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Histological studies on the gonads of the Indian scad *Decapterus russelli* (Ruppell, 1830) from Maharashtra waters, north-west coast of India

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ABSTRACT

Histological studies on the gonads of *Decapterus russelli* were undertaken to understand the mode of gonad development and to describe the maturity stages. Based on the histological studies, six stages of oocyte development were identified. Histological sections revealed that ovarian development in *D. russelli* is of asynchronous type. The presence of many large yolk vacuoles in the cytoplasm of oocytes indicated pelagic nature of the eggs. Maturation of sperm occurs along the entire length of the seminiferous tubules and was found to be unrestricted spermatogonial testis. Within each seminiferous tubule, cysts containing germ cells at different stages of development were seen; however germ cells within each cyst were at the same stage of development. This is the first report on the gonadal development of *D. russelli* from Indian waters.

Keywords: *Decapterus russelli*, Gonads, Histological studies, Maturity stages, Ovaries, Testis

Introduction

Decapterus russelli is caught throughout the year along the north-west coast of India mainly in trawlers at depth range of 100-200 m (Tamhane, 1996). It is a commercially important fish in the states of Kerala, Andhra Pradesh, Karnataka and Gujarat (Poojary, 2009). Fish histology has been studied by several authors (Hoar and Randall,1969; Patt and Patt, 1969; Harder, 1975; Ali and Anctil, 1976; Welsch and Storch, 1976; Roberts, 1978), and detailed descriptions on fish histology were published by Anderson and Mitchim (1974) on trout; Grizzle and Rogers (1976) on channel catfish and Groman (1982) on striped bass. Though several papers on fish histology with respect to pathology have been published, studies on reproductive biology are meager and there is no published report available on the histology of gonads of *D. russelli*. Gonadal developmental study of the species was taken up to provide inputs for the management of the fishery of *D. russelli*. Besides evaluating reproductive health of the species it would also help in assessing effects of endocrine modulating chemicals and other environmental stressors.

Materials and methods

Gonads (ovaries and testes) from fresh specimens of *D. russelli* (20 samples each) in different stages of maturity, collected over a period of one year were preserved in 10% neutral buffered formalin. Based on macroscopic examination of ovaries and microscopic structure and size of ova, the maturity stages of female gonads were identified. Based on the morphology of testes and oozing of milt, stages in males were identified. For better penetration of the fixative, mature ovaries and testes were cut into 7 - 8 mm pieces and immature gonads were fixed whole. After 24 h, the fixative was replaced with fresh 10% neutral buffered formalin. After fixation, the tissues were dehydrated in ascending grades of ethyl alcohol and cleared in xylene and were embedded in paraffin wax and sections of 5µm thickness were cut on a rotary microtome. The sections were mounted on glass slides and were stained by routine haematoxylin and eosin method (Mayer, 1896; Lillie, 1965).

Results and discussion

Ovary

Morphology: Paired ovaries of *D. russelli* are bilateral elongate lobes oriented longitudinally within the abdominal cavity, as found in most of the teleost fishes. They are suspended from the ventral surface of the gas bladder by mesenteries termed mesoovaria (Harder, 1975). The gonadosomatic index (GSI) of females ranged from 0.96 to 2.63. The following maturity stages were identified by macroscopic examination of the ovary and microscopic examination of ova.

Immature (Stage I): Ovaries are thin, thread like, translucent, pale in colour and occupy very little portion of the body cavity. Ova are not visible to the naked eye.

Early maturing (Stage II): Ovaries are thin but not thread like, light pink in colour and occupy less than $^1/_4$ of the body cavity. Ova are not visible to the naked eye. On microscopic examination, presence of spherical closely packed transparent ova is evident. Yolk formation not started. Ova are in the range 0.02 to 0.2 mm in dia.

Late maturing (Stage III): Ovaries are slightly larger and thicker, occupying ½ of the body cavity. Granular ova are visible to the naked eye. Ova are in the range of 0.2 to 0.3 mm in dia.

Mature (Stage IV): Ovaries become larger, thicker and occupy $^2/_3$ to $^3/_4$ of the body cavity. Yellow yolked opaque ova are visible through the thin ovary wall. Maturing ova are in the range of 0.3 to 0.4 mm in dia.

Late mature (Stage V): Ovaries in this stage are bright yellow in colour, longer, broader and thicker, extending up to the full length of the body cavity. There is marked increase in the volume and weight of the ovary. Blood vessels are seen on the dorsal side of the ovary. Ova are deep yellow in colour and visible through the ovarian wall. Under microscope, ova appear completely opaque with transparent periphery and fair amount of yolk. Mature ova lie in the range of 0.4 to 0.6 mm dia.

Ripe (Stage VI): Ovaries are broad with prominent blood vessels, turgid and yellow in colour. They attain maximum size and occupy full length of the body cavity. Blood supply increases considerably. Both translucent and opaque ova with transparent periphery are present. Ripe ova about to spawn are transparent with fat globules. Size of ova ranges from 0.55 to 0.97 mm in dia. Mature ova are predominant than maturing ova. Small immature transparent ova are also present. Running ovary is very loose and vascular. Ovarian wall is very delicate and at slight pressure, eggs ooze out.

Spent (Stage VII): Ovaries are shrunken and flaccid with thin wall. Vascularisation decreases considerably. Ovary looks pale and sometimes reddish to brown. Immature, maturing and mature ova as well as a few unspawned ripe ova are present.

Histology: Ovaries are enclosed in a fibrous connective tissue called tunica albuginea (peritoneal or thick mesothelial covering) which is continuous with mesoovaria as found in largemouth bass, bluegills (James, 1946) and in striped bass (Groman, 1982). The luminal surface of the tunica albuginea folds into ovigerous lamellae arranged perpendicular to the long axes of each ovarian lobe (Fig. 1). The thin lamellar wall contains germinal epithelium, follicular epithelium, blood vessels and connective tissue stroma.

Oogenesis from germinal cell occurs within the folds of follicular epithelium (Harder, 1975; Grizzle and Rogers, 1976). Development of ova from germinal cell to mature ova is divided into six stages defined according

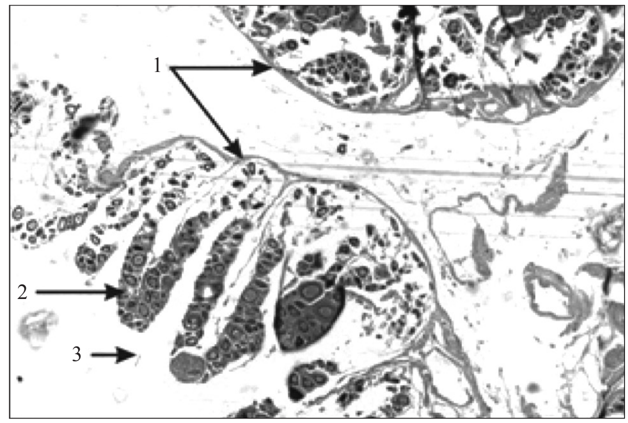


Fig. 1. Photomicrograph of histological section of ovary of *Decapterus russelli* (H & E; X50) (1) Tunica albuginea, (2) Ovigerous lamellae with immature oocytes, (3) Lumen of ovary

to morphological characteristics of nucleus, oviplasm and follicular wall. These stages observed for *D. russelli* in the present study are similar to those described for Gulf menhaden (Coombs, 1969); striped bass (Groman, 1982) and fathead minnow (Yonkas *et al*., 2000).

Stage I oocyte: Mitotic proliferation of germinal cells produces cluster of stage I oocytes (undifferentiated oogonia). Stage I oocytes are nested within the germinal epithelium of the lamellar stroma.

Stage II oocyte: Stage II oocytes are larger and have basophilic cytoplasm and a large central nucleus with diffuse chromatin (Fig. 2). These primary oocytes represent the initial stage (prophase) of meiosis (Harder, 1975).

Stage III oocyte: As the oocytes mature to stage III, they are enveloped by simple squamous follicular epithelium. Provitelline nucleoli become evident in the karyoplasm (Fig. 3). Stage III oocytes are seen in all the maturing stages of the ovary throughout the year. Similar observations were made by Coombs (1969) in Gulf menhaden (*Brevoortia patronus*) and Groman (1982) in striped bass. Wallace and Selman (1981) referred to this, as the primary growth stage or chromatin nucleolar stage in oocyte maturation. Yolk granule formation is not seen at this stage.

Stage IV oocyte: In stage IV, yolk granules and fat vacuoles are seen in the oviplasm. At this stage, euvitelline nucleoli appear at the periphery which are formed by the migration of the provitelline nucleoli. Euvitelline nucleoli are believed to be involved in ribonucleic acid synthesis

(Groman, 1982). In the oviplasm, two distinct acidophilic and basophilic layers are seen (Fig. 4). A distinct vitelline envelope (chorion) appears beneath the follicular epithelium.

Stage V oocyte: In stage V oocyte, yolk vesicles increase tremendously and only few distinct euvitelline nucleoli are seen in the acidophilic nucleus (Fig. 5). The nuclear membrane and karyoplasm begin to degenerate in late stage V oocytes. Fig. 6. shows differentiation of the follicular layer.

Stage VI oocyte: Stage VI oocyte contains oviplasm which is filled with larger fat vacuoles and acidophilic yolk granules. Stage VI and atretic oocytes are shown in Fig. 7a and b. In many teleosts the conclusion of maturation is thought to be preceded by the migration of nucleus to the peripheral position near the zona radiata

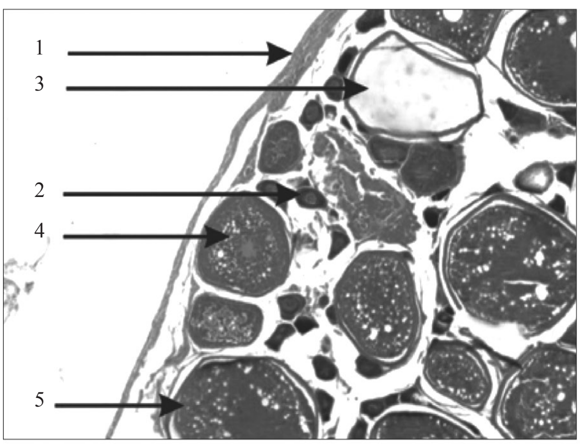


Fig. 2. Section of ovary showing different stages of oocyte (H & E; X50). (1) Tunica albuginea, (2) Stage II oocyte, (3) Empty follicle, (4) Stage IV oocyte, (5) Stage VI oocyte (ripe egg)

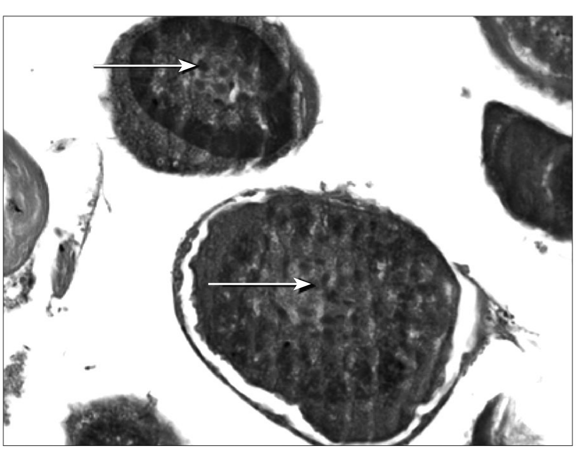


Fig. 3. Stages III oocyte (H & E; X400). Arrow: Provitelline nucleoli

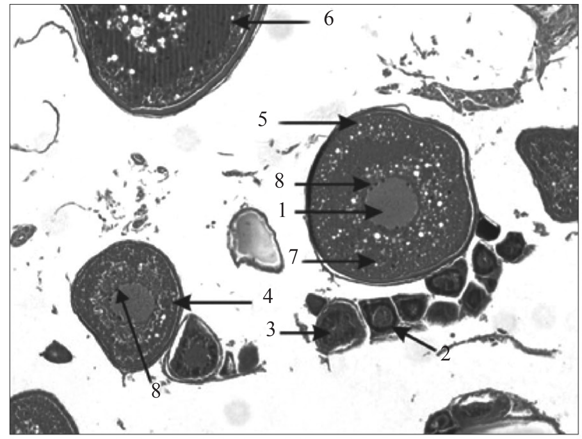


Fig. 4. Oocytes at various stages of development (H & E; X 100). (1) Nucleus, (2) Stage II oocyte, (3) Stage III oocyte, (4) Stage IV oocyte, (5) Stage V oocyte, (6) Stage VI oocyte (ripe egg), (7) Cytoplasm of oocyte (oviplasm), (8) Euvitelline nucleoli

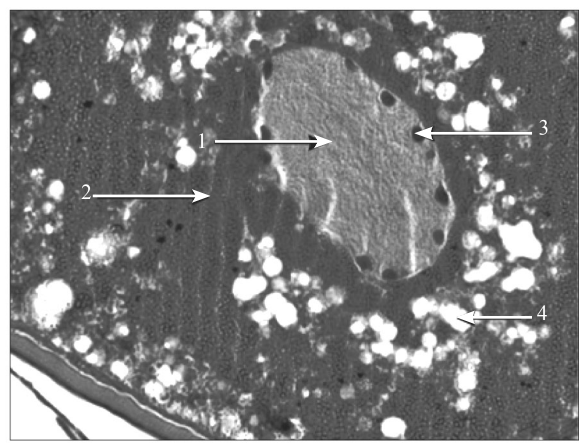


Fig. 5. Late stage V oocyte (H & E; X1000). (1) Nucleus, (2) Yolk granules, (3) Euvitelline nucleoli, (4) Yolk vacuole

and micropyle (Groman, 1982). In *D. russelli* migration of the nucleus was not seen. Interstitial tissue containing macrophages and epithelial elements that may represent endocrine tissue between developing oocytes was seen.

It is evident from the histological sections that post-ovulatory follicles coexisted with ova at stage IV and V indicating that the species is a continuous spawner. The large fat vacuoles in the pelagic eggs help in floating. Oocytes at different stages were found simultaneously in most of the ovaries, indicating asynchronous development of oocytes.

Testis

Morphology: D. russelli has a pair of bilobed elongate testis located within the abdominal cavity, attached to the gas bladder by mesenteries termed mesorchia. The mesorchia

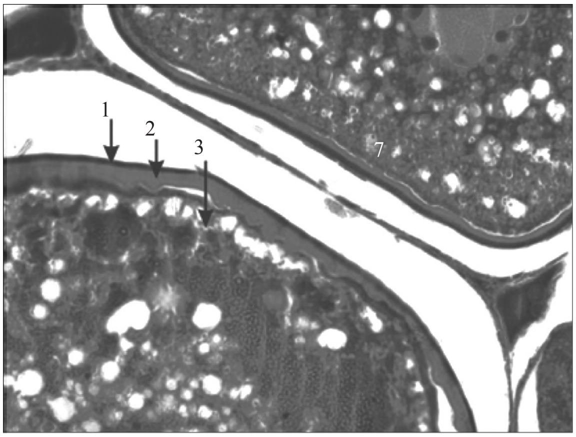
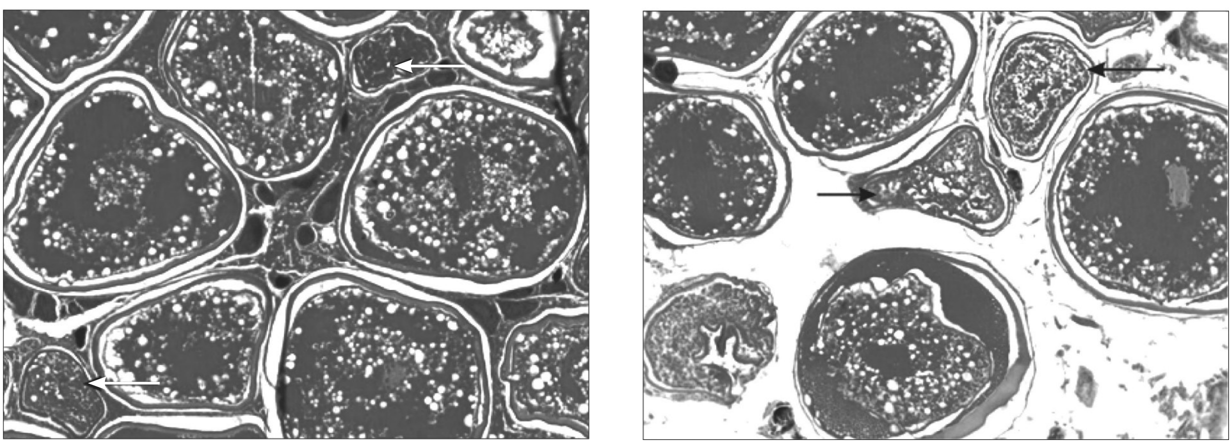


Fig. 6. Details of follicular wall of two adjacent ripe oocytes (H & E; X1000). (1) Follicular cell layer, (2) Vitelline envelope (chorion), (3) Cytoplasm of oocyte (oviplasm)



(a) (b)

Fig. 7 a & b. Histological section of ripe ovary (H & E; X 400). Follicular cell wall, well differentiated stage VI oocyte (ripe egg) and follicular cells embedded in fibrous connective tissue stroma are seen. Arrow: Atretic oocyte

encase a thin tunica albuginea which in turn encase the testicular lobes. Gonadosomatic Index of males ranged from 0.7 to 1.05. By observing morphology of the testis and oozing of milt, 5 maturity stages were identified in males.

Immature (Stage I): The testes are long, slender, thread like structure, pale in colour extending upto $^{1}/_{3}{}^{rd}$ of the body cavity.

Maturing (Stage II): Testes are long, ribbon like bands, white or creamy in colour, extending from $^{2}/_{3}{}^{rd}$ to $^{3}/_{4}{}^{th}$ length of body cavity. Morphological appearance though similar to stage I, slight increase in weight and volume was noted.

Mature (Stage III): Thick, ribbonlike testes extending upto ¾ length of body cavity. They are broader, thicker and opaque in appearance, white or creamy in colour.

Ripe (Stage IV): Testes are turgid and pink in colour. Millt oozes out, on applying slight pressure on the abdomen.

Spent (Stage V): Testes are flaccid, blood shot and ribbon like, extending upto $^{2}/_{3}{}^{rd}$ of the body cavity. The weight and volume of the testes reduced considerably and no milt oozed out, when pressure applied on the abdomen.

Histology: Each testicular lobe comprised of a longitudinally oriented collecting duct (ductus deferens) along the length of which radiated seminiferous tubules. The thin capsule walls of these tubules are formed by invagination of the tunica albuginea (Harder, 1975). Spermatogenesis takes place within the seminiferous tubules. After maturation, the sperms are stored in tubule lumen and within the ductus deferens. Within the luminal margins of the seminiferous tubules were cysts like structures, each containing sperm cells of similar maturity. Within each cyst, germ cells developed synchronously. Germ cells progressed through 6 distinct cytological stages during spermatogenesis *viz*., primary spermatagonia, secondary spermatogonia, primary spermatocyte, secondary spermatocyte, spermatid and spermatozoa as described for striped bass (Groman, 1982).

Primary spermatogonia: The germ cells located within the stroma of the tubule wall give rise to primary spermatogonia. These are large cells with eosinophilic cytoplasm (Fig. 8). A section of maturing testis is shown in Fig. 9.

Secondary spermatogonia: Primary spermatogonia undergo a series of mitotic division and produce a cluster of secondary spermatogonia within the cyst (James, 1946). According to Grier (1981) these cysts are formed by sertoli cells in all teleost testes. In cysts, mature stages of sperm

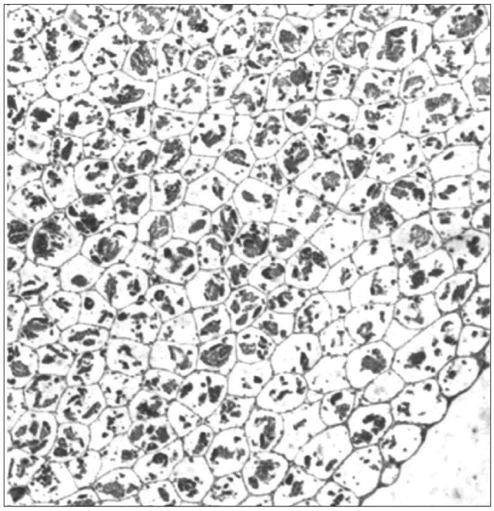


Fig. 8. Histological section of immature testis (H&E;X50). Germinal cells seen in the lobules

cells are located towards the inside of the tubule. Clusters of cells resulting from divisions of the original germ cell maintained a consistent stage of development within the cyst. Secondary spermatagonia were smaller than primary spermatogonia (Fig. 10) with lightly basophilic nuclei.

Primary spermatocytes: A second series of mitotic division results in the formation of cysts full of primary spermatocytes (Harder,1975). They are still smaller with basophilic nuclei (Fig 11 a and b).

Secondary spermatocyte: They are smaller than primary spermatocyte with less cytoplasm and increasingly basophilic nucleus. (Fig. 10 and 11).

Spermatids: Secondary spermatocytes undergo second meiotic division and spermatids are formed which are condensed, intensely basophilic and have very little cytoplasm (Fig. 12).

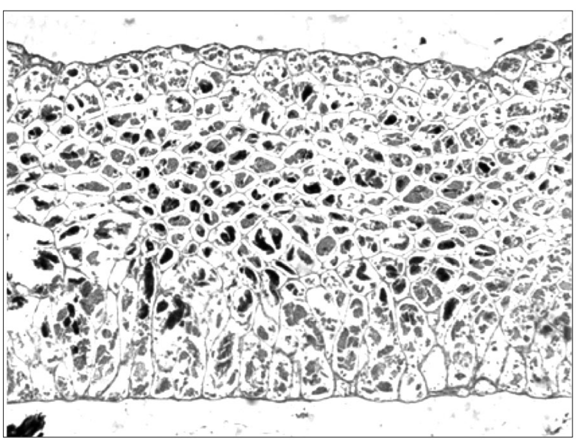


Fig. 9. Histological section of maturing testis (H&E; X50)

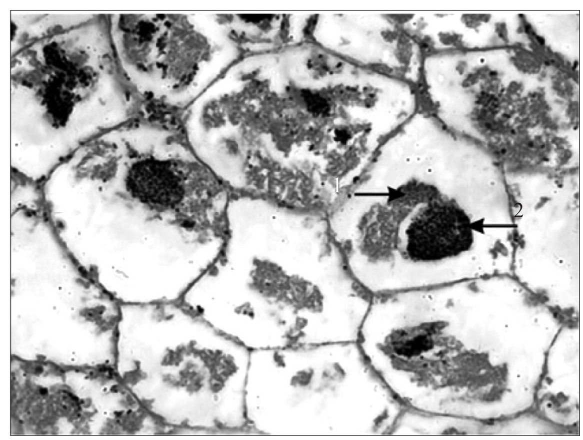
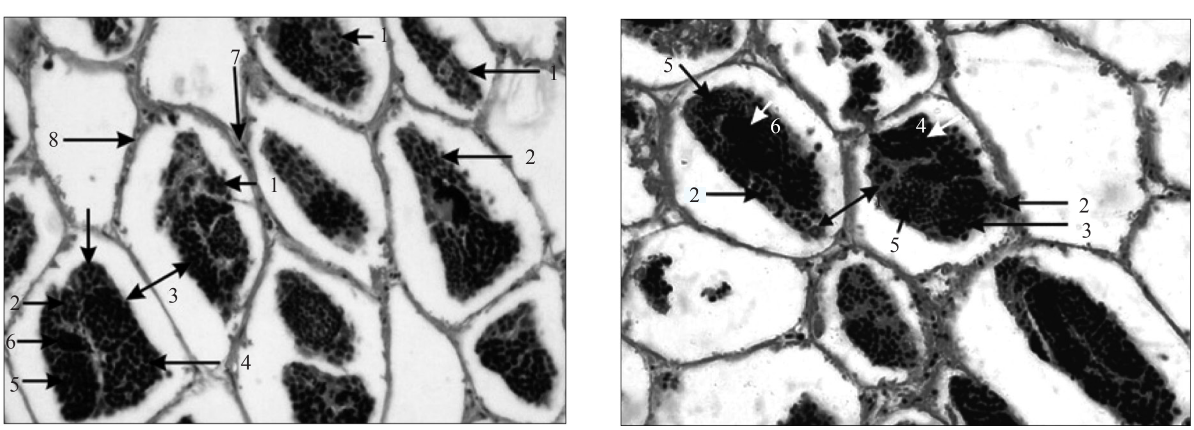


Fig. 10. Photomicrograph of maturing testis (H&E;X400). (1) Primary spermatogonia, (2) Secondary spermatogonia



(a) (b)

Fig. 11. Late maturing testis (H&E; 400). (1) Primary spermatogonia, (2) Secondary spermatogonia, (3) Nest of primary spermatocytes, (4) Nest of secondary spermatocytes, (5) Spermatids, (6) Spermatozoa, (7) Interstitial cells, (8) Seminiferous tubule (lobule)

Spermatozoa: The cysts rupture releasing the spermatids into the lumen where final maturation takes place (Cooper, 1952). Each spermatid further develops into spermatozoa (Fig. 11, 12 and 13) Mature and ripe testes are shown in (Fig. 14, 15 and 16 and spent testes in (Fig. 17).

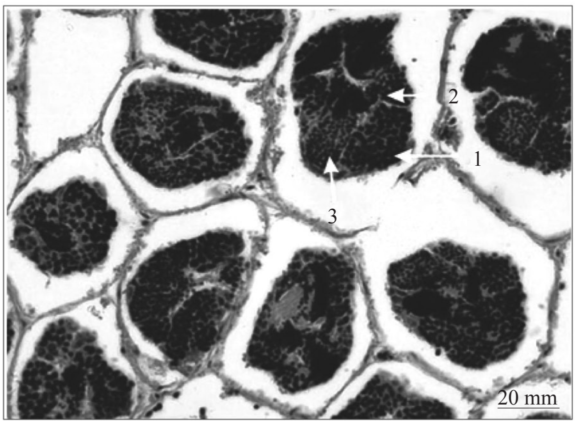


Fig. 12. Histological section of late maturing testis (H&E; X400). (1) Nest of primary spermatocytes, (2) Nest of secondary spermatocytes, (3) Spermatids

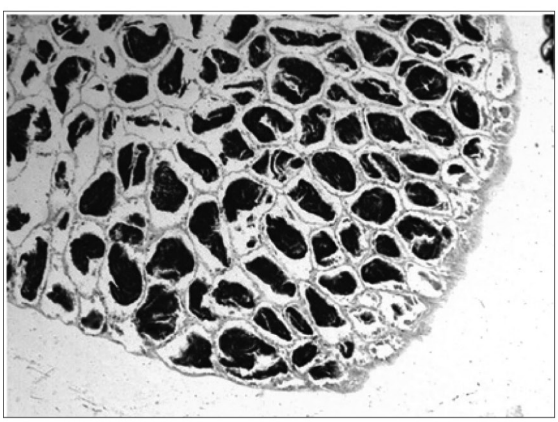


Fig. 13. Photomicrograph of histological section of late maturing testis (H&E; X100)

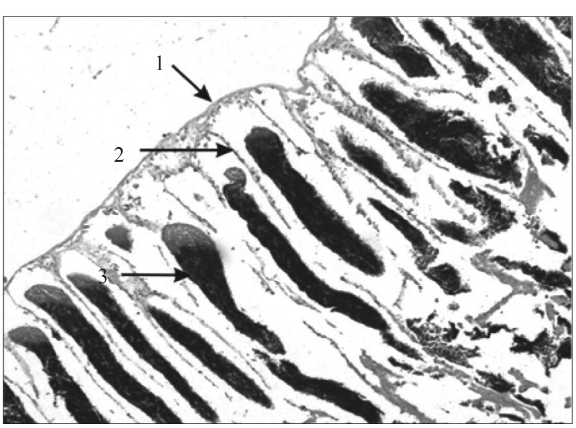


Fig. 14. Histological section of a mature testis (H&E; X100)

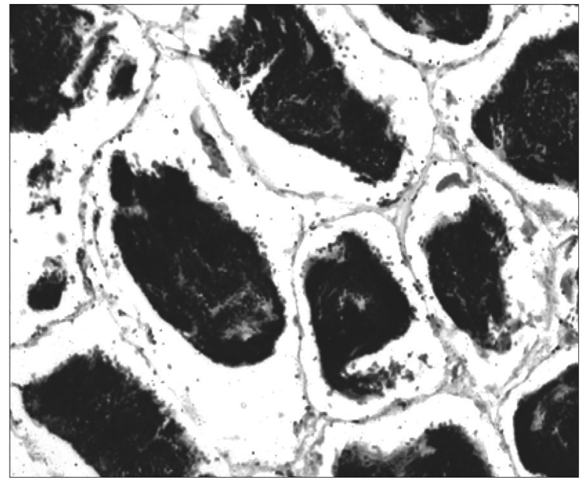


Fig. 15. Histological section of ripe testis showing seminiferous tubules packed with spermatozoa

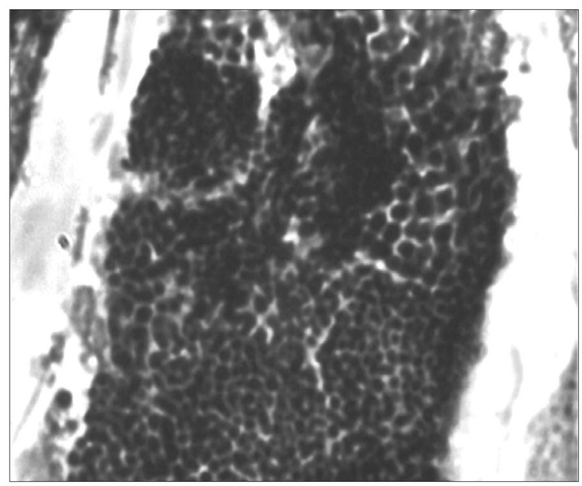


Fig. 16. Histological section of ripe testis showing seminiferous tubule packed with spermatozoa (H&E; X1000)

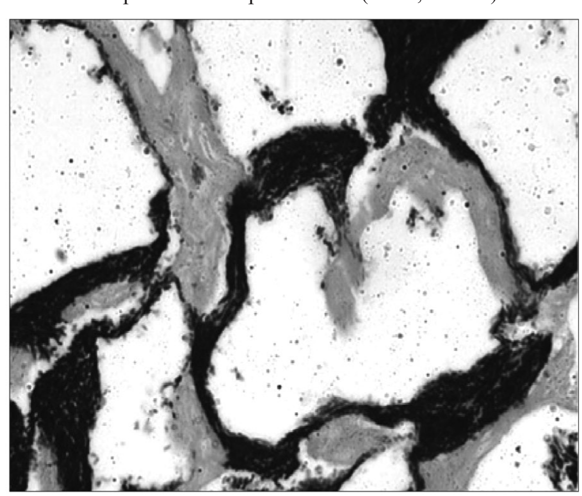


Fig. 17. Photomicrograph of cross section of spent testis (H&E; X1000)

Maturation of sperm in *D. russelli* occurred along the entire length of the seminiferous tubules and appeared to be unrestricted spermatogonial testis as classified by Grier (1981). As the germ cells progressed, their size diminished. No cell fitting the description of sertoli cells or Leydig cells were seen. Groman (1982) failed to histochemically identify sertoli cells or Leydig cells in striped bass. Drance *et al*. (1976) found that in rainbow trout, sertoli cells can be identified by their angular contour and irregularly shaped nucleus. Patt and Patt (1969) indicated that sertoli cells are present in all vertebrate testes and Grier (1981) reported that both cell types are common to all teleosts. Though histologically the above mentioned cells were not seen, their presence cannot be ruled out.

Under favourable spawning conditions, *D. russelli* was found to spawn many times in a year. This study would serve as useful input for management of fishery of the species and in the assessment of abnormal or pathological changes under stressful environmental conditions.

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