season is, therefore, intimately associated with the transfer of various nutrients from the body muscle and liver as well as with the proliferation of various germ line cells formed by the active process of gametogenesis [10, 11].
In the present study, an attempt has been made to study the events of oogenesis and the annual cyclical changes in the ovary and the variations in the histological aspects of the liver in relation to growth, maturation, spawning, post-spawning and resting phases of *Mystus vittatus* (Bloch, 1790).

2. Materials and Methods
Adult living female specimens of *M. vittatus* (length 10-12 cm and weight 30-50 g) were procured from the local freshwater body of Burdwan, West Bengal, India during the second week of every month from January to December 2012. The fishes were acclimatized for five days by feeding oligochaetes daily. Data on total body weight and ovarian weight of 10 fishes were taken to calculate the mean gonadosomatic index (GSI) using the following formula:

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

2.1. Histological methods

After decapitation of the fish the liver and ovaries were removed carefully and fragments of liver and ovaries were fixed in aqueous Bouin's fluid for 18h. Subsequent to dehydration the tissues were embedded in paraffin wax. All the tissues were serially sectioned at 4 μm and stained with iron-alum and/or Delafield's haematoxylin-eosin for ovary and only Delafield's haematoxylin for liver tissues. From the histological preparations of the ovaries, the diameter of various oogenetic cells and their nuclei were measured with the help of reticulo-micrometer and ocular micrometer respectively.

3. Results

3.1. Gonadosomatic index

In the present study it was observed that the values of gonadosomatic index (GSI) in *M. vittatus* followed a regular cyclical change during growth, maturation, spawning post-spawning and resting phases. The results have been presented in Table 1. The lowest GSI value (0.42 ± 0.71) was noticed during the end of post-spawning phase in October. During November i.e. in resting phase the mean GSI value increased to 0.75 ± 0.15. However, during the onset of growth phase in December slight increment of GSI (0.98 ± 0.13) was noticed. During the period of growth phase i.e. in January and February the GSI value was found to increase gradually (1.08 ± 0.04 to 1.67 ± 0.87). Subsequently, from March onwards when the ovary might have entered into the maturation phase GSI gradually increased to 2.15 ± 0.13 and in May GSI rose sharply to 9.48 ± 0.14. In June the ovary was full of mature follicles and the GSI was recorded to be 10.5 ± 0.07. The GSI rose up to a peak value (11.22 ± 0.12) in July but in August it showed a declining trend (8.25 ± 0.24). In the post-spawning period i.e. in September the yolky follicles were reabsorbed and the ovaries suffered from a regression state. The GSI value was recorded as 3.41 ± 0.36 in September.

Table 1: Seasonal variations in the gonadosomatic index (GSI) of female *Mystus vittatus*

Maturity stages of ovary	Months	Mean GSI ± SE
Growth Phase	December	0.98 ± 0.19
	January	1.08 ± 0.04
	February	1.67 ± 0.87
Maturation Phase	March	2.15 ± 0.13
	April	5.51 ± 0.05
	May	9.48 ± 0.14
Spawning Phase	June	10.5 ± 0.07
	July	11.22 ± 0.l2
	August	8.25 ± 0.24
Post-Spawning	September	3.41 ± 0.36
	October	0.42 ± 0.71
	November	0.75 ± 0.15

3.2. Histology

3.2.1. Liver

Histologically the liver of *M. vittatus* was composed of a parenchyma covered by a thin capsule of connective tissue. Hepatocytes vary from polyhedral to round shape and were irregularly arranged surrounding a central vein. Each hepatocyte contained a large, round, and central nucleus with a deeply stained nucleolus and granular cytoplasm (Fig. 1). Hepatocytes showed changes according to different reproductive phases.

3.2.2. Oogenesis

Histologically the inner wall of the germinal epithelium of ovary projected into the ovarian cavity was termed as ovigerous lamellae where development of new crops of oogonia took place. However, the sequence of oocyte maturation in *M. vittatus* had 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).

3.2.2.1. Oogonia (Stage I) (5 ± 7 μm to 12 × 10 μm)

Oogonia were present either singly or in small nests within the ovigerous lamellae. An oogonium was made up of a large nucleus with chromatin threads and basophilic cytoplasm (Fig. 2). An oogonium passed through a number of maturation stages in order to become a mature ovum.

3.2.2.2. Early perinucleolus oocyte (Stage II) (23 × 30 μm to 44 × 28 μm)

This stage consisted of basophilic cytoplasm and a large oval centrally placed nucleus with a diameter ranging from 10 μm × 24 μm and contained about 8-10 basophilic nuclei at the periphery of nucleus together with fragmented chromatin (Figs. 2 and 3). The cytoplasm was basophilic in nature.

3.2.2.3. Late perinucleolus oocyte (Stage III) (68 × 80 μm to 105 × 90 μm)

This stage was characterized by the appearance of cortical alveoli along the peripheral region of the ooplasm. Nucleus was large and spherical having condensed chromatin materials. A thin follicular layer enclosing the zona radiata was also appeared in this stage (Fig. 5).

3.2.2.4. Yolk vesicle oocyte (Stage IV) (145 × 152 μm to 270 × 280 μm)

The cortical alveoli of Stage III oocyte finally covered the entire ooplasm of stage IV oocyte. Most of the yolk vesicle oocytes were empty but some of them were filled with homogenous material which took a faint haematoxylin stain (Figs. 5, 6 and 7). The oocyte was enveloped with a thick zona radiata (porous layer), the middle multinucleated layer the zona granulosa and outermost theca made up of connective tissue (Figs. 5, 6 and 7).

3.2.2.5. Yolk granules oocyte (Stage V) (300 × 350 μm to 400 × 450 μm)

Formation of yolk globules took place in the yolk granules oocytes stage and as a result the cell volume and diameter increased rapidly. During this stage migration of germinal vesicle from the center of the egg towards the periphery was started. The granulosa cells were more prominent having distinct nucleus (Fig. 6).

3.2.2.6. Mature follicle (Stage VII) (500 × 550 μm to 558 × 600 μm)

In these vitellogenic oocytes, the yolk granules coalesced and remain tightly packed with each other so as to form an yolk mass (Fig. 10). The nucleus sometimes remained invisible and when visible, it was eccentric in position with irregular outline (Fig. 9). The thickness of the theca, zona granulosa and zona radiata reduced considerably (Figs. 9 and 10). Such mature follicles were

ready for final extrusion.

3.2.3. Atretic oocytes
Sometimes the developing oocytes failed to attain maturity were called atretic oocytes. The atretic oocytes were characterized by irregular shaped, disintegrated nuclei of the zona granulosa and liquidified yolk granules. Granulosa cells were also proliferated.

3.2.4. Discharged follicle
The post ovulatory corpus luteum developed from the follicular cells immediately after discharge of mature ovum. The shape of discharged follicles was irregular and the granulosa layer showed definite changes in structure (Fig. 10).

3.3. Sequential changes in the liver and ovary during different reproductive phases
In the present observation histoarchitecture of hepatocytes, morpho-histological characteristics and gonadosomatic index of ovary, the frequency of various oogenetic cells, were found to undergo changes during growth, maturation, spawning and post-spawning phases.

3.3.1. Growth phase (December to February)
In this phase the cytoplasm of the liver cells of female accumulated considerable amount of granules. Nuclei were found in the centre of the hepatocytes. The sinusoid endothelium was present in between hepatocytes (Fig. 1). Primary oocytes at all stages were present in the ovary. This phase was dominated by chromatin nucleolus stage (Fig. 2). However, the percentages of late perinucleolus oocytes increased during the end of this period (Figs. 3 and 5).

3.3.2. Maturation phase (March to May)
During this period i.e. yolk deposition stage the hepatocytes was found to slightly enlarge in size and the basophilic cytoplasmic granules increase. Nucleoli were hypertrophied. The binucleate hepatocytes were found to increase in females. The sinusoids were well vascularized (Fig. 4). Different stages of vitellogenic oocytes were characteristically present (Fig. 5). However, majority of the developing oocytes were in the form of yolk granules stage (Figs. 5 and 6). From March onwards the yolk granules continued to coalesce and migrate centripetally. Prominent zona radiata and follicle granulosa cells were prominent (Fig. 7). Subsequently with the advancement of maturity, the immature oocytes were decreased in number (Fig. 6).

3.3.3. Spawning phase (June to August)
In the spawning phase the hepatocytes of female liver showed momentous changes than growth and maturation phases. The hepatocytes were enlarged with depleted cytoplasm having hypertrophic nuclei (Fig. 8). However, some of the hepatocytes were vacuolated with reduced nuclei (Fig. 8). The vascularisation of hepatic sinusoids were also reduced (Fig. 8). The ovaries of this stage were full of ripe ova. In July mature follicles became larger and irregular in shape, the yolk globules broke up and the mature follicles were provided with eccentric germinal vesicle (Fig. 9). At the end of this phase some of the mature follicles collapse inward and zona granulosa and zona radiata were broken in several places. A few discharged follicles were also observed (Fig. 10).

3.3.4. Post-Spawning phase (September to November)
The liver of *M. vittatus* showed hepatic parenchymal arrangement consists of hepatocytes, which were provided with centrally placed nuclei and prominent nucleoli. The basophilic granules started to accumulate in the hepatic cytoplasm (Fig. 11). During post spawning phase mature ova were very few in numbers. By the end of September oogonia and early perinuclear oocyte were appeared below the ovarian wall. In the month of October and November the proliferation of oogonia were started and the developing oocytes of stage III are also found in between primary oocytes (Fig. 12).

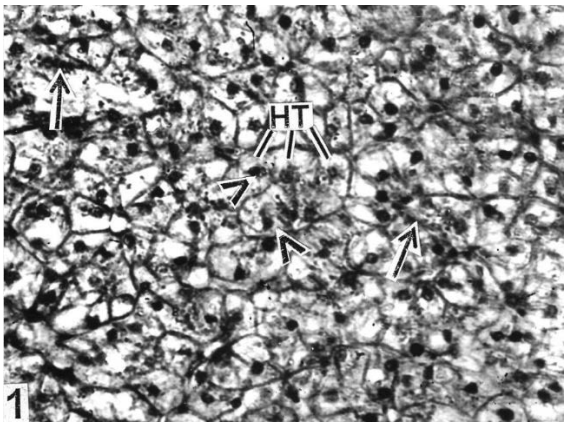

Fig 1: Showing hepatic cells (HC) (arrow heads) with centrally placed nucleus and granular cytoplasm during growth phase. Note hepatic sinusoids (solid arrows) in between hepatic cells (H&E) × 400.

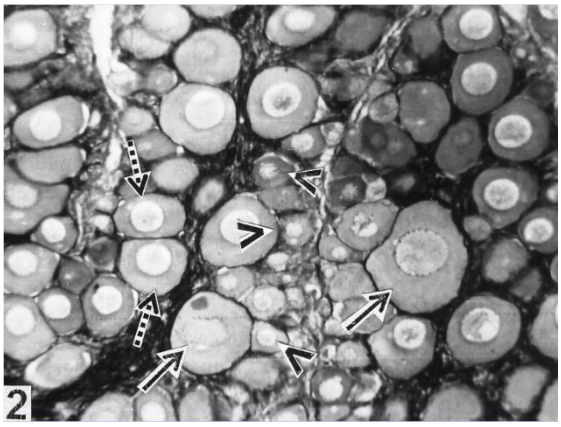

Fig 2: Presence of oogonia (OO) (arrow heads), Oocyte I (OI) (broken arrows) and early perinucleolus oocyte (OII) (solid arrows) during growth phase (H&E) × 400.

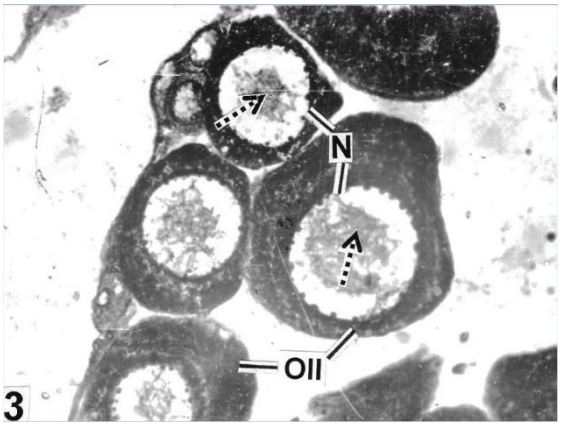

Fig 3: Showing early perinuclear oocyte (OII) with minute cortical alveoli during late growth phase. Note the presence of nucleus (N) with prominent nucleoli along the periphery of N and chromatin materials (broken arrows) (IA) × 600.

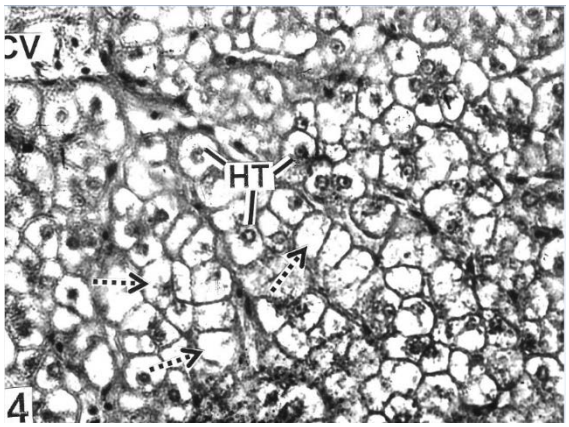

Fig 4: Hepatocytes (HT) encircling the central vein (CV) with granular cytoplasm (broken arrows) during maturation phase. Note vascularized hepatic sinusoid (solid arrow) in between hepatocytes (H&E) × 400.

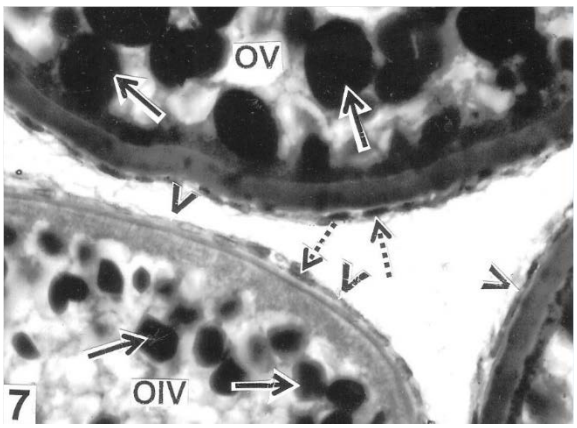

Fig 7: Higher magnification of oocyte IV (OIV) and oocyte V (OV) during maturation phase with prominent yolk globules (solid arrows), theca layer (arrow heads) and granulosa cells (broken arrows) (IA) × 600.

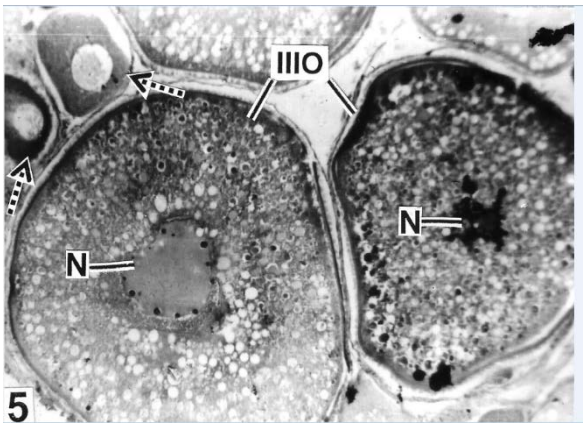

Fig 5: Showing oocyte III (OIII) with prominent cortical alveoli and oocyte IV (OIV) stage with follicular layers. Note the presence of yolk vesicle in the entire ooplasm with middle prominent nucleus (N) in OIV during maturation phase (H&E) × 400.

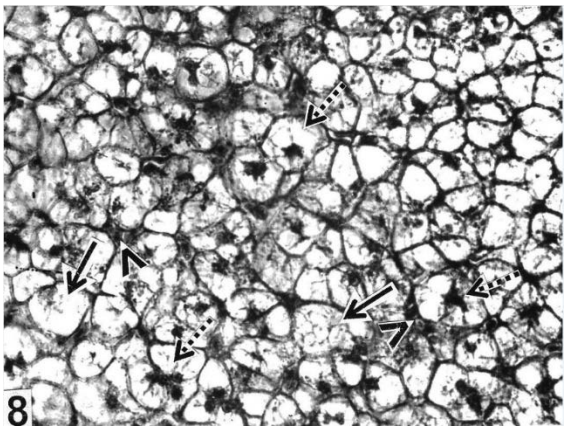

Fig 8: Showing enlarged hepatocytes (broken arrows) with scanty cytoplasm and hypertrophic nuclei during spawning phase. Note some vacuolated hepatocytes having reduced size of nuclei (solid arrows). Arrow heads indicate sinusoid having less vascularisation (H&E) × 400.

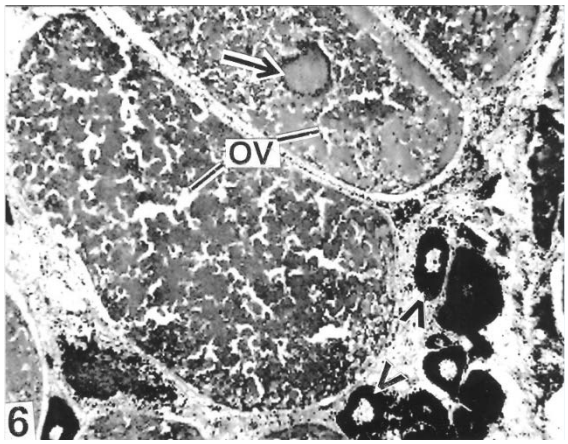

Fig 6: Showing oocyte V (OV) with yolk granules (YG) during late maturation phase. Note migration of germinal vesicle (solid arrow) towards the periphery of oocyte. Arrow heads indicate early oocyte stage (H&E) × 400.

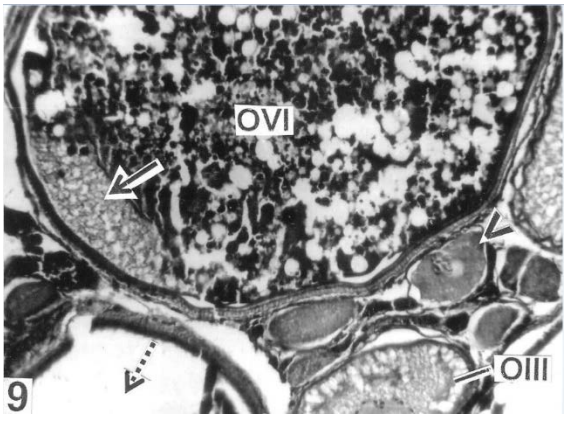

Fig 9: Showing oocyte VI (OVI) with full of yolk granules during spawning period. Note eccentric germinal vesicle (solid arrow), empty discharged follicle (broken arrow), oocyte III (OIII) and immature oocyte (arrow head) (H&E) × 600.

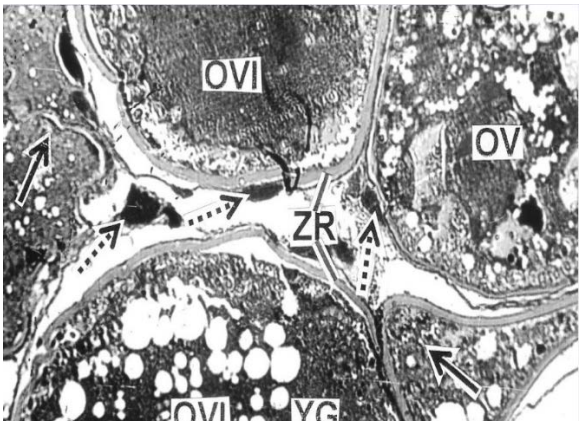

Fig 10: Showing mature oocyte (OVI) stage during spawning phase having coalesced yolk granules (YG) and zona radiata (ZR). Note the presence of blood vessels (broken arrows) adjacent to mature oocyte. Note also oocyte V (OV) and discharged follicles (solid arrows) in between mature ova (H&E) × 400.

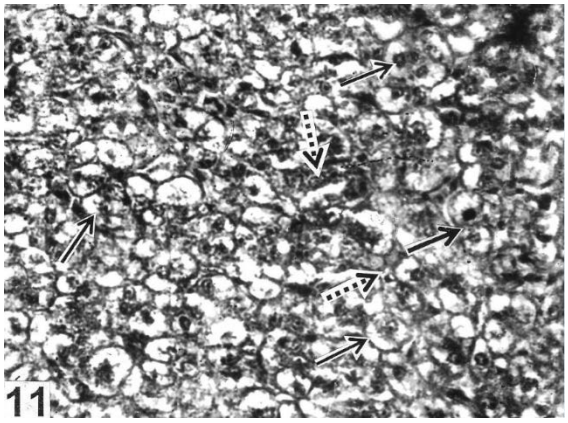

Fig 11: Showing parenchymal arrangement of liver having prominent hepatic cells (solid arrows) with distinct nuclei and granular cytoplasm during post-spawning phase. Broken arrows indicate hepatic sinusoids (H&E)× 400.

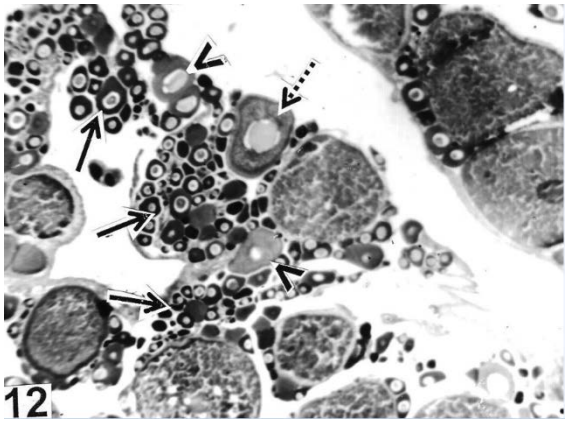

Fig 12: Ovigerous lamellae with oogonial mass (solid arrows) and early perinuclear oocytes (arrow heads and broken arrows) during post-spawning phase. Note some regressed mature ova in between early oocytes (H&E)× 150.

Figs 1-12: Photomicrographs of sections of liver and ovary during growth, maturation, spawning and post-spawning phases in *Mystus vittatus* (Delafield's haematoxylin-eosin: H&E, Iron alum haematoxylin: IA).

4. Discussion

In the present study on *M. vittatus* the GSI value was comparatively lower during the end of the post-spawning phase when the ovaries were found in regressed condition containing mainly primary oocytes. Garg and Jain [12] observed that in *Channa punctatus* ovarian regression occurs at a time when day length began to shorten and temperature reached to its seasonal minimum. GSI increased marginally but remained almost stationary during the growth phase. This might be due to the gradual proliferation of early and late perinuclear oocytes in the ovary. Thakur [13] reported that after post-spawning period the GSI in female fish remained stationary for a prolonged period indicating the dormant phase of ovary. However, from the end of the maturation phase GSI increased rapidly and continued up to spawning phase due to maximum proliferation of vitellogenic oocytes and slow accumulation of yolk granules in the oocytes. Thus, it suggested that adequate food availability helped the fish in recruitment of vitellogenic oocytes and in maintaining the maturation process in the ovary [12, 14, 15]. However, GSI value declined prominently from September onwards due to discharge or reabsorption of yolky oocytes.

In the present investigation, liver of *M. vittatus* showed hepatic parenchyma to consist of hepatocytes which are arranged around a central vein. This histological structure of the liver resembles that described for the stripped weakfish *Cynoscion guatucupa* [16] and for rainbow trout *Oncorhynchus mykiss* [17].

Oogenesis occurred in two stages, the division of oogonia and the transformation of the resting oocytes into mature oocytes. In *M. vittatus* it was observed that the early developmental stages of oogonia arised from the germinal epithelium on the ovigerous lamellae. Each oogonium passed through a number of maturation stages before it became a mature ovum. This involved complex changes in the cytology of the nucleus and cytoplasm. The formation of yolk globules in the late perinucleolus oocyte begins in the periphery of the developing ooplasm; later the wave of the yolk deposition moves subsequently to the centre of ovum. Bisht and Joshi [18], Kapoor [19] observed similar pattern of yolk deposition in *Schizothorax richardsonii* and *Puntius ticto*. In *M. vittatus* the development of ovary can broadly be divided into two broad phases. In the first or previtellogenic phase growth was slow and comparatively few cytoplasmic changes occurred. The second or vitellogenic phase was characterized by rapid growth and deposition of large amount of yolk in the cytoplasm. The terminology and criteria of staging the oocyte were in conformity with the description of Mayer *et al.* [20]. In the present study the yolk vesicle oocyte, yolk granule oocyte and mature ova were enveloped by having radial striations, the zona radiata, the multinucleated zona granulosa and outermost theca. In the late developing oocytes, it might be assumed that the radial striations are relatively active in the transport of essential substances from granulosa nursery cells to the oocyte cytoplasm for building up the cytoplasm the oocyte. Similar observation has also been made by Bromage and Cumaranatunga [21], Shabanipour and Heidari [22] in the mature ovary of rainbow trout and *Liza aurata*. Migration of the germinal vesicle having invisible nuclear membrane was an event associated with the onset of final maturation of oocyte. Mylonas *et al.* [23] reported the dissolution of nuclear wall in mature oocyte in American shad, *Alosa sapidissima*.

In the present investigation the maturational activity in the ovary reached to the highest during late maturation and early spawning phase when oocyte diameter as well as GSI rose up. At the same period the hepatocytes were enlarged with depletion of cytoplasmic granules. This feature might be due to the uptake and accumulation

of nutrients in the oocytes and yolk precursor vitellogenin from hepatocytes. de Vlaming *et al*. [24] reported that principal events responsible for the growth of the oocytes involved sequestration of hepatically derived protein precursor, vitellogenin which takes part in the formation of yolk protein. The liver cells of *M. vittatus* showed an activated state following ovarian maturation. This suggested that probably the liver cells were the site of the synthesis of glycoprotein probably released into the blood and then transported into the oocytes during the process of ovarian maturation.

Aida *et al*. [25] opined that the liver cells in female Ayu were the site of FSPP (female specific plasma protein) synthesis and upon synthesis in the liver cells, this protein was conveyed to the oocytes for final ovarian maturation. The high frequency of binucleate cells in the liver of *M. vittatus* was an interesting phenomenon, suggesting a hyper function of nucleus and nucleolus. Hypertrophy of nuclei and nucleoli and the great increase of RNA in the cytoplasm of the liver of maturing female Ayu suggested that protein synthesis was activated by the commencement of vitellogenesis in the oocytes [25].

5. Acknowledgements

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