6.1. Introduction

Studies on reproductive biology comes first and foremost in fish biological studies and it involves the study of oogenesis, size at first maturity, spawning frequency, fecundity, sexual dimorphism, and early embryonic development. A complete knowledge on reproductive system and biology of fishes are critical to understand the reproductive strategy and annual reproductive cycle of any fish species (Unver & Unver Saraydin, 2004). Reproductive strategies of fish are extremely varied such as typical male or female patterns; self-fertilizing hermaphrodites and gynogenesis; seasonal and continuous breeders; single or multiple spawners; livebearers and fish that naturally undergo sex reversal (Breder & Rosen, 1966; Balon, 1975; Blazer, 2002). Therefore, a detailed study on the
biological features of the threatened species will be very valuable in implementing any programme on conservation of the fish genetic resources (Virjenhock, 1998).

The identification of different stages of gonadal development in fishes is considered a vital part in reproductive studies (West, 1990) and its development can be monitored on the basis of macroscopic and microscopic appearance of gonad (Wang et al. 2003). The gonad of fishes differs largely intra-specifically and inter-specifically depending on many factors including their morphology, anatomy and environmental conditions (Rahemo & Al-Shatter, 2012). The usefulness and importance of histology techniques in reproductive studies have been widely illustrated for fish species (Tyler & Sumpter 1996; Blazer, 2002). The importance of histological description of gametogenesis was emphasised by Booth & Weyl (2000) and Rutaisire (2003), who noted that macroscopic staging must be validated, if errors in the estimation of maturity and seasonality are to be minimised. According to West (1990), histological studies provide precise information on oocyte development, but are unfortunately slow to undertake and also expensive because it involves complex laboratory techniques. Even though, histological analysis of gonadal development is considered as the most accurate method to determine the reproductive pattern in fishes (Wallace & Selman, 1981; West, 1990). Histological studies provide very accurate information on oocyte developmental stages, but their interpretation is confusing because different authors use different terms for the same stage (Booth & Weyl, 2000).

Oocyte developmental stages have been extensively described in numerous species with some differences depending on the species and the classification criteria. Oogenesis stages have been extensively described in numerous freshwater fish species. Earlier general structure and morphology of ovary has been studied by Yamamoto (1956) on Liopsetta obscura; Yamamoto & Yamazaki (1961) on Carassius auratus; Hoar (1969); Siddique et al. (1976) on Labeo gonius; Nagahama
(1983); Guraya (1986); Kobayashi et al. (1988); West (1990); Degani & Boker (1992); Selman et al. (1993) on Brachydanio rerio; Stoumboudi et al. (1993) on Barbus longiceps; Kurup (1994) on Labeo dussumieri; Palmer et al. (1995); Encina & Granado-Lorencio (1997) on Barbus scdater; Coward & Bromage (1998); Maddock & Burton (1999); Merson et al. (2000); Arocha (2002); Guiet al. (2003) on Carassius auratusgibelio; Cek et al. (2001) on Puntius conchonious; Smith & Walker (2004); Selman & Wallace (2005) on Fundulus heteroclitus; Euphrasia (2004) in Osteobrama bleekeri; Sunesh Thamby (2009) in Garra surendranadhinii; Lakshmi (2009) in Horabagrus brachysoma; Teji (2010) in Amplypharyngodon meletinus; Augustine et al. (2013) in Pethia pooladensis and Indira et al. (2014) in Chela fasciata. Gonadal maturation in Sahyadria denisonii has not been studied at morphological and histological level, and their sexual maturation is still poorly understood. Previous assessments were based exclusively on macroscopic observations (Radhakrishnan & Kurup, 2008; Solomon et al. 2012; Mercy et al. 2013), they analysed seasonal gonad variations only in terms of macroscopic examination (gonado-somatic index). In the present study to understand the reproductive mechanism in S. denisonii, macroscopic as well as microscopic investigations were carried out.

6.2 Materials and Methods

6.2.1. Stages of Maturity Observation- Morphological

Gonads were taken out and their length and weight were noted down. Stages of maturity of gonads were determined on the basis of morphological appearance based on macroscopic as well as microscopic observations, by following the standards laid down by international council for exploration of sea (Lovern & Wood, 1937). After assessing the stage of maturation, the ovary was preserved in 4% formalin for further reproductive studies. Quantification of maturity stages was done following morphological characteristics of the gonad such as appearance, colour, degree of distension, relative space occupied in the body cavity and histological
observations such as the size of the ova and their yolk content (Mc Bride et al. 2002). Based on the scheme proposed by El-Boray & El-Gharabawy (1999) was used to classify ovarian maturity stages. Atretic oocytes were classified according to Lambert (1970) and Hunter & Macevicz (1985) and post-ovulatory follicles were followed by Elorduy & Ramirez (1994).

6.2.2. Light Microscopy Observation- Histological

For the histological studies, fresh tissue of testis were used, small pieces of the testis at different stages of maturity were fixed in Bouin’s fluid. The bouin’s fixed tissue was later processed with dehydration schedule and embedded in paraffin, cut into 5 m serial sections and stained with haematoxylin-eosin by following standard procedures (Euphrasia, 2006; Sunesh Thamby, 2009). Testicular development were examined and photographed with a stereo microscope- LAS EZ (Leica Application Suite).

6.2.3. Scanning Electron Microscopy Observation- Ovum

Fresh samples of mature ova were pre-fixed with 1% glutaraldehyde and 3% paraformaldehyde in a buffer of 0.1M phosphate buffer (pH 7.3) for 24 hours and then were post-fixed with 1% osmium tetroxide in same buffer for 2 hours. After dehydrating in an ethanol series, critical point drying, and coating with gold, samples were examined and electro-micro graphed with Scanning Electron Microscope (JEOL Model JSM-6390LV).

6.3. Results

6.3.1. Female Reproductive System in Sahyadria denisonii

The female reproductive system of S. denisonii is characterised by cystovarian type with a pair of ovaries lying suspended from the sides of the body cavity by mesovarium below the air bladder (Image. 6.1 & Image. 6.2). The two
lobes of each ovary were elongated, were connected along their dorsal surface by mesentery, from which they were suspended in the abdominal cavity. The ovary has a broad anterior part, which tapered towards posteriorly and its morphology varied in accordance with the size, age and maturation stage of fish. The two ducts extending from the posterior ends of ovary united to form a common oviduct leading to the urinogenital pore.

**Image 6.1.** General structure of ovarian system in *Sahyadria denisonii*

**Image 6.2.** Histological section (T.S) of *Sahyadria denisonii* showing position of ovarian system
6.3.2. Morpho-Histological Examination of Ovary

The ovary of *S. denisonii* was bi-lobed occupying a large part of the abdominal cavity; the middle portion of the ovary was broader than the anterior and posterior region. Eggs were golden yellow colour in the ripe of ovary and it has been found that the shape, size, and colour varied with different stages of maturity. In the present study, eight maturation stages were identified in *S. denisonii* following El-Boray & El-Gharabawy (1999) and maturation stages observed as, Stage-I (Immature virgin); Stage-II (Early developing); Stage-III (Late developing); Stage-IV (Mature); Stage-V (Ripe); Stage-VI (Spawning); Stage-VII (spent) and Stage-VIII (Developing recovery).

**Stage-I: Immature virgin stage** (Image.6.3a): These are the first stage that has not yet spawned found in young individual. Differentiation of the gonads had just taken place, small, thread like and ova were not visible by naked eye. A detailed examination under the microscope was required, to differentiate ovary from testis. All the ova in this stage were irregular in shape and transparent with a nucleus.

**Stage-II: Early developing stage** (Image.6.3b): In this stage, ovary usually pale white in colour, was translucent occupying about ¼ of the body cavity and the two lobes were equal in length. The ova were visible by naked eye.

**Stage-III: Late developing stage** (Image.6.3c): Ovary was pale yellow in colour, occupying ¾ of the body cavity. The ovary was turgid in nature; ova being visible through the extremely thin ovarian wall.

**Stage-IV: Mature stage** (Image.6.3d): The ovaries almost extended the entire length of the body cavity, lobes were equal in length. Ova were larger, densely yolk-laden and were not free within the ovary.

**Stage-V: Ripe stage** (Image.6.3e): Ovary was in yellow colour and filled the entire length of body cavity. Ovarian wall was thin, ova quite distinct, spherical in shape.
and opaque due to huge amount of yolk present. Ovarian lobes were quite stout and equal in length. With slightest pressure on the abdomen the ova started oozing out, the ovary was now ripe and ready for spawning.

**Stage-VI: Spawning stage** (Image. 6.3f): Ovary at this stage was slightly flaccid, pale yellowish in hue not as firm as the ripe ovaries but still retaining a number of residual ripe ova/atretic ova after the spawning.

![Image 6.3. Different maturity stages in Sahyadria denisonii](Image)

**Stage-VII: Spent stage** (Image.6.3g): The ovary at this stage was much shrunk, blood-shot, occupying ¼ or more of the body cavity and it contained few larger and numerous small eggs. Presence of atretic oocytes distinguished spent ovary from ovaries of early maturity stages.

**Stage-VIII: Developing recovery stage** (Image.6.3h): Ovary usually off white/creamy in colour, occupying less than ½ of the body cavity. This stage may also be called as
spent recovering stage for fishes that have at least spawned once. Ovary was usually with transparent ovarian wall and ova quite visible to the naked eye.

6.3.3. Light Microscopy Examination- Oogenesis

The process of oocyte development in *S. denisonii* followed the same basic progression as that described for other species. Ten separate oocyte developmental stages were identified, and each oogonium in *S. denisonii* passed through ten stages to form a ripe ovum (Image. 6.4). These oocyte developmental stages includes,

I. Oogonia  
II. Chromatin nucleolus  
III. Early perinucleolus  
IV. Late perinucleolus  
V. Early yolk vesicle  
VI. Late yolk vesicle  
VII. Early yolk globule  
VIII. Late yolk globule  
IX. Migratory nucleus  
X. Ripe ova

![Image 6.4. Oogenesis stages in Sahyadria denisonii](image)

1. **Oogonia stage (Image 6.5.I):** Oogonia are the smallest of stock of oocytes, small round cells characterized by a single conspicuous nucleolus in the nucleus. Oogonia have proliferated and produced the oocytes and their growth period varied from species to species is initiated mainly by the accumulation of yolk. Oogonia cells were seen as batches, situated deep within the germinal epithelium and were present in the ovary throughout the year.

2. **Chromatin nucleolus stage (Image 6.5.II):** Chromatin nucleolus stage is characterized by a visible nucleolus and chromatin appeared in the form of a few
thick filaments within the nucleus. Two or more darkly staining nucleoli were observed adhering to the nuclear wall.

**Image. 6.5.** Oocyte developmental stages in *Sahyadria denisonii*

3. **Early perinucleolus stage (Image 6.5.III):** The oocytes appeared as larger than chromatin nucleolus stage, more spherical due to accumulation of more cytoplasm. Nucleoli were found mainly scattered in the chromatin meshwork. The chromatin material was found to show less staining affinity and a fewer seen to fuse together leading to the formation of the nucleoli, while the existing nucleoli and nucleolus has started to move towards periphery. *Yolk nucleus* or *Balbiani bodies* appears first as a small spherical body in close to one side of the nucleus and then migrates to the periphery of the cytoplasm, where it finally disintegrates and disappears.

4. **Late perinucleolus stage (Image. 6.5.IV):** More nucleoli moved to the periphery of the nucleus in the late perinucleolus stage, some were still in the centre of the
nucleus. Late perinucleolus oocytes became more spherical in shape. Oocyte cytoplasm also became less basophilic and gradually loses its good affinity to haematoxylin. A small number of yolk vesicles or cortical alveoli were visible in the late perinucleolus stage.

5. **Early yolk vesicle (Cortical alveoli) stage (Image. 6.5.V):** The size of the oocytes increased compared with that of previous stages. In this stage, cytoplasm was slightly basophilic, turns reddish with eosin. The nucleoli were distributed adjacent to nuclear membrane and nucleolar bits were deeply basophilic, while chromatin material remained poorly basophilic. The major identity of this stage was the first appearance of yolk vesicles or cortical alveoli adjacent to periphery of the cytoplasm. A well-developed cell layer is now visible around the oocyte.

6. **Late yolk vesicle (Cortical alveoli) stage (Image. 6.6.VI):** In this stage, the yolk vesicles increased in size and number. The size of the oocytes increases compared with that of early yolk vesicle stage. The nuclear walls became undulating in outline margin and the nucleoli become reduced in size and number. The outer membrane of the oocytes became thicker in this stage and composed of a non-cellular inner layer called *zona radiate*, surrounded by *zona granuloza* and a thin external layer, *thecal* cells as shown in Image 6.6.VI.D.

7. **Early yolk globule stage (Image. 6.6.VII):** In this stage yolk vesicles in the cytoplasm united to form yolk globules. Appearance of yolk in the form of minute granules can be frequently observed and were seen in the peripheral region. Oocytes began to loose their characteristic shape but were mostly oval or spherical. The germinal vesicle was oval or elliptical in shape and central in position with an irregular, wavy nuclear membrane. The nucleoli and the nuclear membrane were deeply basophilic, while the chromatin material was faintly basophilic.
8. **Late yolk globule stage (Image. 6.6.VIII):** This stage is characterised by the extensive deposition of the yolk globules in the whole of ooplasm and oocytes were still irregular in shape on account of the pressure from the adjacent oocytes. The yolk globules were greatly increased in number and size, which almost completely filled the cytoplasm. The size of the nucleus is very much reduced at this stage. The yolk formation in this stage began with the accumulation of yolk globules in the periphery of the oocytes below the vitelline membrane. At the final stage of vitellogenesis, some globules fused to form larger ones. The oocyte is surrounded by well-developed innermost **zona radiata**, middle **follicular epithelium** and the outermost **thecal** layer.

![Image. 6.6. Oocyte developmental stages in Sahyadria denisonii](image)

9. **Migratory nucleus stage: (Image. 6.6.IX):** The distinguishing feature of migratory nucleus stage was the peripheral migration of the germinal vesicle and the liquefaction of nucleolar material. This is accompanied by reduction in size of
nucleus and gradual dissolution of nuclear membrane. The yolk globules almost completely filled the cytoplasm.

10. Ripe egg stage (Image. 6.6.X): The oocyte was now mostly spherical or oval in shape and covered by a fine outer sheath in this stage. The yolk vesicles were few and scattered; their interspaces were filled with yolk globules, and granules. Maturation is completed by the peripheral migration and dissolution of the germinal vesicle (GVBD), thus nucleus became invisible at this stage. The nucleus moved towards the micropyle region in animal pole and when the nucleus reached micropyle, nucleus started to disappear which is called germinal vesicle break down (GVBD). When ovulation takes place, follicular membranes rupture leading to the release of ripe ova into the ovarian lumen for spawning.

General pattern of oogenesis in *S. denisonii* conformed to that of most other teleost. Analysis of histology of ovary in *S. denisonii* revealed that, there are ten stages of ova development in *S. denisonii* (Image. 6.4), the first four stages of oocytes were small, non yolky in nature (Image. 6.5. I to IV) while, next six oocyte stages were yolky (Image. 6.5. V to X). The frequency distribution of ova in different stages of gonads indicated that, size range of mature ova being nearly half the range of total size of entire intra-ovarian eggs (see chapter section 4.3) was indicative of extended period with fractional spawning characteristics. According to De Vlaming (1983) ‘*fractional spawning*’ is used for species that spawn part of an ovulated clutch, while ‘*multiple spawning*’ generally refers to more than one spawning in a season. The ova diameter ranged from 32 to 224µm in the immature stocks. The ripe stock had ova diameter range of 928-1440µm. The maximum diameter of ova recorded during the present study in the ova class of 1440-1504µm. There was always the presence of large percentage of immature stock and a distinguishable stock of nearly half the range of total size ripe ova in the ovary (Image 6.9). This type of oocyte distribution corroborates with category-B of
spawning frequency classification by Prabhu (1956). The relatively high proportion of reserve oocytes in the ovaries of barbs has also been noted earlier (Al-Daham & Bhatti, 1979). This ova-stock distribution is indicative of seasonal with extended spawning period.

6.3.4. Atretic Stages

Atresia or degeneration of oocytes is a common event in the fish ovary. Following spawning a small number of post-vitellogenic oocytes fail to undergo maturation or ovulation, these subsequently degenerate, become atretic. In *S. denisonii* atresia affects the vitellogenic and matured oocytes (Images 6.7). Follicular layer of oocytes gradually lost their compactness and strength and became thin considerably and the oocyte is also invaded by phagocytic cells.

6.3.5. Post-ovulatory Follicles

The follicular membrane remains in the ovary after the discharge of ova, collapsed due to the mechanical pressure. This structure is referred as the
postovulatory follicles, discharge follicles, ovulation scar or corpus luteum of ovulation. In *S. denisonii*, these structures were observed during period of spawning and spent phase. Postovulatory follicles in *S. denisonii* had a wide irregular lumen, surrounded by hypertrophied follicular cells and a theca (Image 6.8).

**Image 6.7.** Different types of atretic stages in *Sahyadria denisonii*

**Image 6.8.** Postovulatory stages observed in *Sahyadria denisonii*
6.3.6. Scanning Electron Microscopy- Ovum

The unfertilized mature ova of *S. denisonii* were circular in shape (Image. 6.10a) have an average diameter of 1220µm. The surface of the *zona radiate* was wavy, uneven with a uniform distribution of almost round pores with a uniform distribution of polygonal protuberances (Image. 6.10b). In the present study, it was found that the unfertilized eggs of the *S. denisonii* possessed only one micropyle at the animal polar region (Image. 6.10c). The micropyle region was not flat; it was circular or oval in shape and belonged to type III micropyle (Image. 6.10d). The Ovum surfaces did not have any special attachment structures (microvilli) (Image. 6.11) and whose eggs appeared completely smooth. The outer opening of the micropyle canal have circular outline.

![Image 6.10](image-url)

**Image 6.10.** Scanning electron microscopy of egg surface structures of *Sahyadria denisonii*: whole view of egg (a-c); Micropylar region (d); higher magnification of egg surface (e); higher magnification of micropyle region(f).
Image 6.11. Scanning electron microscopy of egg surface structures of *Sahyadria denisonii*: Higher magnification of ova surface (a-c); higher magnification of surface showing round or oval accessory pores without microvilli (d) Regular distribution of round pores on the egg surface (e-f).

6.4. Discussion

In the present study, histological analysis is being used to describe the reproductive pattern of *S. denisonii*, an important targeted indigenous freshwater species for ornamental fisheries. Studies of gonad morphology are important for understanding the species reproductive biology (Martins *et al.* 2012). Generally teleost display two types of ovaries - gymnovarians and cystovarians (Helfman *et al.*
The cystovarian type in which the ovarian lumen is continuous with the gonoduct is a common feature for most teleosts (Nagahama, 1983). While in gymnovarian type, ovary lacks a part of the ovarian capsule and therefore, ovulated eggs are discharged directly into the abdominal cavity and spawned through the genital pore (Jalabert, 2005; Kagawa, 2013). The female reproductive organs of *S. denisonii* are built on the general teleostean pattern as observed in other teleosts. In teleost fishes, the paired ovary is either fused along the entire length or completely separate or fused posteriorly (Kagawa, 2013), however in *S. denisonii* ovary was united at the posterior region to form the oviduct as reported similarly in many freshwater fishes such as *Carassius auratus* (Yamamoto & Yamazaki, 1961); *Tor tor* (Rai, 1965); *Channa gachua* (Sanwal & Khanna, 1972); *Schizothorax richardsonii* (Bisht & Joshi, 1975); *Ambassis commersonii* (Grimes & Huntsman, 1980); *Horaglanis krishnani* (Mercy et al. 1982); *S. plagiostomus* (Agarval, 1996); *Brachydanio rerio* (Selman et al. 1993); *Barbus longiceps* (Stoumboudi et al. 1993); *Labeo dussumieri* (Kurup, 1994); *Pethia conchonius* (Cek et al. 2001); *Carassius auratus gibelio* (Gui et al. 2003); *Fundulus heteroclitus* (Selman & Wallace, 2005); *Dwakinsia filamentosus* (Mannan et al. 2010); *Pethia pookodensis* (Augustine et al. 2013); *Chela fasciata* (Indira et al. 2013); *Pseudosphromenus dayi* (Athira & Jayaprakash, 2014). The results of the present study in *S. denisonii* are supported by the findings of the above authors.

The assessment of maturity stages of gonad development in individual fish is an important component in reproductive biology of fish (West, 1990). The identification and classification of maturity stages are used according to different geographical and environmental areas (Crossland, 1977; Peerez et al. 1989; Millan, 1999; Ferreri et al. 2009). The general pattern of developmental stages of ovary in *S. denisonii* conformed to that of most teleosts with slight modification; eight maturation stages were identified by following El-Boray & El-Gharabawy (1999)
classification as immature, early developing, late developing, mature, ripe, spawning, spent and developing recovery. Monthly analysis of the distribution of the maturity stages revealed that *S. denisonii* has an extended spawning period from October to late February (See chapter 4). This result corroborates with the earlier reports by Solomon *et al.* (2012) and Mercy *et al.* (2013), while contrary to the Radhakrishnan & Kurup (2005) that *S. denisonii* was reported to spawn during June-August. Based on the developmental stages of ovary in fishes, 3 to 14 stages of gonadal maturity were recognised (Ferreri *et al.* 2009). Three stages of maturity recognised by Azadi & Mamun (2004); four stages of gonad ripeness by Nayak (1959), Hilge (1977) and Goodbred *et al.* (1997); five stages maturity by Belsare (1962) and Nunez & Duponchelle (2009); six stages observed by Htun-Han (1978); seven stages by De Silva (1973) and Borg & Van Veen (1982); eight maturity stages were described by Hoda (1995), El-Boray & El-Gharabawy (1999); nine stages by Treasurer & Holliday (1981) and ten stages by Yamamoto & Yamazaki (1961). Karekar & Bal (1960) have drawn up fourteen maturity stages for the same ovary described maturation with four stages by Nayak (1959). Therefore there is no standardized method for staging ovary in teleost (Blazer, 2002) and maturity scales, determination of maturity stages may vary considerably among peoples and labs (Ferreri *et al.* 2009). In the present study, gonadal development of *S. denisonii* has been studied and maturity stages were classified subjectively into eight stages based on appearance of fresh gonads, sizes and changes in GSI by following El-Boray & El-Gharabawy (1999). Eight stages of maturity were recognised such as immature, early developing, late developing, mature, ripe, spawning, spent and developing recovery.

For various reasons the fish ovary has attracted the attention of the research workers during the past many decades. According to Lubzensa *et al.* (2010), there is a knowledge gaps remains in understanding the dynamic processes associated with
oogenesis from the time that germ cells turn into oogonia, until the release of ova during spawning in teleost’s. The teleostean ovary is composed of follicles derived from the germinal epithelium, where oogonia developed into ova (Guraya, 1986; Selman & Wallace, 1989) and also producing different stages of oocytes within the same ovary (Wallace & Selman, 1981). Oogenesis consists of proliferation of oogonia from the germinal epithelium that covers the ovigerous lamellae, which is divided by mitosis originating oocytes (Wallace & Selman, 1981; Grier, 2000; Grier et al. 2007). Oocyte maturation characterized by the changes in the nucleus, ooplasm, yolk and the surrounding layers (Guimaraes et al. 2005). Important works on the ovarian histology of teleosts are those of Wallace (1903); Wheeler (1924); Padmanabhan (1955); Yamamoto (1956); Dutt & Govindan (1975) in Anabas scandens; Ritakumari & Padmanabhan (1976) in Etroplus suratensis; Grimes & Huntsman (1980) in Ambassis commersoni; Mercy et al. (1982) in Horaglanis krishnani; Saksena & Raizada (1984); Selmen & Wallace (1988) in Fundulus heteroclitus; Gopalakrishnan (1991) in Mugil cephalus; Selmen et al. 1993) in Brachydaniio rario; Veena et al. (1995); Grier (2000); Kurian & Inasu (2002); Euphrasia (2006) in Osteobrama bleekeri; Sunesh Thamby (2009) in Garra surendranadhinii; Lakshmi (2009) in Horabagrus brachysoma; Teji (2010) in Amplypharyngodon meletinus; Augustine et al. (2013) in Pethia pookodensis and Indira et al. (2013) in Chela fasciata.

Oogonia development in fishes varies with the species, and several standards have been employed for staging the process of oogenesis based on the size, amount and distribution of various cell inclusions like nucleus, nucleolus, yolk nucleus, yolk vesicles, yolk granules and lipid globules (Wallace & Selman, 1981; West, 1990). In majority of fishes, the process of oogenesis may be divided to four to thirteen stages (West 1990; Nagahama, 1983; Fishelson et al. 1997 in Capoeta damascina; Unal et al. 1999; Gokce et al. 2003). Thirteen stages were noticed by Gopalakrishnan (1991)

The development of oocytes as described in *S. denisonii* was similar to that observed in many fish species. In *S. denisonii*, nest of oogonia were observed in addition to immature oocytes, therefore it can be speculated that the germinal epithelium is responsible for the new crop of oogonia, similar to the finding of Aravindan & Padmanabhan (1972). While some authors observed that, oogenesis started from the follicle cells as well as from the residual oogonia (Wheeler, 1924; Yamamoto, 1956). In chromatin nucleolus stage, size of the nucleus is large and then decreases towards progressive stages. Present study recorded the existence of yolk-nucleus in *S. denisonii* oocytes, yolk nucleus was first appeared in the early perinucleolus oocyte, and it was also seen in the late perinucleolus stage of oocyte. The yolk nucleus first appears as a concentration of bodies around the nucleus, which later detaches itself from the nucleus, becomes spherical, and moves towards the peripheral region of the oocyte, where it disappeared similar to other cyprinid
species. While, Rita Kumari & Padmanabhan (1976) and Mercy et al. (1982) assumed yolk nucleus originated *de novo* in the cytoplasm of oocyte. In the present study, the yolk nucleus disappeared from the oocyte before they enter into vitellogenic stages and hence assumed that it has no role in vitellogenesis. Stock (1961) reported yolk nucleus is also used for taxonomic classification; Cyprinids are characterised by yolk nucleus with a granule in a vacuole. Hence, yolk nucleus origin, structure and its functions in *S. denisonii* is yet to be investigated.

During oogenesis, the size of the oocytes increased considerably due to a progressive accumulation of lipid and protein-yolk within the cytoplasm by vitellogenesis (Montchowui *et al.* 2012). In present study the yolk vesicles were appeared at the end of perinucleolus stage. Wallace & Selman (1981) reported that in most teleost species the yolk vesicles appeared just before the lipid droplets and yolk granules appear. In fishes, the *zona radiata* of the chorion of eggs played an important role during development and the structure of the chorion differs in different species depending on the nature of the substratum where they incubate eggs (Nagahama, 1983). A thicker *zona radiata* was observed in *S. denisonii*, it may be to provide a mechanical protection against the abrasion of the bottom as noted in *Carrasius auratus*, *Salmo gairdneri* (Nagahama, 1983) and *Danio rario* (Cakici & Ucuncu, 2007). Yolk vesicles were the first type of inclusions appearing in the cytoplasm of oocytes and then vesicles united to form yolk globules. Yolk globules were first appeared peripherally (Early yolk globule stage), later they fuse with each other to form a single mass of yolk (late yolk globule stage). During the final part of oocyte development in *S. denisonii*, the germinal vesicle or nucleus migrated to the animal pole where the micropyle is located (migratory nucleus stage). These sequential developmental events commonly reported in fish species in general (Nagahama, 1983; Euphrasia, 2006; Augustine *et al.* 2012; Montchowui *et al.* 2012). However, the progressive appearance of these reserves materials (Yolk vesicles,
granules, globules) varies with fish species (Nagahama, 1983; Sarasquete et al. 1996).

The frequency distribution of ova in final maturity stages of *S. denisonii* recorded the presence of nearly half the range of total mature eggs in ova frequency distribution. Present results supported the view of Prabhu (1956); Hickling & Reutenberg (1936); El-Greisy (2000), who hypothesized that this kind of oocyte distribution is an indicative of an extended seasonal spawning characteristic. According to Perres & Klippel (2003), presence of partially spawned females or running-ripe females or several oocyte stages in the same histological section, are evidence that the species have extended spawning and they may discharge their ripe ova in batches during spawning period. Observations on captive breeding of *S. denisonii* at Government Fisheries hatchery, Thrissur (Kerala, India) have shown that individual *S. denisonii* bred two times in a season under controlled conditions (Personal communication). This kind of breeding is also indication that individual fish may be able to spawn more than once in extended spawning period. After spawning, residual oocytes and unwanted materials are reabsorbed in a process known as atresia (Hunter & Macewicz, 1985). Atresia is characterized by the disintegration of the nucleus, breakdown of vitelline envelope, liquefaction of yolk globules, cell material phagocytosis, and degeneration of the follicular cells (Ball, 1960; Kamel, 1990; Blazer, 2002; El-Gamal, 2003). Oocytes not ovulated were subject to a degenerative process called follicular atresia (Wallace & Selman, 1981), this process can occur during any phase of the oocyte development (Unver & Unver Saraydin, 2004). In the present study, atretic stages were noticed only in vitellogenic oocytes. The follicle collapses after the oocyte has been released to form structures called post-ovulatory follicles (POFs) and in *S. denisonii* POFs had a wide irregular lumen, surrounded by hypertrophied follicular cells and a theca.
Ultra structural characteristics of the ovum surface and micropyle of teleost eggs differs in different species, and have recently been considered as a criterion for identification of eggs (Ohta et al. 1983; Wallace & Selman, 1990; Chen et al.1999). The envelope of teleost egg has a key role in their reproductive success. The egg surface has been studied using scanning electron microscopy by Ohta et al. (1983); Johnson & Werner (1986); Esmaeili & Johal (2005); Chen et al. (2007); Costa & Leal (2009) and Esmaeili & Gholamifard (2012). Occurrence of single micropyle on unfertilized egg of S. denisonii was similar to the cyprinid species reported by Bless & Riehl (2002); Lahnsteiner (2003) and Esmaeili & Johal, 2005). According to Riehl (1993), generally three type of micropyle are noticed in fishes such as, Type-I with a deep pit and short canal; Type-II with a flat pit and corresponding longer canal and Type-III having a funnel shaped vestibule from the bottom of which a cylindrical micropylar canal extended. Present study showed that the micropyle of unfertilized egg of S. denisonii may classify as type-III. Teleostean eggs are known to possess a number of pores or knobs on the surface of the egg membrane (Riehl & Patzner, 1994; Riehl et al. 1995; Esmaeili & Gholamifard, 2012). While, in S. denisonii egg surface do not have any special attachment structures, but possessed pores scattered uniformly over the egg surface. Presence of pores is a usual feature of many species like Tor tambroides (Kaman et al. 2014); Onchorhynchus gorbuscha, Hypophthalmichthys molitrix (Shabanipour & Hossayni, 2010). Normally several structures are noticed over the egg surface of teleost’s like, filaments, globules, villi, fibrillar net, adhesive discs and jelly coat (Johnson & Werner, 1986; Bless & Riehl, 2002; Rizzo et al. 2003; Esmaeili & Johal, 2005).

The oocytes growth of S. denisonii were similar to the other fish’s and in most of the teleost’s the progress of oogenesis. The oocytes development in S. denisonii displayed a series of changes, which their division into ten stages. The spawning type is determined by the interaction between the dynamics of oocyte
development, the frequency of spawning within a breeding season and the frequency of this event during his life (Araujo, 2009). Asynchronous developments of oocytes with ten histological stages in the Cystovarian ovary which passed through eight morphological maturity stages and a single seasonal with protracted spawning was noted characteristics of the ovarian cycle in *S. denisonii*. The ova distribution indicated that spawning taking place only once a year, during a longer period, size range of mature ova being nearly half the range of total size of entire intra-ovarian eggs. Determining annual reproductive cycles, spawning frequency and timing of spawning are the main challenges in fish reproductive biology studies. Confirmation of the types of spawning frequency and spawning interval in this barb awaits further studies by using transmission electron microscopy. However, results of the present study hopefully would contribute knowledge to the research on the process of the oogenesis of *Sahyadria denisonii*.

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