5.1. Introduction

Reproductive strategies of fishes are extremely diverse and include male/female patterns; self-fertilizing hermaphrodites and gynogenesis; seasonal and continuous breeders; single-spawners or multi-spawners; livebearers and fish that naturally undergo sex reversal (Breder & Rosen, 1966; Balon, 1975; Potts & Wooten, 1984; Blazer, 2002). A complete knowledge of the reproductive system and the reproductive biology of fishes are critical to understand the reproductive strategy, annual reproductive cycle (Unver & Unver Saraydin, 2004) and gonad development stages of any given species (West, 1990). Therefore, a detailed study on the biological features of the threatened species will be very valuable in
implementing any programme on conservation of fish genetic resources (Virjenhock, 1998).

The gonadal morphology of fishes differs largely on intra-specifically or inter-specifically depending on many factors including morphology, anatomy and environmental conditions (Rahemo & Al-Shatter, 2012). The identification of different stages of gonad development is considered an essential part in reproductive studies, and histological studies provide accurate information on oocyte development, but it involves complex laboratory techniques (West, 1990). The study of histology in reproductive studies minimized the errors during macroscopic estimation of gonadal maturity and reproductive seasonality (Tyler & Sumpter, 1996; Booth & Weyl, 2000; Blazer, 2002; Rutaisire, 2003). Hence histological analysis of gonadal development is considered as the most accurate method to determine the reproductive pattern in teleosts (Wallace & Selman, 1981; West, 1990).

Histological studies provide very precise information on the spermatogonia developmental stages, but their interpretation is confusing, because different authors use different classification for the same structures (Blazer, 2002). Males are in general more difficult to stage than females and may give a less defined estimate of the spawning season and spawning frequency (Fairbridge, 1951). Some of the notable works on male reproductive system are those of Yamamoto (1956) on Liopsetta obscura; Yamamoto & Yamazaki (1961) on Carassius auratus; (Bhatti & Al-Daham (1978) on Barbus luteus; Afroze & Hossain (1990) on Amblycephalomyzon mola; Selman et al. (1993) on Brachydanio rerio; Afroze (1996) on Puntius gonionotus; Cek et al. (2001) on Puntius conchonius; Matsumoto et al. (2002) on Cyprinus carpio and Carassius cuvieri; Glaser et al. (2003) on Ctenopharyngodon idella; Gui et al. (2003) on Carassius auratus gibelio; Selman & Wallace (2005) on
Spermatogenesis stages have been extensively studied in numerous species with some differences depending on the species and the classification criteria. The testicular morphology and histology in teleost’s has been studied by Wallace (1903); Padmanabhan (1955); Yamamoto (1956); Yamamoto & Yamazaki (1961); Hoar (1969); Siddique et al. (1976); Dodd (1977); et al. (1982); Nagahama (1983); Guraya (1986); Kobayashi et al. (1988); West (1990); Gopalakrishnan (1991); Selman et al. (1993); Stoumboudi et al. (1993); Kurup (1994); Veena et al. (1995); Coward & Bromage (1998); Maddock & Burton (1999); Grier 2000; Merson et al. (2000); Lucano-Ramirez et al. (2001); Dasgupta (2002); Kurian & Inasu (2002); Arocha (2002); Glasser et al. (2003); Gui et al. (2003); Goswami & Dasgupta (2004); Smith & Walker (2004); Selman & Wallace (2005); Vicentini et al. (2010); Rahemo & Al-Shatter (2012). Gonad maturation in *S. denisonii* has not been studied at the morphological and histological level, and their gonadal maturation is still poorly understood. Previous assessments were based exclusively on macroscopic observations and gonado-somatic index. In the present study, to understand the reproductive mechanism in *Sahyadria denisonii*, macroscopic as well as microscopic investigations were carried out.

5.2. Materials and Methods

5.2.1. Assessment of Maturity Stages

Samples of *S. denisonii* were collected from September 2012 to August 2013 from River Valapattanam. For each fish, total length and total weight of fish and testes were recorded to nearest to mm and mg. After assessing the stage of maturation, the testes were preserved in 5% formalin for further studies. Quantification of maturity stages was done by following morphological characteristics of the testis such as
appearance, colour, degree of distension, and relative space occupied in the body cavity (Mc Bride et al. 2002) and scheme proposed by EL-Boray (2001).

5.2.2. Tissue Processing for Histology

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).

5.2.3. Scanning Electron Microscopy Observation- Spermatozoa

Milt was stripped off from species by manual pressure over the abdominal region of fish and fixed in 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 teroxide 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).

5.3. Results

5.3.1. Male Reproductive System

The male reproductive system of S. denisonii which is characterized by a pair of testis is soft and elongated structures lying in the body cavity and ventral to the swim bladder (Image. 5.1). It leads posterio-ventrally as two vas deferens, which unite to form a spermatic duct opening to the exterior through the urogenital aperture. Each testis is attached to the dorsal body wall by the connective tissue, mesorchium and composed of numerous thin walled seminiferous lobules. Within
the lobules, cells in various stages of spermatogenesis were seen in distinct nests of cells, more advanced germ cells lie towards the centre of the testis. The testes had a broad anterior part which tapered posteriorly. The length of the testis varied with the size of the fish. The length of *S. denisonii* testes varied in from 1.6 to 4.1 cm and mean weight from 0.026 to 0.834 gm. The testes of *S. denisonii* were found to be in different colours with the development of maturity, opaque and creamy white in colour at the mature stage. Sexual maturation was characterized by enlargement, and change in colour with the testes turning milky white. The testes having two equal lobes were cylindrical and folded structure, but not branched. Testes of maturing and mature individuals had a turgid texture and folded structure (Image. 5.1), while spent individuals had a loose texture.

![Gonadal system in male *Sahyadria denisonii*; DS-digestive system](image)

**Image 5.1.**

### 5.3.2. Morpho-histological Examination of Maturity

The testes of *S. denisonii* were bi-lobed fleshy structure occupying a large part of the abdominal cavity; the middle portion of the testis was broader than the anterior and posterior region. Based on shape, size, colour, texture and histological differentiations, eight maturity stages were recognized in *S. denisonii*, 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-VII (Developing recovery). A description of different maturity stages were given below,

**Stage- I: Immature virgin** (Image 5.2a)

These are the first stage of testes development, which have not yet spawned and found in young individual. They were thin, thread-like, and pale in colour, occupied a very small proportion of the body cavity and left lobe slightly longer than the right one. In this first stage, all lobules of the testis are formed of primary spermatogonia (SG₁) of different sizes and are found in single or group.

**Stage- II: Early developing** (Image 5.2b)

Testes became enlarged, firm, developing its creamy white colour, increase in size occupying about ¼ of body cavity and broadened at the anterior region and tapered towards the posterior side. The testes exhibited active spermatogenesis, where different stages of spermatogenetic cells, including spermatogoniums and primary spermatocytes were found inside the lobules.

**Stage- III: Late developing** (Image 5.2c)

Testes became white, firm, occupying about ½ of the body cavity. This stage of maturation is characterized by the formation of the lobule lumen. The secondary spermatocytes are predominant in this stage along with primary spermatocysts, spermatogoniums and few spermatids.

**Stage- IV: Mature** (Image 5.2d)

Testes became creamy white, firm, lobulated with irregular outer margin occupied ¾ of the body cavity. In this stage, the spermatids and spermatozoa are predominant over all the earlier spermatogenetic cells. The spermatozoa near the wall
of the lobules form parachute shaped clumps, where as those found in the lumen of the lobules become separately free.

**Stage- V: Ripe** (Image 5.2e)

The testes became creamy white, soft lobed and reached their maximum size. Milt could be easily extruded upon exerting slight pressure on the belly. Testis contained abundance of spermatozoa and spermatid in the outer portion of gonad and lobules were packed with spermatozoa. The spermatogium, primary spermatocysts cells are very few in numbers and were found attached to the wall of the lobules.

Image 5.2. Maturity stages recognized in male *Sahyadria denisonii*

**Stage VI: Spawning** (Image 5.2f)

The testes were very extensive, milky white in colour and milt could be easily extruded upon exerting slight pressure on the belly. Anterior portion of testis lobes started too shrunken. At this stage, considerable quantities of sperms are
discharged. The spermatogium, primary spermatocysts cells were very few in numbers and found attached to the wall of the lobules.

**Stage VII: Spent** (Image 5.2g)

Testes seemed to be dorso-ventrally flattened, became flabby, thin and dull white in colour. The wall of the lobules became very thick, blood cells were found scattered and some lobules contained residual sperms or not.

**Stage VIII: Developing recovery** (Image 5.2h)

In this stage, the spent testes entered the regeneration phase with well-defined seminiferous lobules with spermatogonia and spermatocytes. Anterior and posterior region of testes were broadened than early developing stage.

### 5.3.3. Light Microscopy Examination- Spermatogenesis

The testes of *S. denisonii* were paired, elongated structures and composed of numerous seminiferous lobules separated from each other by means of stromal layer and surrounded by visceral peritonium and thick layer of tunica (Image 5.4e). The interlobular stroma contained loose connective tissue, blood capillaries and interstitial cells (Leydig cells) (Image 5.4g). The internal testicular structure of *S. denisonii* was lobular type, in which the seminiferous tubules are grouped in many cysts, where spermatogenesis occurs. The lobular testis in *S. denisonii* is unrestricted spermatogonia being dispersed along the length of the lobule. Active spermatogenesis was observed in *S. denisonii* and the process of spermatogenesis occurred progressively during the annual reproductive cycle. Large amounts of spermatozoa were accumulated in the central system of ducts and after completion of spermatogenic process spermatozoa were released into the lumina of lobules. With the release of spermatozoa the structures of the lobules changed. Their function changed from sperm production to sperm storage.
Each spermatogonium in testis passed through different stages to form mature spermatozoa (Image 5.4.A-L). In testes lobules were filled with discrete nests of spermatogenetic cells in various stages of maturation. Each nest of cells contained one spermatogenetic stage and cell size decreased gradually by development to spermatozoa from Spermatogonia (Image 5.3).

**Image 5.3.** Transverse section through the testis showing spermatogenesis, Spermatogonia (SG), Primary spermatocytes (SC₁), Secondary spermatocytes (SC₂), Spermatids (ST) develop in cysts that ruptured to release spermatozoa (SZ) into the lumen of the lobule. H&E-40x

The six stages of spermatogenesis were recognized in the testis of *S. denisonii* and they are,

**1. Primary spermatogonia (SG₁)** (Image 5.5.A-B)

Spermatogonia were the first group of cells to appear during the process of spermatogenesis and were most packed near the germinative zone of testes. Primary spermatogonia were large single cells, more or less spherical in shape and are distributed all along the germinal epithelium. They could be found either as individual cells or as groups of cells and they were the largest cell compared to other
stages in spermatogenesis. Primary spermatogonium cells under went further mitotic divisions to form secondary spermatogonium.

**Image 5.4.** Transverse section (TS) of testis showing various stages of spermatogenesis

2. **Secondary spermatogonia (SG2)** (Image 5.5.A-B)

Secondary spermatogoniums were produced by the mitotic division of primary spermatogonia (SG₁). They were smaller than the primary spermatogonia (SG₁) and have a centrally placed nucleus. The secondary spermatogonia were found in clusters, ovoid cells with clear cell membrane, nuclei stained intensely. They were similar to primary spermatogonia except in size and also darker than the primary spermatogonia (SG₁).
3. Primary spermatocytes (SC₁) (Image 5.5.C)

Secondary spermatagonia divided mitotically to give rise to primary spermatocytes and were smaller than the secondary spermatogonia (SG₂) with reduced cytoplasm. Nucleolus was not visible in all cells or still distinct and larger compared to cell size. Cytoplasm stained faintly and nucleus purple with the haematoxylin-eosin stain.

4. Secondary spermatocytes (SC₂) (Image 5.5.C)

Secondary spermatocytes were formed by the meiotic division or reduction division of primary spermatocytes (SC₁). They were smaller and darker than primary spermatocytes. Cytoplasm was less and nucleolus no longer visible.

5. Spermatids (ST) (Image 5.5.D)

The spermatids (STs) were produced by the second meiotic division of the secondary spermatocytes (SC₂) and were much smaller, compact dark dot like structures. They appeared as deeply stained with Haematoxylin-eosin. Spermatids undergo spermiogenesis to produce the spermatozoa.

6. Spermatozoan (SZ)(Image 5.5.E)

Spermatozoa were the smallest spermatogenic cells in spermatogenesis with distinct tail and darkly stained nucleus and their transformation from spermatids to spermatozoa was called spermiogenesis. A mature spermatozoon consisted of two regions; head was round shaped with elongated tail. After completing spermiogenesis, the spermatozoa were released into the lumen and vasa efferentia, respectively. The lumen of the seminiferous lobules in ripe males was richly packed with mature spermatozoa.
Image. 5.5. Spermatogenesis in *Sahyadria denisonii*, Primary spermatogonia (SG₁); secondary spermatogonia (SG₂); Sertoli cells (S); Primary spermatocyst (SC₁); Secondary spermatocyst (SC₂); Spermatids (ST); Spermatozoa (SZ); Head (H); Flagellum (F)
5.3.4. Scanning Electron Microscopy (SEM) of Spermatozoa

Scanning electron microscopy was used to investigate the fine structure of the spermatozoa of *S. denisonii*. Spermatozoa of *S. denisonii* were tightly packed in the lumen of the testes lobules (Image. 5.6). Spermatozoa were uni-flagellated and consisted of a head, short mid-piece and flagellum. The head is ovoid shaped with smooth surface, cone-shaped mid-piece and a cylindrical long flagellum. Spermatozoa had no acrosome and are aqua-sperm type.

Image 5.6. SEM photograph of ripe testis showing spermatozoa; H-Head, M-middle piece, F- Flagellum
5.4. Discussion

The fish reproductive physiologists, in general have placed more importance on the study of histological changes in the females than male (Schulz et al. 2010). Fine structural work on spermatogenesis continues to enhance our understanding of germ cell differentiation and provides insights into the relationships between various teleost groups. Generally, gonadal development was monitored on the basis of their microscopic and macroscopic appearance and nowadays gross morphological changes were studied in number of freshwater fish species (Wang et al. 2003). Analyses of gonad morphology are important for understanding the species biology and have been extensively applied to the teleostei (Martins et al. 2012). Despite a great diversity in their reproductive strategies, the testes of numerous teleost species show a similar general structure. The testes of *S. denisonii* had two equal lobes which were cylindrical and folded structure but not branched, similar to other cyprinid fishes (Al-Daham & Bahatti, 1979; Bardakci et al. 2000). In fishes, the paired testes are either fused along the entire length or completely separated or fused posteriorly (Selman & Wallace, 2005). In *S. denisonii* testes were united at the posterior region to form the spermatoduct, as reported similarly in many freshwater fishes such as *Carassius auratus* (Yamamoto & Yamazaki, 1961); *Barbus tor* (Rai, 1965); *Labeo fimbriatus* (Bhatnagar, 1972); *Schizothorax richardsonii* (Bisht, 1974); *Horaglanis krishnani* (Mercy et al. 1982); *S. plagiotomus* (Agarval, 1996); *Brachydanio rerio* (Selman et al. 1993); *Barbus longiceps* (Stoumboudi et al. 1993); *Labeo dussumieri* (Kurup 1994); *Barbus scateri* (Encina & Granado-Lorencio, 1997); *Ctenopharyngodon idella* (Glasser et al. 2003); *Carassius auratus gibelio* (Gui et al. 2003); *Pethia conchonius* (Cek et al. 2003); *Osteobrama bakerii* (Euphrasia, 2006); *Amblypharyngodon melellinus muriyadensis* (Teji, 2010); *Pethia pookodensis* (Augustine et al. 2013) and *Chela fasciata* (Indira et al. 2014).
Present study shows that, testis of *S. denisonii* exhibits remarkable cellular changes in different months of the year. The testis of *S. denisonii* was elongated paired organs attached to the dorsal body wall, and similar type of gonadal arrangement has been described by EL-Maghraby et al. (1981); Zaki et al. (1994); Asem (1995) and EL-Boray (1997). The testis of *S. denisonii* was covered with tunica albuginea which composed of connective tissue layer and a smooth muscle layer. Generally testis is composed of two main compartments, *inter-tubular* (interstitial) containing Leydig cells, blood vessels, connective tissue cells and *tubular* compartment being continuous with the tunica albuginea as reported by Koulish *et al.* (2002) and Schulz *et al.* (2010). Histological examination of testis of *S. denisonii* revealed that the tunica albuginea consisted of a mesothelium, a few layers of connective tissue and blood vessels. The protrusion of tunica albuginea into the testicular parenchyma completely divides it into lobular structure consisting of numerous seminiferous lobules. This is a common pattern found in family Cyprinidae (Koulish *et al.* 2002; Schulz *et al.* 2010; Teji, 2010; Montchowui *et al.* 2012).

The anatomy of testes of *S. denisonii* was found to be similar with other freshwater Cypriniformes that have external fertilization such as *Brachydanio rerio* (Selman *et al.* 1993); *Barbus longiceps* (Stoumboudi *et al.* 1993); *Labeo dussumieri* (Kurup, 1994); *Barbus scateri* (Encina & Granado-Lorencio, 1997); *Ctenopharyngodon idella* (Glasser *et al.* 2003); *Carassius auratus gibelio* (Gui *et al.* 2003); *Pethia conchonius* (Cek *et al.* 2003); *Osteobrama bakerii* (Euphrasia, 2006); *Systomus sarana* (Chakraborthy *et al.* 2007); *Amblypharyngodon melettinus* (Teji, 2010); *Pethia pookodensis* (Augustine *et al.* 2013) and *Chela fasciata* (Indira *et al.* 2014). Commonly, fishes have three distinct testicular types namely, *anastomosing tubular testis, lobular restricted spermatagonial testis* and *lobular unrestricted spermatagonial testis* (Parenti & Grier, 2004). In the *anastomosing tubular testis* the germinal compartment and tubules form loops that are interconnected, from the periphery to the testicular duct
region (Grier, 1993). In the lobular restricted spermatogonial testis the spermatogonia are confined in the distal lobes in the testicular periphery. However, in the lobular unrestricted spermatogonial testis the spermatogonia are distributed along the entire length of the testicular lobules (Parenti & Grier, 2004). For internal structure, *S. denisonii* had lobular unrestricted type testis, in which the seminiferous tubules were grouped in many cysts, where spermatogenesis occurs.

The testes undergoing reproductive activity exhibit six stages of spermatogenetic cells were identified in *S. denisonii*, namely primary spermatogonia (SG\(_1\)), secondary spermatogonia (SG\(_2\)), primary spermatocyte (SC\(_1\)), secondary spermatocyte (SC\(_2\)), spermatid (ST) and spermatozoa (SZ). Most authors divide the spermatogenesis process in teleost fishes into four to six stages of development, depending on the species and the choice of criteria used (Nagahama, 1983; Joshi & Joshi, 1989; West, 1990; EL-Boray, 1997; Rutaisire *et al.* 2003; Unver & Unver Saraydin, 2004; Montchowui *et al.* 2012). The formation of these cells occurred as asynchronous process in the lobules, where all these cells were found in one lobule. This phenomenon and presence of gonadal developmental stages at different maturity in the same period during spawning, in addition to the discharge of sperms intermittently may lead to the conclusion that *S. denisonii* has a prolonged spawning season. However, the duration of the spermatogenesis and the degree of testicular enlargement in teleost varied with the species and geographic location (West, 1990; Fraile *et al.* 1992; Rutaisire *et al.* 2003; Schulz *et al.* 2010). Spermatogenesis in *S. denisonii* appeared to be similar to that reported for many other teleosts with six developmental stages in *Mystus seenghala* (Sathyanesan, 1959); *Opsanus tau* (Hoffmann, 1963); *Tilapia nilotica* (Latif & Saady, 1973); *Clarias macrocephalus* (Mollah, 1986); *Puntius dukai* (Joshi & Joshi, 1989); *Schizothorax plagiostomus* (Agarval, 1996); *Rhabdosargus haffara* (EL-Boray, 1997); *Osteobrama bakerii*
Spermatogenesis is a highly organized and coordinated process, in which diploid spermatogonia proliferated and differentiated to form mature spermatozoa (Schulz et al. 2010). In many tropical species, reproduction is a seasonal or cyclic event related to environmental signals (Billard & Breton, 1978; Nash, 1998) and their active spermatogenesis may take place in summer, in spring, or may begin in autumn and finish in spring (Billard & Breton, 1978; Billard, 1986). The duration of spermatogenesis is usually shorter in fish than in mammals and is also influenced by the water temperature (Nobrega et al. 2009). Spermatozoa within the seminiferous tubules are immotile and may lack fertilization capacity (Nagahama, 1983; Billard, 1986; Sajan et al. 2013). Sperm motility is initiated when the milt is diluted with an activating medium such as freshwater, saline media, pH, or ovarian fluid (Scott & Baynes, 1980). While Morisava & Morisava (1986) stated fish spermatozoa acquire their motility when passing through the duct, in a process that seems to occur in a relatively short time. Spermiogenesis consists of series of morphological changes that lead to the differentiation of spermatids into spermatozoa, and these changes includes nuclear condensation, elimination of organelles and cytoplasm, flagellum formation, and the rearrangement of cellular organelles along the spermatozoon cytoplasm (Billard & Breton, 1978; Jamieson, 1991; Nash, 1998; Schulz et al. 2005; Schulz et al. 2010). Generally, in fishes three types of spermiogenesis have been described viz., type I (characterized by a perpendicular flagellum in relation to the nucleus with nuclear rotation); type II (flagellum develops parallel to the nucleus without nuclear rotation) and type III (flagellum is central without nuclear rotation) (Quagio-Grassiotto & Oliveira, 2008).

Scanning electron microscopy (SEM) was used to investigate the fine structure of the spermatozoa of S. denisonii. Spermatozoa of S. denisonii were
characterized by a head, a short mid piece, single flagellum, and absence of acrosome. Spermatozoa of *S. denisonii* were uni-flagellated, anacrosomal, aqua-sperm type, which is typically found in external fertilizing fish (Jamieson, 1991). Generally, shape of the head, mid-piece and flagellum of spermatozoa are highly variable between teleosts (Mattei, 1991; Maricchiolo *et al.* 2004; Leal *et al.* 2009).

The spermatozoan head of family Cyprinidae is usually in spherical to ovoid shape (Baccetti *et al.* 1984) and *S. denisonii* had atypical cyprinid spherical to ovoid shape. Spherical type heads were described for the *Esox lucius* (Roheli *et al.* 1950), ovoid shaped ones for *Apogon imberbis*, (Lahnsteiner, 2003) and banana-shaped ones for *Anguilla anguilla* (Todd, 1976). A short type mid piece in *S. denisonii* is common in teleosts with external fertilization (Nicander, 1970), while internal fertilization is linked to elongation and complexity of the gamete (Jamieson, 1991).

In teleost, spermatozoa generally have no acrosome, and the impenetrable chorion is pierced by a micropyle that gives access to the membrane of the oocyte. The spermatozoa of the *S. denisonii* lack acrosome, assumed by the presence of micropyle in the ova. Basically, spermatozoa of fish have been categorized into acrosomal type (with an acrosome) and anacrosomal type (lacking an acrosome) (Jamieson, 1991). Teleost spermatozoa lack an acrosome, which occurs in all other vertebrate groups. This may be related to the presence of a micropyle in teleost eggs, which assures sperm penetration (Grier, 1981; Nagahama, 1983). In most vertebrates, the acrosome contains some enzymes, which were released to the environment to hydrolyse the zona pellucida of the oocyte to fuse with the oocyte. While if the acrosome reaction happens in a large water area, the enzymes released would be useless since they are diluted quickly by water (Hu *et al.* 2005). Adapting to this, the micropyle is a channel structure through which an anacrosomal sperm can enter the egg without proteolytic decomposition of the zona pellucida of the egg (Amanze & Iyvengar, 1990). Thus the present study indicated that micropyle-dependent...
fertilization is the approach by which this species prevent the egg from poly sperm in *S. denisonii*. Moreover, fish spermatozoa can be classified into two forms, aqua-sperm and intro-sperm, according the external or internal mode of fertilization, respectively (Jamieson, 1991), so spermatozoa of *S. denisonii* are anacrosomal, aqua sperm type.

In *Sahyadria denisonii*, the testes of different individuals caught during the same period were exhibiting different spermatogonic activities. Spermatogenesis takes place progressively during the annual reproductive cycle and the spermatozoa were discharged gradually from the seminiferous lobules. The annual testicular cycle consists of successive stages of relaxation and rehabilitation, spermatogenesis, activation and depletion. From the morphological and the histological studies, it may be concluded that the spawning season of *S. denisonii* to extend from October to March. Finally, future studies of gonadal development using transmission electron microscopy (TEM) and evaluation of the reproductive cycle in line with environmental factors are necessary to better comprehend the spermatogenesis in *Sahyadria denisonii*.