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HISTOLOGICAL AND ULTRA STRUCTURAL ANALYSIS (SEM) OF GONADAL MATURATION OF *Monopterus cuchia* (HAMILTON, 1822) UNDER THE CLIMATIC CONDITION OF MEGHALAYA, INDIA

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Abstract: ***Monopterus cuchia*** **(Hamilton)** ***is*** **one of the most expensive species due to its high nutritional and medicinal values. Analysis of gonadal maturation of *M. cuchia* was done to understand the reproductive biology as well as to identify its peak maturity for breeding. Monthly variation of Gonado-Somatic Index (GSI) reflects the gonadal maturing progression. Different stages of oogenetic development were examined microscopically. Based on the histological characteristics of testis and ovary, four stages of development were described. Scanning electron microscope (SEM) study was used to analyze and further supplement the findings by histology. This paper provides new information on histological analysis and ultra structures of maturation of gametes showing different developmental stages of both male and female species of *M. cuchia* in Meghalaya, India. Therefore observation of gonads was done to ascertain the actual pattern of maturation of gonads and eventually helps in determining period for actual induced breeding of the species which breeds once in a year.**

Keywords: **Gonadal maturation, GSI, *M. cuchia*, Micropyle, SEM, Spawning period.**

I. INTRODUCTION

Monopterus cuchia is one of the most economically important fish species having high market as well as nutritional values. It is a fresh water species with a snake-like appearance and it is locally known as '*Khabsein*' in Khasi language (Meghalaya, India). This species is belonging to the family Synbranchidae of the order Synbranchiformes and it is available only in selected area of the state. The species is an economically important freshwater fish, with distribution in India, Nepal, Bangladesh, Pakistan and Myanmar (Menon, 1999) (Mirza and Alam, 2002) and (Zhou *et al*., 2002). The IUCN, Bangladesh (IUCN, 2000) enlisted *M. cuchia* as a vulnerable species in Bangladesh. But due to bad water management policy for irrigation, over exploitation and various ecological changes in its natural habitat, this species is now under threat (Disaster, 1990) (Chakraborty and Nur, 2009).

Histological study is an essential tool to understand the features of the reproductive biology and a thorough study of gonad morphology, anatomy and histology is required for proper management of the fishery (Mahmoud, 2009). Both Gonado-Somatic Index and histological examination of gonads were used to determine the spawning season of the Blue

Sprat *Spratelloides gracilis* in the waters around Penghu, Central Taiwan Strait (Weng *et* al., 2005). Moreover, regular histological and histochemical examination of reproduction system could categorically define the size and age of a fish at first maturity, as well as reproductive cycle in natural and controlled system (Agarwal, 1996). Reproductive studies of the teleost fishes require knowledge of the stage of the gonad development (Koc *et al*., 2008). Reproductive development in fishes is well understood by histological studies, which are the most convenient method to decide the reproductive state of fishes (West, 1990). The histological studies demonstrated that there are two developmental phases of the oocyte namely, the primary growth phase and secondary growth phase. Also, these two phases were investigated by many authors (Latif and Saddy, 1973) (Guraya *et al*., 1975) (Ramadan *et al*., 1978) (EL-Gharabawy and Abdel-Aziz, 1988) (EL-Gharabawy, 1996) and (El-Halfawy *et al*., 2007). The role of climatic condition of an area, particularly the environmental temperature, on sexual maturation and breeding of fish has been reported by several investigators (Hora, 1945) (Chaudhuri, 1960) (Popma and Lovshin, 1996) (Donelson *et al*., 2014). (Bhuyan *et al*., 2002) have recorded that the rate of spawning and fertilization in *Labeo gonius* (Hamilton, 1822) is low in colder climatic conditions at mid-altitudinal region of Meghalaya. Moreover (Pillay, 1990), reported that research on environment and related aspects of aquaculture and biology of the fish at ultra-structure level is still scanty and is yet to become an integral part of farming system development.

Scanning Electron Microscope (SEM) studies are an important pre-requisite to obtain further insight into the fine structures of teleost eggs and the scanning electron microscope was used to investigate the ultra structure of the egg membrane surface (unfertilized egg) of an endemic cyprinid fish, *Cyprinion tenuiradius* (Esmaeili and Gholamifard, 2011). During fertilization a single sperm enters the micropyle, then the inner part of the micropylar canal becomes narrower and a plug-like blockage quickly forms on the micropyle to prevent polyspermy (Chen *et al*., 2007). (Bhuyan *et al*., 2003) reported on the Scanning Electron Microscope (SEM) study of ovary, egg surface structure and hatchling of *Labeo gonius* with reference to induced breeding and revealed that the structure and shape of micropyle was affected by low temperature leading to less fertilization of eggs at the mid-altitudinal region. On the other hand, the micropyle and its microstructures in unfertilized eggs are important characters in gamete recognition and fish egg identification, therefore its morphology may be species specific (Ginsburg, 1968) (Kobayashi and Yamamoto, 1981) and (Chen *et al*., 2007).

Several workers have been reported on the studies of reproductive biology, histological analysis and ultra structure study of various fish species at different levels. However, a report on gonadal cycle and ultra-structural variations in the gonad development of *M. cuchia* is not available. Hence the present work was undertaken to find out the natural reproductive cycle of both male and female species of *M. cuchia* based on Gonado-Somatic Index (GSI) and histological sections. It is also very important to analyze and assess the breeding cycle of *M. cuchia* in order to make a successful breeding culture and practices. Therefore the present study describes the Gonado-Somatic Index (GSI), reproductive cycle, histological and ultra structural study of *M. cuchia* from Meghalaya with the objectives to determine the monthly variation in development of reproductive organs and its peak reproductive season which will eventually help in development of protocol for artificial breeding of the fish.

II. MATERIALS AND METHODS

(A). Histological analysis of gonads: The Fish specimens of *M. cuchia* were collected from different Districts of Meghalaya. The experiments were conducted in the month of January 2016 at the Department of Fishery Science, St. Anthony's College, Shillong (25° $34^{'}11^{"}$ N; 91° $51^{'}$ $21^{"}$ and altitude 1487 MSL) and repeated in the following year for confirmation of the result.

The freshly collected gonads samples were fixed in Bouin's- Allen's fixative (Gabe, 1976) and kept for overnight. Thereafter, the samples were washed with running water till yellow colour of Bouin's disappeared. The cleared samples were dehydrated with different grades of alcohol (30% to 100%) for fifteen minutes each and followed by two minutes treatment in xylene. The dehydrated samples were treated with xylene-wax for at least three hours in an oven and then embedded in pure paraffin wax (58ºC- 60ºC congealing point) overnight at 60ºC. After block preparation, the samples were sectioned at 8μm in compliance with accepted histological procedures (Luna, 1967) (Humason, 1972) (Gabe, 1976). The slides were then deparaffinised in xylene for about 30 minutes (time adjusted depending on necessity). The stretched sample sections were processed through grades of alcohol and stained with a regressive Harri's Hematoxylin and Eosin (H&E) stain (Luna, 1967). The stained slides were dipped in xylene and mounted with DPX. The preparations were then examined under motic high resolution microscope mounted with a camera.

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The Gonado-Somatic Index (GSI) was also calculated to compare the development of gonads along with histological study. The Gonado-Somatic Index (GSI) was calculated as follows:

GSI= Weight of the Gonad (g)/ Weight of the Fish (g) X 100.

(B). Ultra-structure analysis of gonads with Scanning Electron Microscopy (SEM): Experiment on Scanning Electron Microscopy (SEM) was conducted at the Electron Microscope Laboratory, Sophisticated Analytical Instrument Facility (SAIF), North-Eastern Hill University (NEHU), Shillong. For SEM specimens processing, glutaraldehyde was used as a fixatives so that the samples will not get decomposed. The samples were then washed with sodium cacodylate buffer and kept inside the refrigerator. For desiccation, different grades of acetone were used and after 95% acetone, dry acetone was used to dehydrate the samples. Dry acetone contains 99.8% acetone and it is the purest grade of acetone. Moreover, dry acetone contains copper sulphate and hence it is used to remove any traces of water. For further drying of the samples, Tetramethylsilane (TMS) was used. Tetramethylsilane has a very low boiling point and can evaporate or boil even at room temperature. Even though Tetramethylsilane evaporate the samples, but it will not affect the samples. Along with it, Tetramethylsilane will evaporate all the acetone present in the target samples. The detailed standard protocol for analyzing and assessing biological fixation includes the primary fixation in which gonads of *M. cuchia* were fixed in 2.5% glutaraldehyde prepared in 0.1 M sodium cacodylate buffer at pH 7.2 to 7.4 for 4 h at 4°C, washed in buffer overnight and then dehydrated with grades of acetone (i.e. 30%-100%). The dehydrate samples were dried by Tetramethylsilane (TMS) drying technique of (Dey *et* al., 1989). The dehydrated samples were immersed in Tetramethylsilane (TMS) at 4ºC for 10 minutes. A change for another 10 minutes in TMS at 4°C was followed. The samples from TMS were placed in a glass slide and dried at room temperature (25-26ºC). The samples were then secured horizontally to brass stub (10 mm x 12mm) with double coated adhesive tape connected via a patch of silver paint to ensure charge conduction. A conductive coating was applied to the sample using JFC 1100 (Jeol) ion sputter coater. A relatively low vacuum (10^{-3}tor) was established in the sputtering chamber, and gold was used as the "target" material. The preparations with scanning electron microscope (JEOL JSM-6360) using the secondary electron emission mode at an accelerating voltage of 15kv (SAIF, NEHU, Shillong).

III. RESULTS

(A). Gonado-Somatic Index and Histological analysis of gonads: The Gonado-Somatic Index of *M. cuchia* was calculated and analyzed to identify its peak breeding season for the fish. In males, highest GSI value of 3.4±0.8 was observed during the month of May and lowest value of 0.02±0.1 was observed during the month of September. Similarly in case of females, highest GSI value of 7.33±0.081 was observed during the month of May and the lowest GSI value of 0.27±0.089 was observed during the month of September. This indicates the maturity of the species which corresponding to the breeding period. Moreover, this study implies that highest GSI value in May is indicative of the fact that *M. cuchia* may have peak breeding season in May. This is as shown in Fig-1.

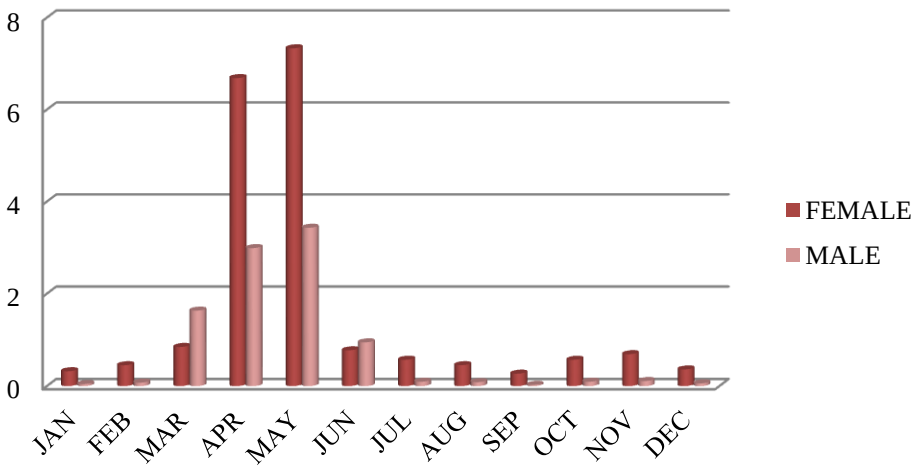

Fig-1: Monthly variation of Gonado-Somatic Index (GSI) of *M.cuchia*

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Analysis of microscopic structure of histological sections of *M.cuchia* gonads were performed and divided into four stages as follows:

Stages	Degree of maturity	Months of availability
I	Immature	Spent (Oct-Dec)
II	Developing	Pre-Breeding (Jan-Feb)
III	Mature	Breeding (Mar-Jun)
IV	Spent	Post-Breeding (Jul-Sep)

(a). Stage I- Immature (Oct-Dec): In the immature stage, small sizes of the gonads were observed in both male and female. In case of female, single lobe of ovaries was present and the colour of the ovaries changes from reddish brown to yellowish in colour. Photomicrograph of histological sections of ovaries shows the presence of good number of blood cells and it also revealed the presence of nucleoli in the pre-mature oocyte (Fig-2a). In case of testes, presence of two equal, very thin, narrow and long sperm ducts were observed and the colour of the testes changes from whitish to dull white in colour. The photo-micrographs of testes revealed that the testicular wall is thick and number of blood vessels present is comparatively less. It has been observed that in this stage, numerous seminiferous tubules are present (Fig-3a).

(b). Stage II- Developing (Jan-Feb): The analysis of the ovaries in the early maturing stage during the month of February reveals that the surface feature of the ovary is a thick-walled structure with 1.05 µm. In most of the developmental stage, the oocytes were observed to be attached to each other. Photomicrograph of the histological sections revealed that primary and secondary vitellogenic oocytes, cortical alveolar, as well as some atresia was also present (Fig-2b). In case of male gonads, development of spermatids was observed besides the presence of both primary and secondary spermatogonia. In testes, primary spermatocyte, secondary spermatocyte and spermatozoa were also found to be present (Fig-3b).

(c). Stage III- Mature (Mar-Jun): In the matured stage, ovary expanded and occupied the whole cavity. In histological sections of ovaries, nucleus was small in size with nucleoli and they began to migrate towards the periphery. Oocytes were enlarged in size and were fully compact with yolk granules (Fig-2c and 2d). The testes of the species during this stage were observed to be fully packed with matured spermatids ready to be released. Moreover, tubule diameter was observed to be very large filled with spermatids and spermatozoa. During the matured stage of testes, large numbers of blood vessels were also observed which indicates rich supply of blood (Fig-3c and 3d).

(d). Stage IV- Spent (Jul-Sep): In the spent stage, the ovaries were found to be flaccid (Fig-2e). Moreover, the ooplasm gradually shrunk and the gap between the oocyte membrane and nucleus appeared. Histological analysis also revealed that atresia, post-ovulatory follicle complex, cortical alveolar and vitellogenic oocytes were also present. Photomicrograph of histological section of the male gonads, observed that the testes were small and flaccid. Lumen of lobules irregular and very less sperm were observed in the lumen of the lobules i.e. empty lobules with some residual sperms (Fig-3e).

Figure-2: Histological stages of ovary of *M.cuchia*

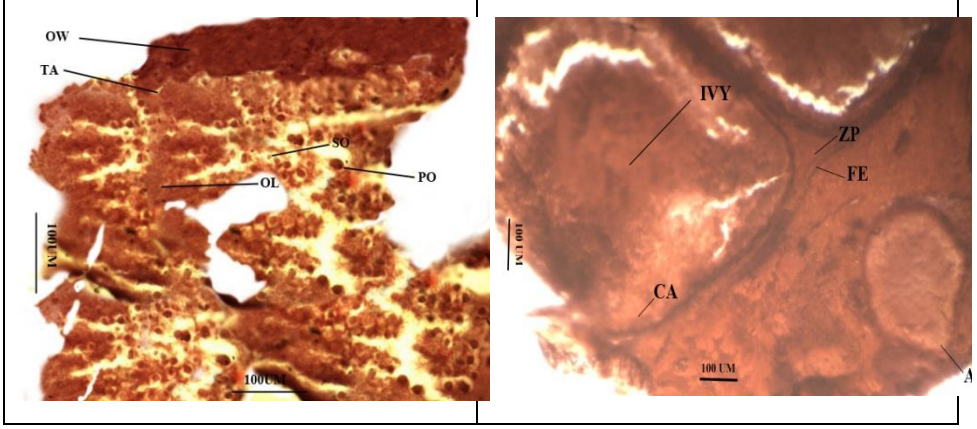

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Fig-2(a): Stage I (Immature) 40X	Fig-2(b): Stage II (Developing) 40X

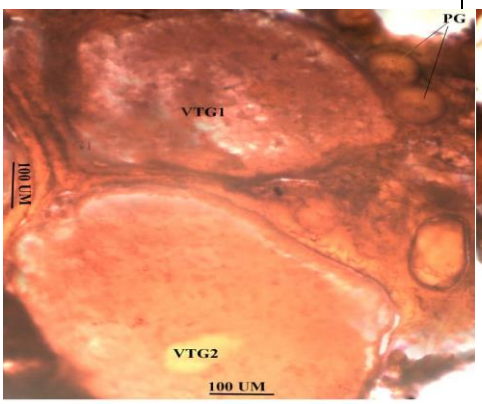
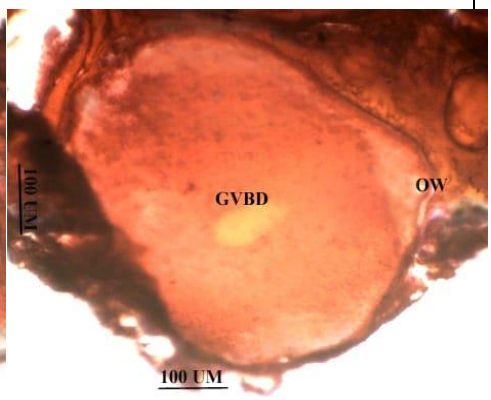

	

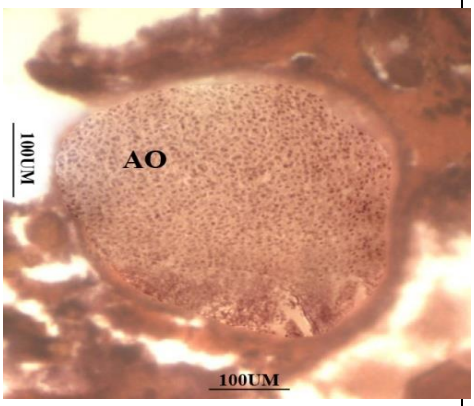
Fig-2(c): Stage III (Mature) 40X	**Fig-2 (d): Stage III (Mature) 40X**
	**Fig-2: PO**: Primary Oocytes, **SO**: Secondary Oocytes, **OW**: Ovarian Wall, **OL**: Ovarian Lamella, **TA**: Tunica Albuginea, **IVY**: Intra Vesicular Yolk, **FE**: Follicular Epithelium, **ZP**: Zona Pellucida, **CA**: Cortical Alveoli, **AT**: Atresia, **PG**: Primary Growth oocyte, **VTG1**: Primary Vitellogenic oocyte, **VTG2**: Secondary Vitellogenic oocyte, **GVBD**: Germinal Vesicle Breakdown, **AO**: Atresia Oocytes.
Fig-2 (e): Stage IV (Spent) 40X	

Figure-3: Histological stages of Testis of *M.cuchia*	

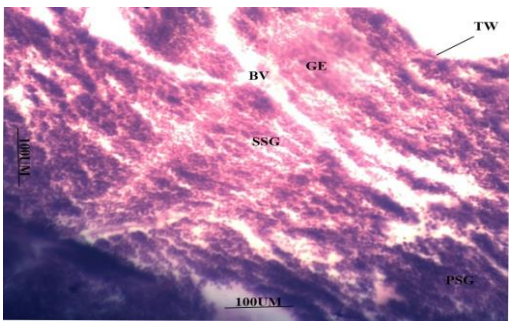
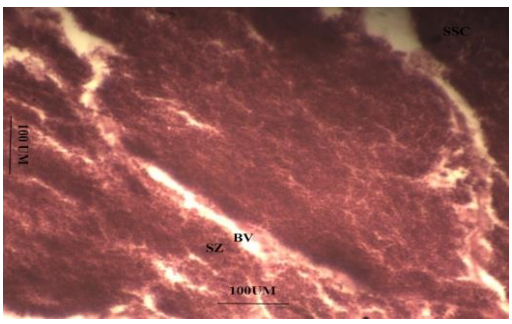

	

Fig-3(a): Stage I (Immature) 40X	**Fig-3(b): Stage II (Developing) 40X**

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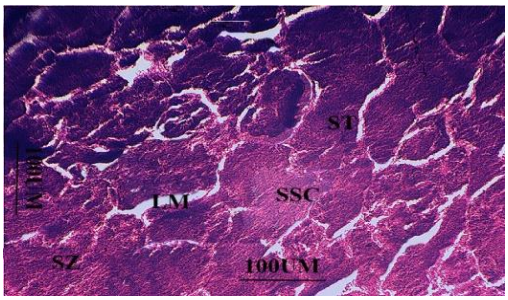
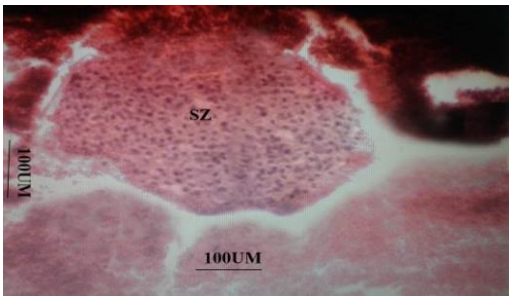
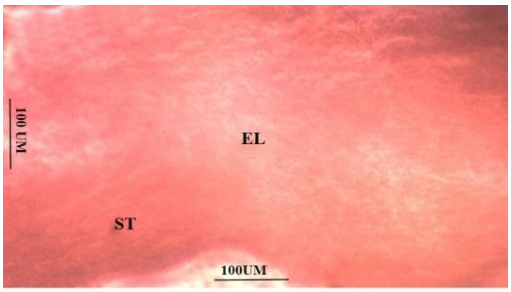
<tr><td> 
</td><td> 
</td></tr>
<tr><td>Fig-3(c): Stage III (Mature) 40X</td><td>Fig-3(d): Stage III(Mature) 40X</td></tr>
<tr><td> 
</td><td rowspan="2">Fig-3: BV: Blood Vessel, GE: Germinal Epithelium, TW: Testicular Wall, PSG: Primary Spermatogonia, SSG: Secondary Spermatogonia, SSC: Secondary Spermatocyte, SZ: Spermatozoa, LM: Lamellae, ST: Spermatids, EL: Empty Lumen.</td></tr>
<tr><td>Fig-3(e): Stage IV (Spent) 40X</td></tr>
</table>

(B). Ultra-structure analysis of gonads with Scanning Electron Microscopy (SEM): Ultra-structure study of the ovary (Fig-4) and testis (Fig-5) of *M.cuchia* have been done using Scanning Electron Microscope (SEM) and four stages of development were identified and compared with the corresponding stages of histological sections. The observations are as follows:

(a). Stage I- Immature (Oct-Dec): Normal folding of germinal epithelium could be distinctly differentiated in immature stage of ovary. Large number of blood vessels was seen and this indicates the good supply of nutrients for the future eggs. In the early stage of development, the immature female reproductive cells assemble and each primary oogonium arises from the germinal epithelium cells. The size of the germinal epithelium was found to be 0.80µm (Fig-4 (a).

(b). Stage II- Developing (Jan-Feb): Few yolk globules were seen in this stage and presence of undulation was also observed on the surface of the ovary. Sectional view revealed that primary oocytes were also seen but in some sections stages of secondary oocytes in the form of yolk vesicle were observed in the developing stage of ovary (Fig- 4 (b) and (c). It has been observed that primary and secondary oocytes were attached to each other. Moreover, the egg surface feature was found to be smooth and no honey-comb structure was observed in this stage. The size of the primary oocyte was found to be 4.33µm and the secondary oocyte size was 5.88µm.

(c). Stage III- Mature (Mar-Jun): During this stage, the size of matured ova was found to be 1.78µm and the ovigerous lamellae were observed to attach to the ova. Moreover, the fully matured fertilized ova were covered with a thick membrane (Fig-4d). Distinct undulations throughout the surface of the ova were observed. Unlike most fishes the micropyle was found to be open but in this case matured ovary with closed micropyle was observed and this indicates that the egg was already been fertilized (Fig-4e). Micropyle was not totally circular when compared with other fishes and the width of the pit was 1.40µm.

(d). Stage IV- Spent (Jul-Sep): Ovary starts to degenerate and become flaccid and the unreleased oocytes gradually absorbed in the germinal epithelium. Shrinkage of ooplasm and follicle layer was also observed (Fig-4f).

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Figure-4: Photomicrograph of ovary of *M.cuchia*

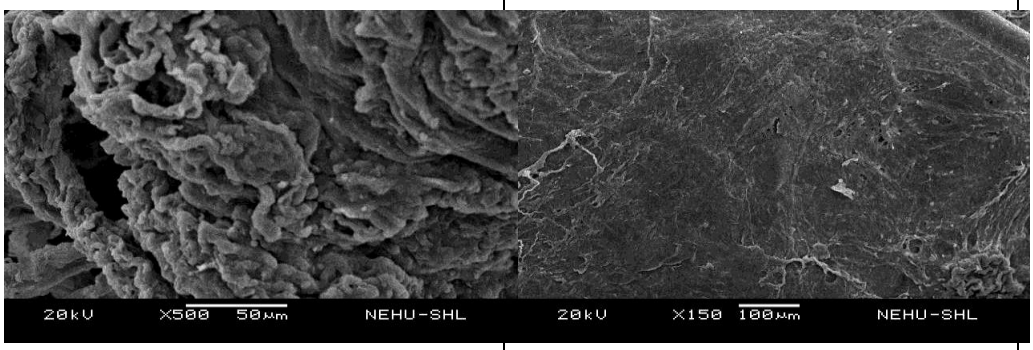

Fig-4 (a): Folding of germinal epithelium in immature ovary (Bar=50µm)

Fig-4 (b): Surface feature of the ovary in immature stage (Bar=100µm)

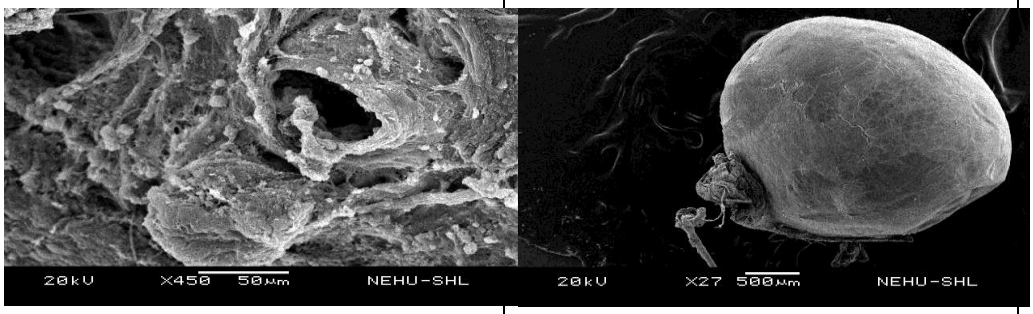

Fig-4 (c): Primary and Secondary oocytes were observed at the developing stage of ovary (Bar=50µm)

Fig-4 (d): Fully developed fertilized egg (Bar=500µm)

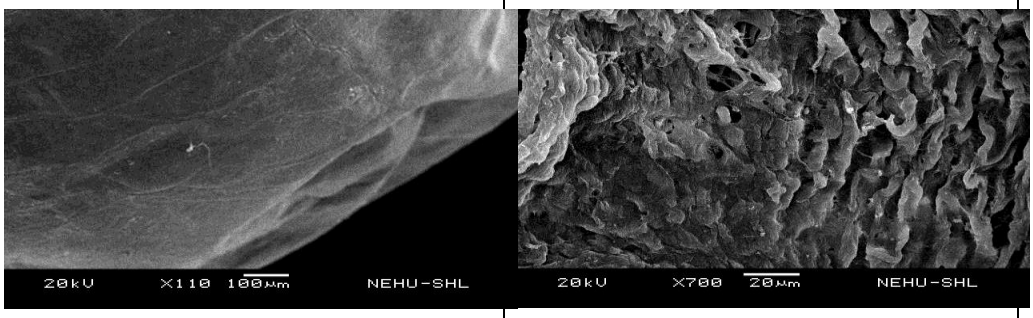

Fig-4 (e): Matured ovary with closed micropyle (Bar=100µm)

Fig-4 (f): Follicles of germinal epithelium during spent stage (Bar=20µm)

Scanning Electron Microscope (SEM) analysis of testis was done and compared with corresponding histological sections to confirm the developmental stages. The findings are depicted below:

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(a). Stage I- Immature (Oct-Dec): Spermatogonia, spermatocyte and normal folding of germinal epithelium with size of 2.04µm can be seen in the immature stage of testis. The size of spermatocyte was found to be 5.26µm and spermatogonia size was 8.33µm and this is as shown in Fig-5(a). In this case it was observed that there was a large folding of testicular wall which acts as a substratum for future sperm mother cells.

(b). Stage II- Developing (Jan-Feb): The size of the germinal epithelium was found to be 1.99µm. In this stage it was observed that the germinal epithelium showing developed sperm mother cells with size of 2.56µm. Moreover, large number of blood vessels was also seen .Spermatocytes as well as spermatogonia was also present in this stage and the range of spermatogonia was 2.35-5.00µm. Moreover, undulations are also developed in the germinal epithelium and this is as shown in Fig-5(b, c and d).

(c). Stage III- Mature (Mar-Jun): Spermatozoa and / or spermatids are dominant. In this stage, Spermiation with an average size of 1.75µm started and formation of sperms (size=1.78µm) was also observed in matured stage of testis. During this stage, spermatogonia increased in number and it undergone spermiation leading to transformation into matured sperm. During this maturation process, blood supply also increases (Fig-5e and 5f).

(d): Stage IV- Spent (Jul-Sep): Testis is dominated by spermatozoa. Testis at the spent stage revealed shrinkage along the side of the germinal epithelium (size=2.04µm). Moreover, empty pockets or lobules were observed from where sperms are released. Numbers of blood vessels are reduced and the folding of germinal epithelium slowly becomes smooth. Remaining sperms are gradually absorbed in the testicular membrane (Fig-5g).

Figure-5: Photomicrograph of testis of *M.cuchia*

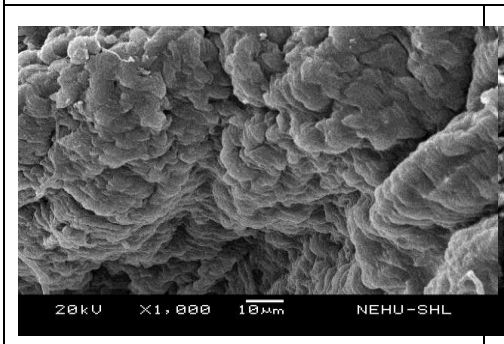

Fig-5 (a): Folding of germinal epithelium in immature stage of testis (Bar=10µm)

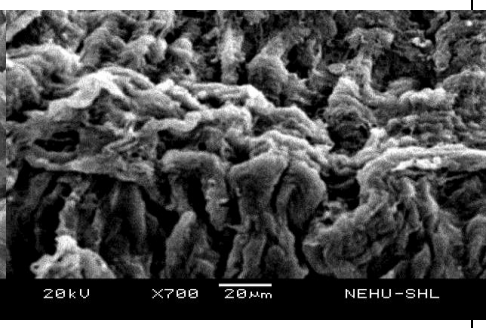

Fig-5 (b): Developed sperm mother cells (Bar=20µm)

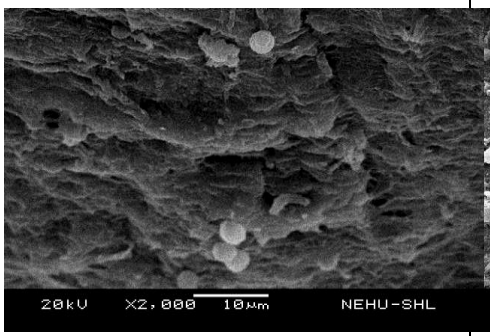

Fig-5 (c): Movements developed in the germinal epithelium (Bar=10µm)

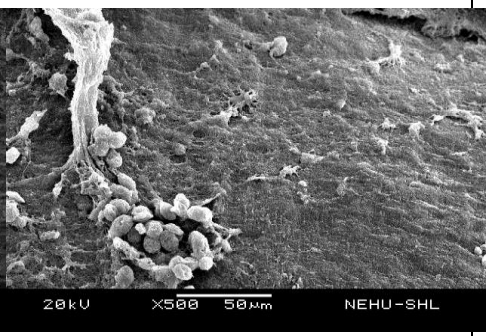

Fig-5 (d): Spermatogonia were observed in the developing stage (Bar=50µm)

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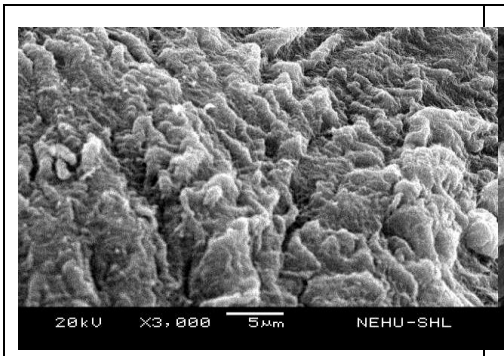

Fig-5 (e): Spermiation started (Bar=5µm)

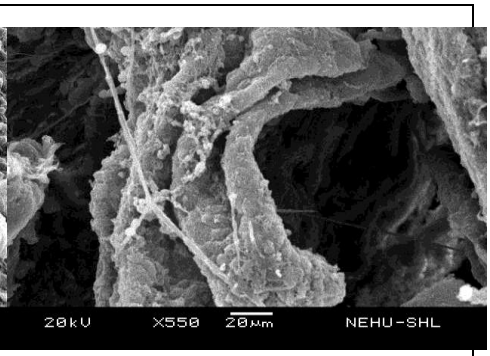

Fig-5 (f): Sperms formation observed in matured stage of testis (Bar=20µm)

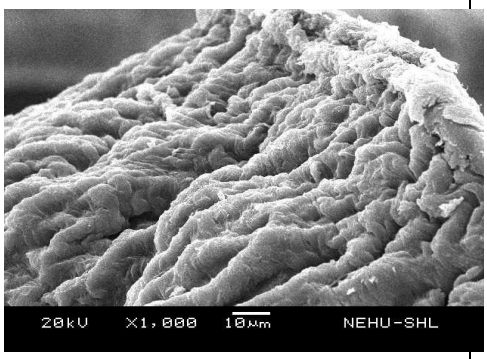

Fig-5 (g): Shrinkage along the side of the germinal epithelium in spent stage (Bar=10µm)

IV. DISCUSSION

Monopterus cuchia is a fresh water fish with a snake-like appearance. Gonado-Somatic Index (GSI) is one of the most important parameter to determine the different stages of maturity in fishes. Monthly variation of GSI reflects the ovarian activity in fishes. (De Vlaming *et al*., 1982) discussed the utility of Gonado-Somatic Index as an indicator of the reproductive activity of a stock. During this period of analysis it was observed that in males, highest GSI value of 3.4±0.8 was observed during the month of May and lowest value of 0.02±0.1 was observed during the month of September. Similarly in case of females, highest GSI value of 7.33±0.081 was observed during the month of May and the lowest GSI value of 0.27±0.089 was observed during the month of September. Moreover, mean GSI of *M. cuchia* was found to increase as the fish reaches the maturity stage and it decreases gradually after the spawning stage. The peak breeding season for the species lies in the month of May and this species breeds only once a year. Highest Gonado-Somatic Index value of *M. cuchia* suggests that the ovary harbours percentage of yolk laden ripe eggs in May which is more or less similar to (Dewan, 1973) (Chakraborty, 2010) (Chakraborty *et al*., 2007). They reported that the spawning period of *Chela phulo*, *Puntius sarana* and *Ompok pabda* lies between June and September. Reproductive potential of a population is one of the basic exigencies to designate the individuals of that population in respect to their gonadal conditions (Jhingran and Verma, 1972).

The paper represents a detailed histological and ultra structural study of both ovary and testes, of *M. cuchia* at mid-altitude region of Meghalaya showing various developmental stages. Histology is the scientific analysis of the microscopic structure of biological tissues. The main aim of histology is to analyze tissues structure and this will enable us to understand their physiological and anatomical functions. The usefulness and importance of histological techniques in reproductive studies have been widely illustrated for fish species (West, 1990) (Tyler and Sumpter, 1996) (Blazer, 2002).

Histology offers a powerful tool for reproductive studies and is routinely used for sex verification, assessment of reproductive phase, or quantification of atresia (Blazer, 2002). Histology is particularly crucial for confirming the sexual pattern in hermaphroditic fishes. This analysis provides new information on histological analysis of maturation of gametes showing different developmental stages of both male and female species of *M. cuchia* in Meghalaya. In fishes, the egg mother cells undergo several changes till it finally becomes matured which will be released outside the body during the period of spawning. In the present analysis, the oocyte development in case of *M. cuchia* was divided into four stages.

During this analysis it was observed that the ovaries and testes showed morphologic changes in response to variation of either of the two environmental cues like temperature. A similar case was also reported by (Bhuyan, 2008). Several authors have reported different developmental stages of oocytes in different fishes (Khanna, 1970) (Groman, 1982) (Agarwal, 1996). This pattern of sexual maturation also influences the somatic growth pattern in fishes. Differences in growth were explained by differences in the timing of the spawning and the relative spawning investment (Hansen *et al*., 2001). Moreover, morphological changes in developing oocytes were observed in different stages of maturity including changes in gonad size and oocyte (Grau *et al*., 2009) (Lubzens *et al*., 2010) (Mohamed, 2010). Primary growth phase of oocytes is characterized by the nucleus undergoing major transformations such as an increase in size and the formation of multiple nucleoli, which generate large quantities of ribosomal RNA (Takashi, 1982) (Bhuyan, 2008). Moreover, in the breeding stage the membrane surrounding the nucleus disappeared in a process called germinal vesicle breakdown. The cortical alveoli were pushed to the membrane of the oocytes. The cortical alveoli play an important role in preventing polyspermy in fish (Guraya, 1982).

The gonad development as well as gametes maturation of *M. cuchia* in Meghalaya was similar in case of *Monopterus albus* (Mei *et al*., 1993) and similar case was also reported by (Miah *et al*., 2013) on the analysis of molecular identification and sexual differentiation of fresh water mud eel *M. cuchia* in Bangladesh. Moreover, the stages of oocyte development of *M. cuchia* are similar to that of the analysis of gold fish by (Yamamoto and Onozat, 1965), white fish *Caulolatilus princes* (Elorduy-Garay and Ramirez-Luna, 1994), *Puntius gonionotus* (Afroz, 1996), *Puntius sarana* and *Ompok pabda* and somewhat similar to that of *Pleuronectes flesus* investigated by (Janseen *et al*., 1995). In *M. cuchia*, the ovaries contained oocytes only in early developmental stages and are small in size which mostly consists of oogonia, early and late perinucleolus stage, cortical alveoli and yolk granule stage. During breeding season, female *M. cuchia* possessed gonads that contained exclusively vitellogenic oocytes. Similar case were also reported by (Chakraborty, 2018) and similar sequence of oogenesis was also noted in *Amblypharyngodon mola* and *Chela phulo* (Dewan, 1973), in case of *Puntius sarana* (Chakraborty *et al*., 2007) and *Ompok pabda* (Chakraborty *et al*., 2010). Spawning was observed from May to June as indicated by the presence of an appreciable number of female berried conditions. May to June formed the major spawning period as evidence by the presence of the maximum number of berried and spent fishes in these months. Moreover, the oocytes in *M. cuchia* did not mature at the same time. Some of fishes became fully matured; on the other hand, other remained under developing condition. The developing oocyte remained under way of vitellogenesis and gained maturation, and released which supported by the findings of (Chakraborty, 2018), (Mustafa, 1991) and (Hora and Pillay, 1962) with mola, *Amblypharyngodon mola*, Rohu, *Labeo rohita* and catla, *Catla catla.*

Maturity stages of testes were divided into four stages. Testis consists of two equal, very thin, narrow and long sperm ducts and the colour changes from whitish to dull white in colour. Testis has the germinal epithelium and the interstitial cells which are separated from each other by a basement membrane (Lo Nostro *et al*., 2003). According to (Bucholz *et al*., 1964), mature spermatozoa are seen aggregated in the lumen of testicular lobules without any regular arrangement, they begin to aggregate in the lumina even at the early stage when many of the germ cells still remain immature and do not reveal any connection with the intra-lobular somatic cell elements or sertoli cells. Moreover, it has been noted that the rhythm of gonadal development depends on various factors such as temperature, photoperiod, along with social and behavioral factors such as visual, olfactory and auditory stimuli (Esmaeili *et al*., 2010) (Asadollah *et al*., 2011) (Keivany *et al*., 2012) (Abaszadeh *et al*., 2013) (Dopeikar *et al*., 2015).

In this study, it was observed that testicular development of *M. cuchia* usually occurs earlier than ovarian development. Similar case was also reported by (Chakraborty, 2018). But testicular development of salmononids occurs later than ovarian development (Nakamura *et al*., 1998) (Guraya, 1994). Thus the results of the present analysis can be stated that the male breeding period of *M. cuchia* varies from April to August. In September, all the individuals were found to be spent. It can also be presumed that the period from September to January is the resting period for male individuals (Chakraborty, 2018). Temperature is closely correlated to mature testis which is also agreed by (Khan and Jhingran, 1975)

during their analysis on male Rohu, *L.rohita*. Similar cases were also reported that *M. cuchia* normally breeds from late April to early July (Chakraborty, 2008), (Chakraborty, 2010) and (Chakraborty *et al*., 2013) who reported that the breeding season of spiny Guchi, *Macrognathus pancalus* and *Monopterus cuchia* starts from late April to early July with peak in May. Therefore histological observation of gonads was done to ascertain the actual pattern of maturation of gonads and also to find out its breeding season.

Moreover, Scanning Electron Microscopy (SEM) study revealed similar stages of the gonads development and this corresponds with the histological study which revealed the presence of large number of blood vessels in immature stage and this indicates a gradual increased in blood supply. In case of testis, less number of blood vessels was observed in the immature stage. In developmental stage, histological analysis revealed the presence of oocytes which are attached to each other. In case of SEM, it showed the presence of primary and secondary oocytes along with few yolk globules. In males, both the SEM and histological study revealed the presence of spermatocyte and spermatogonia. During mature stage, histological analysis revealed that oocytes were enlarged in size. Whereas in SEM study it was observed that the surface features of oocytes was marked by undulations. Presence of closed micropyle indicates that the egg was already been fertilized. In case of testis, large number of blood vessels was observed which indicates an increase supply of blood. The histological and Scanning Electron Microscope study of both male and female gonads in the spent stage revealed that the ovary and testis becomes small and flaccid.

V. CONCLUSION

Results of the present analysis revealed a clear cut four maturity stages in the gonad development of *M. cuchia*. Highest Gonado-Somatic Index value in the month of May indicates the fact that *M. cuchia* breeds during the month of May. Since no report was available regarding the histological and ultra structural analysis of gonad development of *M. cuchia*. Hence, this paper gives some insight about the histological and ultra structural study of *M. cuchia* gonads at mid-altitude region of Meghalaya. Therefore this study will contribute valuable knowledge needed for fisheries management and aquaculture of the species by increasing the knowledge of reproductive biology of this species. Further investigation is required for fine tuning of induced breeding protocol for large scale seed production and culture of this species under captivity as a measure for conservation and sustainable production.

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