



Seasonal Morphohistological Changes in the Activity of the Liver Cells and Its Co-relation with the Ovarian Cycle in the Asian Striped Dwarf Catfish *Mystus vittatus* (Bloch, 1794).

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Abstract:- The morphohistological changes in the liver and ovary of the adult Asian striped catfish, *Mystus vittatus* was studied during different seasons of the year. Depending upon the sizes and cellular morphology, eight different types of oocytic stages were recognised in the ovary of *Mystus vittatus*. Four distinct phases of oogenesis were found to occur and simultaneously the liver was observed to undergo seasonal variation in their histological characteristics indicating changes in physiology and metabolic activities during the seasonal cycle of female reproduction. So, the seasonal changes of the liver histology could be well co-related with the cytological changes in the ovary.

Keywords: Ovary, Liver, *Mystus vittatus*, Morphohistology.

1. **INTRODUCTION**

Mystus vittatus (Bloch, 1794) is a small striped Asian catfish that inhabits the fresh water bodies of the Indian subcontinent including Bangladesh, Pakistan, Sri Lanka, Bhutan and Nepal. It has also been reported from Myanmar, Malaysia, Laos, Vietnam and Cambodia (Froese & Pauly, 2006). The knowledge of the seasonal reproductive cycle of this fish is of great concern since this small, indigenous fish species has a high nutritional value in terms of protein, micronutrients, vitamins and minerals which are not commonly available in other foods (Ross *et al*, 2003).

Several workers have contributed to the study on the ovarian cycle in the teleosts that are seasonal breeders and come up with noticeable histological changes during different seasons of the year (Ferreira *et al*, 2012; Khanna & Sanwal, 1971; Manna & Bhattacharya, 1993; Rastogi, 1963; Singh *et al*, 2008; Yin *et al*, 2012). It is well known now that the liver of some teleosts undergoes seasonal variations in size and in the content of fat and glycogen (Olivereau & Leloup, 1950; Pickford, 1953; Ito *et al*, 1962) Again, the process of oogenesis demands large amount of energy. According to some workers some of the lipids deposited in the oocytes during maturation do not come directly from ingested food, but are transferred from lipid stores in the liver and muscles (Zahind, 1959; Larson, 1974; Htun-Han, 1978; Chupkin *et al*, 1988).

Seasonal histological analysis of ovary provides an opportunity to understand the cellular

kinetics, changes and development of the ovarian cells. Similarly seasonal alterations in metabolic status can also be revealed by the histology of the liver cells since, high metabolic alterations takes place during ovarian development in fishes (Eliassen & Vahl, 1982), and it is evident that there will be a variation in the liver histology due to oogenesis.

The aim of our study was to examine light microscopically the seasonal histological changes in the liver and its relationship with the seasonal morphohistological changes during the process of oogenesis in *Mystus vittatus*.

2. **MATERIALS AND METHODS**

Specimen collection and data analysis

Adult female *Mystus vittatus* (Bloch) were collected fortnightly throughout the year from a particular stocking pond located in Burdwan district of West Bengal, India, in order to avoid ecological variations in different ponds that can affect ovarian and liver development. About 186 female fishes with body weight between 21g to 48g were dissected for our experimental purpose. Every month data on total body weight, liver weight and ovarian weight of 15 fishes were taken to calculate the mean Hepatosomatic index (HSI) and Gonadosomatic index (GSI) respectively using the following formulae

$$HSI = \frac{\text{Hepatic weight}}{(\text{Total body weight} - \text{Hepatic weight})} \times 100 \quad (1)$$

$$GSI = \frac{\text{Gonadal weight}}{(\text{Total body weight} - \text{Gonadal weight})} \times 100 \quad (2)$$

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Histological methods

The liver and ovary were dissected out and cut into small pieces and then fixed in aqueous Bouin's fluid (Costa & Chaves, 1943) for 18 hours. The fixed tissues were placed in 70% ethanol and then dehydrated properly through graded ethanol, followed by acetone and cleared in benzene. Tissues were embedded in paraffin (56^o C-58^o C) and sectioned at 4µm thickness.

The sections of liver and ovary were stained with the haematoxylin-eosin method (Costa and Chaves, 1943). In addition the ovaries were also stained with the iron-alum haematoxylin and Mallory's triple stain. The stained sections were mounted permanently with DPX and examined under binocular microscope.

Histological measurements and statistical analysis

From the histological preparation of the liver and ovaries, the diameters of the various cells were measured with the help of reticulo-micrometer and ocular micrometer. The cell height of the liver cells and oocytes were measured at four points within each cell at 90^o from one another and reported as the mean ± Standard Error of Mean (SEM).

3. RESULTS

Liver histomorphology

The liver as an organ plays a vital role in the storage of energy. It consists of large number of functional units called lobules; at whose centre lie the central veins. The large polygonal hepatic cells radiates out from the central vein. The sinusoids separate the sheets of hepatocytes and empty into the central vein. The hepatocytes are characterised by the presence of a prominent nucleus and mass of cytoplasm around it whose position and proportion varies according to the changing physiological conditions of the fish. Our present study of the hepatocytes reveals that the diameter, number and morphology of the hepatocytes vary during the different seasons of the year. Besides, the fat vacuoles are observed in between the hepatocytes, which act as indicators of storage for the liver cells.

Hepato-Somatic Index (HSI) and Gonado-Somatic Index (GSI)

In our present study it has been observed that the HSI value and the GSI value of female *Mystus*

vittatus varies largely during the different months of the year. It follows a similar qualitative regular cyclical change during growth, maturation, spawning and post spawning stage as shown in figure 1. The maximum value of HSI (1.93 ± 0.09) has been recorded during June whereas the highest GSI value (26.51 ± 0.87) has been noticed during July when the ovaries remained packed with the fully mature follicles. The lowest HSI value (0.82 ± 0.04) has been recorded during November and the lowest GSI value (0.98 ± 0.71) has been recorded during October when the ovary underwent the post-spawning phase. 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 found to become 22.12 ± 0.12 . During the post-spawning period i.e., from September onwards the yolky materials of the ovarian follicles are reabsorbed and the GSI value declines to 3.41 ± 0.36 in September.

Oocyte development stages

A sound knowledge of the gonad morphology is essential for identifying and describing the stages of gonad development (Vazzoler, 1996). According to Gomes *et al*, 2001, an oocyte growth resulting from the developing characters of the germinative cells initiates the process of oogenesis. The sequence of oocyte maturation has been divided by various authors into different stages viz., into four stages in *Danio rerio* (Koc *et al*, 2008), six in *Rutilus frisii kutum* (Saeed *et al*, 2010), seven in *Liza subviridis* (Chan & Chua, 1980), eight in *Oligosarcus hepsetus* (Santos *et al*, 2005) and nine in *Tandanus tandanus* (Davis, 1977). In the present study, the growth of the oocyte of *Mystus vittatus* has been conveniently divided into eight distinct developmental stages (excluding the atretic oocytes) using the optical microscopic observation for the first time as per our knowledge and are shown in (Table-1).

Table 1 Terminology, Size and Characteristics for staging the oocytes of *Mystus vittatus* (Bloch, 1794)

Stage	Terminology	Size	Characteristics
PRE VITELLOGENESIS			
I	Oogonia	8 ± 0.05 µm	Appear singly or sometimes in nests in the germinal epithelium. Ooplasm is chromophobic. Large central nucleus. Nucleoli not clear.
IIa	Chromatin nucleolus oocytes (condensed chromatin cells)	22 ± 0.64 µm	Situated very close to oogonia. Meagre amount of ooplasm. Stain deep. Large nucleus with 2-4 visible central nucleoli.
IIb	Early (Small) perinucleolus oocytes	32 ± 0.42 µm	Thin layer of follicular epithelium. Large oval centrally placed nucleus with an average diameter of 14µm ± 0.6. 5 to 8 basophilic nucleoli. Some fragmented chromatin present. Basophilic ooplasm.
IIIa	Late (Large) perinucleolus oocytes	92 ± 1.18 µm	Ooplasm stains lighter. Centrally located nucleus. Nucleoli 15 to 30 with various sizes. Dispersed chromatin. Appearance of a thin layer of follicular cells enclosing the zona radiata
VITELLOGENESIS			
IIIb	Yolk vesicle	188±1.96 µm	Most of the vesicles are empty. Takes faint haematoxylin stain. Large nucleus with irregular shape. Nucleoli in the peripheral zone. Zona granulosa is clearly visible along with zona radiata. Deposition of yolk (intravesicular) occurs inside the peripheral vacuoles.
IV	Yolk granule (Vitellogenesis)	424±1.08 µm	Yolk vesicles are filled with yolk granules. Central or eccentric nucleus. Ooplasm change from basophilic to acidophilic in staining nature. Zona radiata increase in thickness (8.5µm±0.002). Syncytial granulosa layer becomes thicker (12µm±0.05).
V	Vitellogenic (Mature) oocytes	558± 1.12µm	Yolk laden oocytes. Voluminous cytoplasm with granular appearance. Larger yolk vesicles located towards the periphery. Eccentric nucleus. Zona granulosa (13.5 µm±0.01) and zona radiata (11.5 µm±0.02) thickened to its highest possible limit.
VI	Post-ovulatory follicles (Discharged follicles).	—	Mature follicle discharged. Theca and follicular cells present. Irregular in outline. No definite size and shape.

Atretic follicles (Atretic oocytes)

The growing oocytes sometimes fail to attain maturity and undergo resorption by the phagocytic activity of the invading granulosa cells and gives rise to the atretic oocytes. They are characterised by irregular, disintegrated nuclei and liquefied yolk granules. Some authors have observed this structure in all the phases of development (Santos *et al*, 2005). In our present study the atretic oocytes have been found to occur mostly during the spawning and post-spawning periods (**Fig. 4d, 5a**).

Phases of oogenesis

In our present study on the basis of morphohistological characteristics of the ovary, gonado-somatic index (**Fig. 1**) and the frequency of occurrence of various oocytic cells, the event of oogenesis in *Mystus vittatus* has been divided into the following four distinct phases:

- 1) Growth phase(December to February)
- 2) Maturation phase (March to April)
- 3) Spawning phase (May to August)
- 4) Post spawning or Resting phase (September to November).

Cyclical changes in the liver and ovary during oogenesis

During oogenesis the liver histology of *Mystus vittatus* along with the ovarian features have been found to change noticeably.

- 1) Growth phase (December to February)

Liver histology

The prominent nucleus continues to appear and the cytoplasmic mass slightly increases in volume and hence, the hepatocytic diameter ranges from 12.5µm to 13.5µm (**Fig. 1, inset**). Granules begin to appear in the cytoplasm and the fat vacuoles begins to appear (**Fig. 2a**) and by February the cells become filled with a number of fat vacuoles (**Fig. 2b**). Cytoplasmic vacuolization is an indicator of cell activation and become frequent during the late growth phase.

Ovarian features

This stage consists of ovaries with oogonium, chromatin nucleolus, early and late perinucleolar stages of oocytes (**Fig. 3a, 3b, 3c**). Prominent tunica albuginea can also be seen covering the ovarian wall. The average GSI values ranged between 1.67 ± 0.87 to 2.15 ± 0.13.

The oogonia are few in number and lie within the laminar epithelium. Early primary oocytes are observed which are slightly larger than the oogonium. The percentages of late perinucleolar oocytes are gradually increasing during the end of this phase (Fig 3c, 3d).

2) Maturation phase (March to May)

Liver histology

Most of the hepatocytes have enlarged nuclei with distinct nucleoli. The cytoplasmic content is high and hence the cell appears larger measuring from $16.08 \pm 0.12 \mu\text{m}$ to $18.37 \pm 0.08 \mu\text{m}$ in diameter (Fig. 1, inset). The cytoplasm appears granulated. Large numbers of fat vacuoles appear throughout the tissue indicating very high biosynthetic activity. By the end of May, most of the hepatocytes were found with vacuolated and granular cytoplasm and adjacent blood vessels indicating very high activity (Fig. 2c).

Ovarian features

The yolk vesicle stage is found to dominate all other stages such as chromatin nucleolus, early and late perinucleolus stages during the early maturation phase i.e., march and early april. Besides, the immature oocytes and developing oocytes few oogonial cells are found up to the month of March. From April to May vitellogenesis is found to start and the yolk filled follicles i.e., yolk vesicle stage sharply increases in number (Fig. 4a, 4b, 4c). The inter-follicular spaces are found to be reduced and the ovigerous lamellae become indistinguishable. The average GSI value is found to be between 6.26 ± 0.07 and 19.60 ± 0.17 . Towards the end of May, the number of developing follicles attains maximum abundance while the number of immature oocytes decline to a minimum. The Zona granulosa and Zona radiata become clearly visible and the nuclei within the granulosa cells can be easily identified (Fig. 4c).

3) Spawning phase (June to August)

Liver histology

In June and early July the cellular activation was pronounced as indicated by increased cytoplasmic granulation and vacuolization. The nuclei were enlarged and round with distinct nucleoli. The cellular diameter ranged between $17.92 \pm 0.02 \mu\text{m}$ to $17.04 \pm 0.04 \mu\text{m}$. Enlarged fat vacuoles and highest number of blood vessels are observed in the tissues (Fig. 2d). The fat vacuoles begin to decrease in size and number during the later part of July onwards and the cells manifested less cytoplasmic content and smaller nuclei without prominent nucleoli indicating decrease in the biosynthetic activity (Fig. 2e).

Ovarian features

This phase show almost all the stages of yolk formation-which includes yolk vesicles, yolk granules and also mature ova. In July the oocytes of stage VI become large and irregular in shape. The yolk globules begin to break up. Some of the mature eggs are found to contain an eccentric nucleus (Fig. 5a). The zona radiata and zona granulosa appear prominent and thicker than before (Fig. 5b). Some of the mature follicles collapse inward and zona granulosa and zona radiata break in several places. Atretic oocytes have also been detected (Fig 5a). The average GSI value is in between 24.72 ± 0.08 to 22.12 ± 0.12 .

4) Post spawning or Resting phase (September to November).

Liver histology

The nuclei of the hepatocytes lie close to each other. The nuclei appear very distinct and in between the hepatocytes quite a number of empty spaces are seen with fewer blood vessels (Fig. 2f). The fat vacuoles decrease in size and number indicating a sharp decrease in storage activity of the liver cells. The cytoplasmic mass appears to be homogenous and very less in quantity. The cell size lies within $10.22 \pm 0.08 \mu\text{m}$ to $13.49 \pm 0.06 \mu\text{m}$ in diameter (Fig. 1, inset).

Ovarian features

During early October, oogonia appear in large numbers below the ovarian wall. In late October the oogonia start proliferating and early stages of oocyte (stage-II, III, IV), begin to increase. The atretic follicles and discharged follicles with undulating zona radiata (Fig. 5c, 5d) become more common during September. However, few mature ova are still observed (Fig. 5c). The oocytes with resorption vacuoles and oocytes undergoing resorption are frequently observed in this phase. The nuclei of the zona granulosa appear prominently around some discharged follicles (Fig. 5d). The average GSI value lies between 3.41 ± 0.36 to 0.98 ± 0.71 .

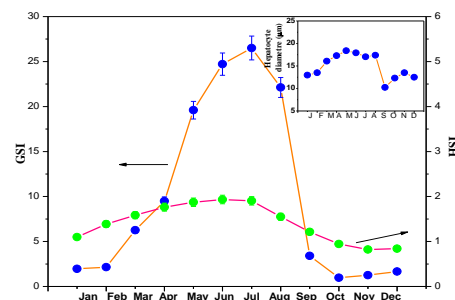


Fig. 1: Seasonal variation of the Hepatosomatic index (HSI) and Gonadosomatic index (GSI) of female *Mystus vittatus* under controlled condition, (inset) Seasonal variation of Hepatocyte diameter of female *Mystus vittatus* under controlled condition.

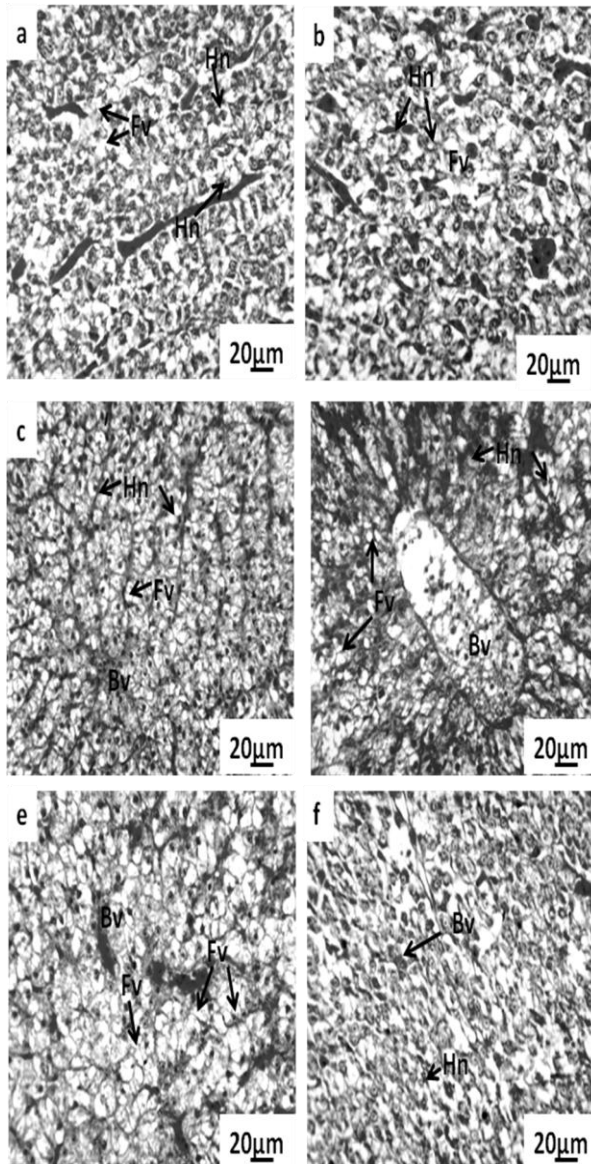


Fig. 2: Optical micrographs of the histological section of liver in female *Mystus vittatus* stained with Haematoxylin-Eosin during different reproductive phases: (a) growth phase (December) showing distinct Hepatic nuclei (Hn) and few emerging small Fat vacuoles (Fv), (b) late growth phase (February) showing distinct Hepatic cells with Hepatic nuclei (Hn) and relatively larger Fat vacuoles (Fv), (c) late maturation phase (May) showing larger Hepatic nuclei (Hn) surrounded by blood vessels (Bv) and Fat vacuoles (Fv), (d) early spawning phase (June) showing Hepatic cells with nuclei (Hn) and larger Fat vacuoles (Fv) and blood vessels (Bv), (e) late spawning phase (August) showing relatively fewer number of Hepatic cells with distinct Hepatic nuclei (Hn), many fat vacuoles (Fv) and Blood cells (Bc), (f) post spawning phase (November) showing smaller Hepatic nuclei (Hc) and fewer Blood vessels (Bv).

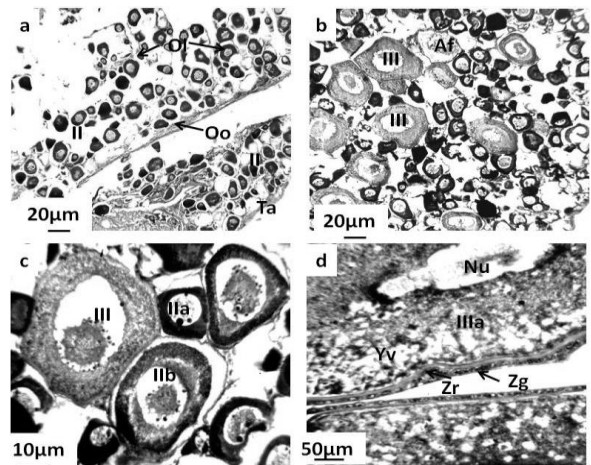


Fig. 3: Optical micrographs of the histological section of ovary in *Mystus vittatus* during growth phase: (a) early growth phase (December) showing Tunica albuginea (Ta), folds of Ooogonia (Oo), nests of Oogonia (Oo), Condensed chromatin cells (II), many Early perinucleolus oocytes (II), stained with Haematoxylin-Eosin, (b) growth phase (January) showing Chromatin nucleolus oocytes (II), Small (early) perinucleolus oocytes (II) and Large (late) perinucleolus oocytes (III) stages stained with iron-alum haematoxylin stain, (c) late growth phase (February) showing the stage IIa, IIb and stage III containing large nucleus with irregular shape stained with iron-alum haematoxylin stain, (d) showing a stage IIIa oocyte with Yolk vesicles (Yv) in which the Zona granulosa (Zg) and zona radiata can be observed, stained with iron-alum haematoxylin stain.

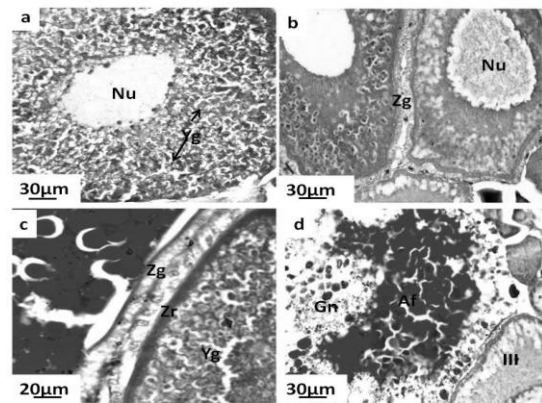


Fig. 4 : Optical micrographs of the histological section of ovary in *Mystus vittatus* during maturation phase: (a) mid maturation phase (April) showing yolk deposition in the yolk vesicles stained with Haematoxylin-Eosin stain; (b) Stage IIIb Oocytes containing prominent, Zona granulosa (Zg) and nucleus (Nu) stained with Mallory's triple stain; (c) Oocyte of late maturation phase (May) showing zona granulosa (Zg) with syncytial nuclei and zona radiata (Zr), oocytes with yolk granules (Yg) stained with Haematoxylin-Eosin stain; (d) magnified view of an atretic follicle (Af), with yolk granules surrounded by zona granulosa nuclei (Gn) and stage III oocyte, stained with Mallory's triple stain.

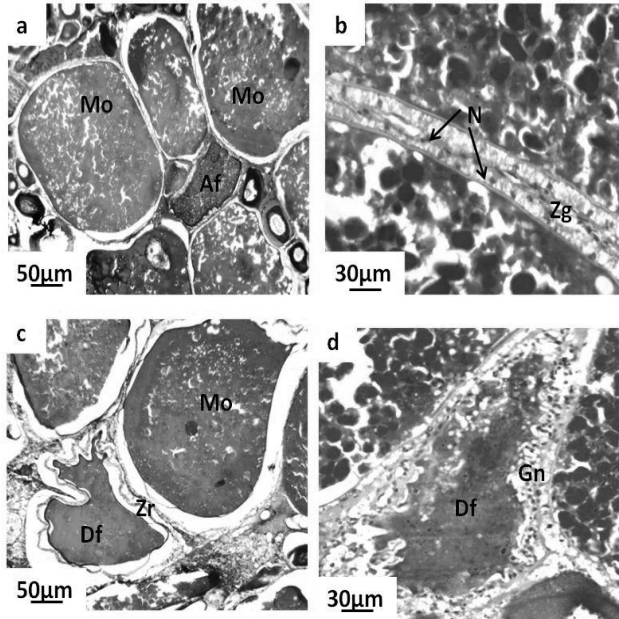


Fig.5: Optical micrographs of the histological section of ovary in *Mystus vittatus* during spawning and post-spawning phase: (a) spawning phase (July) showing mature oocytes (Mo), Atretic follicle (Af) and stage II oocytes stained with Mallory's triple stain, (b) Stage V Oocytes containing prominent, Zona granulosa (Zg) with nuclei and zona radiata stained with Haematoxylin-Eosin stain, (c) Oocyte of post-spawning phase (September) showing discharged follicle (Df) surrounded by undulating zona radiata (Zr), Mature oocytes (Mo) stained with Mallory's triple stain, (d) magnified view of a discharged follicle (Df), surrounded by zona granulosa nuclei (Gn) and stained with Haematoxylin-Eosin stain.

4. DISCUSSION The liver-ovary relationship

As observed from our study, the histology of the female liver greatly varies during different reproductive phases. Again, the dynamics of storage and depletion of lipids and glycogen along with the varying nucleus and cytoplasmic morphology could be seen in the liver histology of *Mystus vittatus*.

The slight increase in cytoplasmic mass and granulation along with distinct nuclear morphology during the growth phase is an indication for the onset of biosynthetic activity of the hepatocytes. This again is supported by the appearance of small to medium fat vacuoles in the liver tissue. This fact is reflected in the increasing value of HSI during this phase.

In March, with the onset of maturation phase, the cell and nuclear size, cytoplasmic granulation and vacuolization in hepatocytes increase gradually and reach their maximum limits by end of May i.e., with the onset of spawning (**Fig. 1, inset**). The increased hepatocytic size is an indication of increased biosynthetic and anabolic activities inside the cells. As a

result, The HSI value increases further and reaches its maximum value making a plateau in the months of May-June (**Fig. 1**). These activities can be correlated with the changes inside the ovary i.e., yolk formation and deposition in the oocytes. It has been shown in *Lipophrys pholis* (Shackley & King, 1977, 1978, 1979) that the yolk incorporates exogenous protein during oocyte development, which was synthesized in the liver. During the late spawning phase i.e., in August, when much of the matured oocytes are released and the yolk deposition is almost completed, the hepatocytes have been found to decrease in cytoplasmic mass and granulation, denoting a rapid fall in the biosynthetic activity of the liver cells (**Fig. 1, inset**) resulting decrease in the average value of HSI (**Fig. 1**). This is due to the complete transfer of the stored materials from the liver cells to the egg yolk in the oocytes.

The cellular size continues to decrease after the spawning period i.e., in the post-spawning phase till November and early December, after which small fat vacuoles reappear and the cytoplasmic mass increases slowly. The smaller hepatocytic size is attributed to the diminished activity of the liver during the post-spawning and oocyte proliferating phase, when the energy needed is sufficiently retrieved from the food alone taken by the fish. Hence, our study confirms that the liver acts as a reservoir or storage organ for the energy needed by the female *Mystus vittatus* during the various oogenetic phases and directly influences the entire female seasonal reproductive cycle.

It can be clearly noticed (**Fig. 1**) that a phase lag has been occurred between the maximum values of HSI (in May) and GSI (in June) during the seasonal variation. This observation can be attributed to the maximum biosynthetic activity of the liver cells at the onset of spawning phase and the maximum storage activity of the matured oocytes which occurs later at the middle of the spawning phase. The time lag is explained from the fact that the biosynthesis of the proteins first occurs in the liver cells and then the yolk of the maturing oocytes incorporates these exogenous proteins from the liver cells.

5. CONCLUSION

The present work gives us valuable information regarding the role of liver in the synthesis and transfer of reserve energy in the form of yolk to the oocytes. The study has provided information about the energy location and transfer during the process of oogenesis in *Mystus vittatus*. However, the histological study alone is not sufficient to study the detailed mechanism and hence, molecular interpretation is required to know the inter-relationship between the liver and ovarian tissue at the specific level.

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