THE STRUCTURE OF THE TESTIS OF THE MULLET, *LIZA DUMERILI* (TELEOSTEI; MUGILIDAE) WITH SPECIAL REFERENCE TO SPERMATOGENESIS

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Accepted: February 1978

ABSTRACT

The macro-morphology and the histology of the testis of *Liza dumerili* are described with special reference to the various types of spermatogenic cells. *L. dumerili* possesses typical teleost testes. The only peculiarity is the conspicuous main sperm duct, which runs on the surface (medio-ventral) along the entire length of each testis. Testicular lobules contain cysts in which highly synchronized spermatogenesis takes place. Three generations of spermatogonia are evident, although further subdivisions are possible. Four types of primary spermatocytes are recognized. Typical interlobular Leydig cells appear in close association with small blood vessels. "Lobule-boundary cells" seem to be present but were only evident in 1 μm Epon sections.

INTRODUCTION

Histological studies of Mugilidae have been confined mainly to females and even here most attention has been paid to the later oocyte stages (Shehadeh & Ellis 1970; Zhitenev *et al.* 1974; Kuo & Nash 1975; Pien & Liao 1975). In contrast, the testes of many teleosts other than the Mugilidae have been studied in detail. Works on testicular structure and seasonal testicular changes include those of Turner (1919) on the perch, *Perca flavescens*, Geiser (1922) on the top minnow, *Gambusia affinis*, and the review of Oslund (1928) on seasonal modifications in the testes of vertebrates. These works established the cystic nature of the teleost testis.

More recently Marshall & Lofts (1956) contributed towards a better understanding of the Leydig cells of teleosts, although much confusion is still apparent as to the occurrence of these cells in the different teleost families.

Some of the more important contributions during the past fifteen years are those of Hyder (1969) on *Tilapia leucosticta*, Hiroi & Yamamoto (1970) on salmonid fishes as well as the reviews by Forbes (1961) and Lofts (1968). Although the above investigations have contributed considerably to our knowledge, it is evident that many key problems have not been solved. For example, much confusion exists as to the development and fate of the teleost spermatogonium, and the morphology of the sperm duct seems to be poorly studied. Moreover, Dodd (1960) stated that the histology of the teleost testis varies considerably in both complexity and arrangement of constituent parts. It would therefore seem to be difficult to generalize on teleost testicular morphology.

In this paper, the structure of the testis of *Liza dumerili*, one of the most abundant fish species to be found in estuaries along the south-east coast of South Africa, is described.

MATERIALS AND METHODS

Approximately 500 *Liza dumerili* (male and female) were captured in the mouth of the Swartkops Estuary (*ca.* 14 km north of Port Elizabeth) from August 1973 to October 1974. Most of these fish were used for studying the breeding cycle (Van der Horst 1976). Fish were taken to the laboratory in estuarine water and there transferred to a 700-litre PVC holding tank also filled with estuarine water. A model DKS 10a Turbelle filtration unit with a combined ultraviolet lamp and protein skimmer were used to filter and aerate the water.

Testes were obtained from *L. dumerili* after anaesthesia in 0,01% MS 222 in sea-water. This allowed adequate time for dissection and fixation.

Testes of 50 males were either fixed in Bouin's Fluid or cut into smaller pieces and fixed overnight in 3% glutaraldehyde (pre-cooled to 4°C). The Bouin's-fixed testes were dehydrated, embedded in paraffin wax, and 5 to 7 μm sections of the anterior, middle and posterior part of each testis were stained with Harris's haematoxylin and eosin Y and mounted in either Clearmount or DPX.

The glutaraldehyde-fixed testes were transferred to phosphate buffer (pH 7,4), cut into smaller blocks (1 mm^3), postfixed in 1% osmium tetroxide for an hour, washed again in buffer and dehydrated. No. 2 gelatin capsules were filled with Epon 812 and the material embedded and polymerized for three days at 50°C. One-μm sections were cut with glass knives using an LKB ultramicrotome and stained with 2% toluidine blue and boric acid over a flame. The sections were allowed to dry and mounted in DPX.

The different spermatogenic cells were measured under oil immersion (1 000 ×). Both bright field and Nomarski differential interference microscopy were used.

RESULTS AND DISCUSSION

Macro-morphology of the testes

The testes of *L. dumerili* are long, flat to slightly folded, structures, situated dorso-lateral to the alimentary canal, and ventral to the large swim-bladder. Each testis is suspended by the mesorchium, which is continuous with the visceral peritoneum of the swim-bladder and which runs along the entire length of the testes, as in *Eucalia inconstans* (Ruby & McMillan 1970). The left testis is always the longer of the two and extends anteriorly to the middle cardiac part of the stomach, to which it is attached by connective tissue. The right testis on the other hand, extends only to the pyloric part of the stomach. Both testes run posteriorly to the hind part of the abdominal cavity, where they unite for a short distance just anterior to the common sperm duct and urinary bladder.

The main sperm ducts are macroscopically visible only during the breeding season. The ducts run along the ventro-medial surface of the testes and unite with one another to form a common sperm duct, which opens into a urogenital pit, just posterior to the anus. The term "sperm duct", also used by Sadleir (1973), is preferred to ductus or vas deferens. The term "vas deferens" implies that it is embryologically derived from the Wolffian duct, which is

only found in the Selachii, Chondrostei and Holostei among fish (Romer 1970). The male "gonoduct" originates from the mesonephros and is therefore not a true vas deferens. A main testicular artery and vein run parallel to each main sperm duct and, respectively, give off and receive several branches to and from the testes.

The course of the main sperm ducts of *L. dumerili* seems to differ from all other teleosts studied. In *Engraulis malabaricus, Punctus colus* (Desphandi & Nadkarni 1973), *Eucalia inconstans* (Ruby & McMillan 1970) and salmonids (Henderson 1967), the main sperm ducts are short and extend superficially from the posterior parts of the testes, unlike those found in *L. dumerili* and probably other Mugilidae. Apart from this and other minor differences, the position and macro-appearance of the testes of *L. dumerili* are very similar to those of other teleosts.

Histology of the testes and spermatogenesis

A fairly thin tunica albuginea, which consists of moderately dense, irregular connective tissue with coarse bundles of collagen, surrounds the testis. The main sperm duct seems to be formed from a specialized evagination of the tunica albuginea (Figure 1), and is open towards the testis over almost its entire length. Although the wall of the duct histologically resembles the tunica albuginea, it is much thicker than the latter and in addition contains smooth muscle fibres.

The main seminiferous tubules or lobules are blind-ending towards the peripheral or lateral sides of the testis and extend over the width of the testis. These lobules run medially into tertiary ducts, which connect with longitudinal collecting or secondary sperm ducts (Figure 1). These secondary sperm ducts run parallel to the primary or main sperm duct. The walls of the lobules consist mainly of collagenous fibres, which are continuous with the tunica albuginea. The lobules do not seem to be extensively branched. Within these lobules and the tertiary ducts, cystic spermatogenesis, which is a typical teleost feature, takes place. I found that spermatogenesis first starts in the anterior and medial parts of the testis and then proceeds towards the peripheral and posterior parts.

The mean diameter of the different spermatogenic cells and their nuclei is given in Table 1.

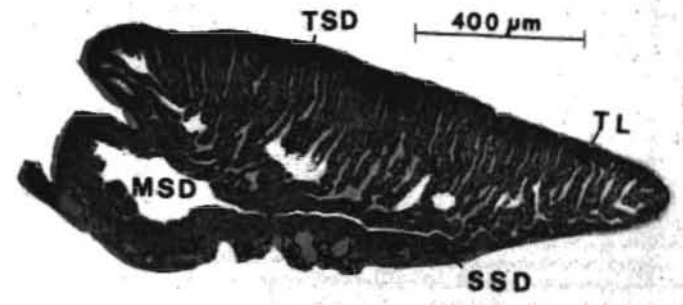
Great confusion is apparent as far as the terminology of teleost spermatogonia is concerned. In a detailed study on *Poecilia reticulata*, Billard (1969) found as many as 14 successive generations of cells between the stem cells (earliest spermatogonia) and the spermatocytes. In *L. dumerili*, however, three distinct generations of spermatogonia were identified on the basis of cell and nuclear diameters and appearance of the cytoplasm, although these might probably be subdivided using the criteria outlined by Billard (1969) for *Poecilia reticulata.* In *L. dumerili* the spermatogonia of the largest generation are aproximately 12 μm in diameter. Each of these cells contains a large spherical nucleus approximately 7,4 μm in diameter with a prominent nucleolus (Figures 2–4: RPS). The cell outlines appear well defined and the cytoplasm contains many small vacuoles as well as inclusions which stain intensely with toluidine blue. These cytoplasmic details could be observed only in the 1–2 μm sections stained with toluidine blue and boric acid. In routine H and E stained sections, the cytoplasm of these cells appears clear. Hyder (1969) and Hiroi &

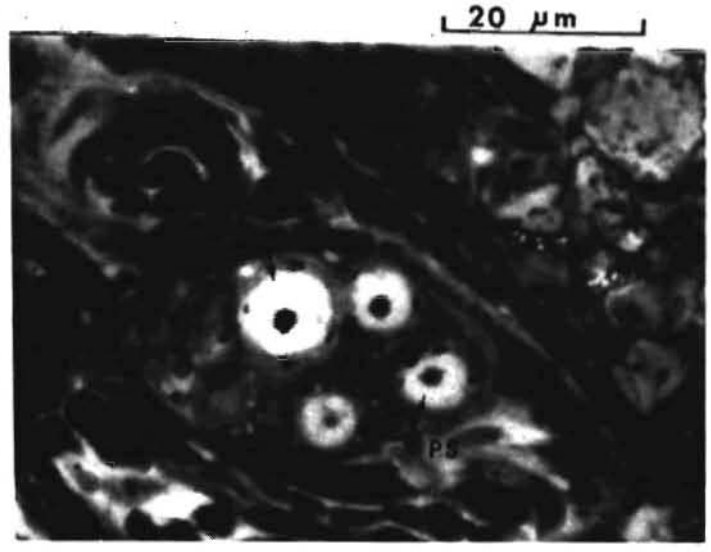
Yamamoto (1970) observed similar cells in the testes of *Tilapia leucosticta* and *Oncorhynchus masou* respectively. Since these cells seem to be present in the testes of *L. dumerili* throughout the year, although much less obvious and difficult to locate during the breeding cycle, they would appear to be the earliest spermatogonia. I term these cells residual primary, or resting primary spermatogonia, because they are evident even when the testes reach their maximum state of ripeness. Testes at this stage are filled to capacity with sperm and the only other cells present are these residual primary spermatogonia. A few of these cells are in close contact with the tunica albuginea and some can be seen along the testicular lobules. It would seem unlikely that any of these cells could give rise to sperm in the same breeding cycle and they therefore seem to be "resting". Hiroi & Yamamoto (1970) also refer to these cells as resting spermatogonia, while Wilson (1925) and Gokhale (1957) term them primary spermatogonia. Hyder (1969) and Hiroi & Yamamoto (1970) found these cells singly in the testes of *Tilapia leucosticta* and *Oncorhynchus masou.* Although they appear as single cells in *L. dumerili*, they were also found in clusters of two to four, or in clusters together with the second type of spermatogonium. This second generation of spermatogonia is morphologically very similar to the residual primary spermatogonia, except that the cells are smaller (8,6–10 μm in diameter) and contain more cytoplasmic vacuoles. Since they resemble the residual primary spermatogonia in great detail, I term them primary spermatogonia (Figures 2, 3: PS). They

TABLE 1.

Average size and size ranges (in μm) of the spermatogenic cells (except sperm) of *Liza dumerili.* (At least 50 cells of each spermatogenic cell type were measured using ten fish.)

	Spermatogonia			Spermatocytes		Spermatids
	Residual primary	Primary	Secondary	Primary	Secondary	
Mean cell diameter	12,10	9,1	7,1	6,1	4,9	4,3
Range	10–15,40	8,6–10	6,2–8,1	5,7–6,5	4,2–5,4	—
Mean nuclear diameter	7,4	6,1	5,5	4,3	2,9	2,3
Range	6,8–8,6	5,7–6,4	4,3–6,0	4,30–4,6	2,9–3,6	2,1–2,9





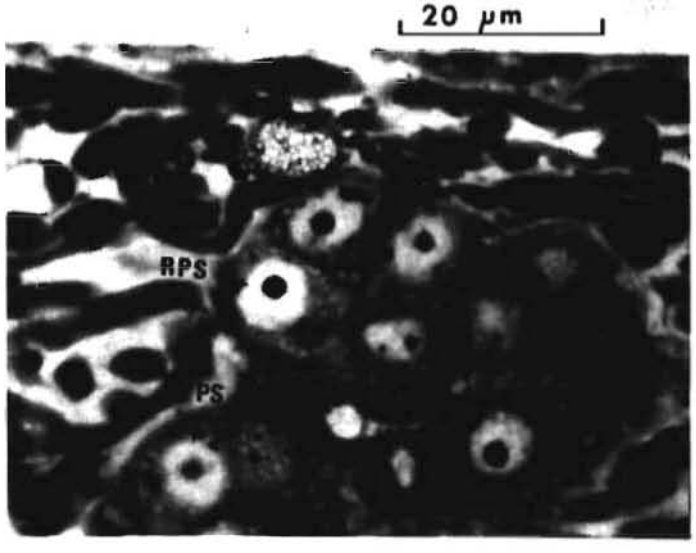


FIGURE 1 (upper left).

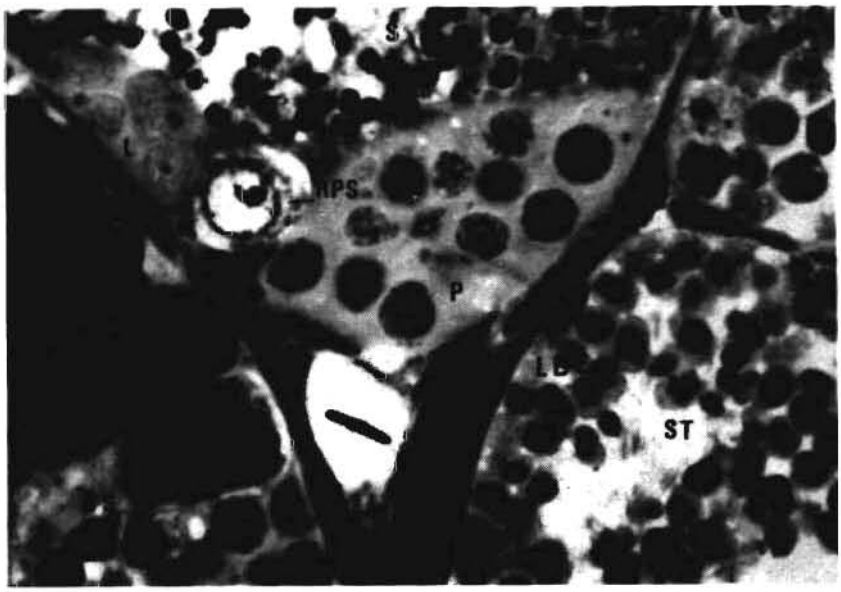
Low power magnification of transverse section through testis showing the various ducts. MSD, main sperm duct; SSD, secondary sperm duct; TL, testicular lobule; TSD, tertiary sperm duct.

FIGURE 2 (upper right).

High power magnification of residual primary spermatogonia (RPS) and primary spermatogonia (PS).

FIGURE 3 (lower left).

Cluster of approximately 10 spermatogonia, RPS, residual primary spermatogonium.



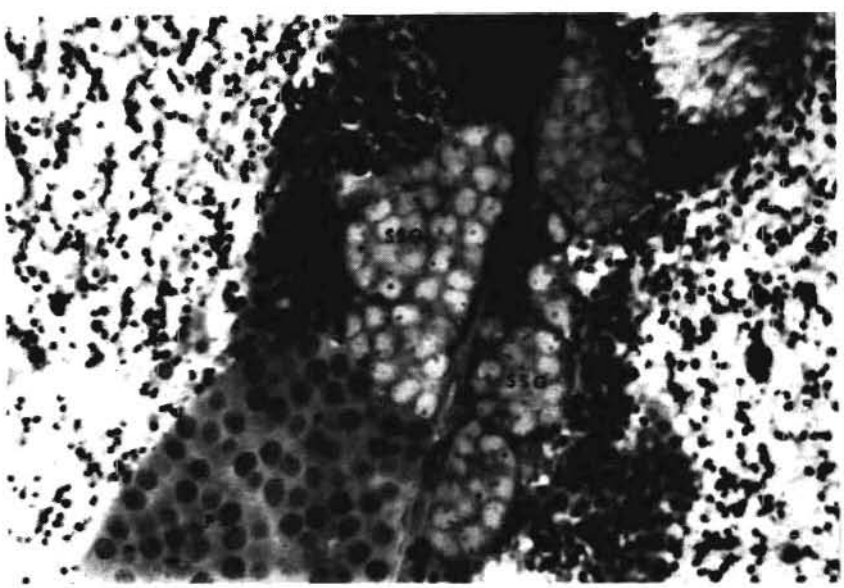


FIGURE 4 (above).

High power magnification of testis showing a residual primary spermatogonium (RPS), leptotene (L) and pachytene (P) primary spermatocytes, secondary spermatocytes (SS), spermatids (ST), mature sperm (S) and lobule boundary cell (LBC).

FIGURE 5 (below).

Section along two lobules showing testicular cysts with secondary spermatogonia (SSG), and leptotene (L) and pachytene (P) primary spermatocytes.

seem to represent the link between the residual primary spermatogonia and the secondary spermatogonia.

Clusters of as many as 16 cells containing both residual and primary spermatogonia were found in the testes of *L. dumerili*, although clusters with only primary spermatogonia were also evident. Hiroi & Yamamoto (1970) refer to cells in *Oncorhynchus masou*, resembling the primary spermatogonia of *L. dumerili*, as mitotic spermatogonia. Spermatogonia of the third generation occur only in cysts along the lobule walls and as many as 24 were noted in a single cyst. These cells seem to be comparable to the secondary spermatogonia of *Gadus merlangus* (Gokhale 1957) and *Tilapia leucosticta* (Hyder 1969) and are therefore named accordingly in *L. dumerili*. These secondary spermatogonia are 7,1 μm in mean diameter (6,1–8,1 μm); their cell outlines are very vague and cannot be distinguished in most cases, while the cytoplasm appears clear and does not readily take up stain. The nucleus contains one or two spherical nucleoli and the density of the chromatin material increases in comparison with the other spermatogonial generations (Figure 5). This feature seems to be common to "later" spermatogonial stages of teleosts as observed by Courot *et al.* (1970). Gokhale (1957) and Hyder (1969) found as many as 16 secondary spermatogonia per cyst in the testes of *Gadus merlangus* and *Tilapia* respectively. They furthermore believe that all these spermatogonia originate from one primary spermatogonium (equivalent to the residual primary spermatogonium of *L. dumerili*), because they never found more than one primary spermatogonium per cyst. Gokhale (1957) also states that at least four generations occur between the primary and secondary spermatogonia. Although 16 secondary spermatogonia per cyst were found in the testes of *Gadus* and *Tilapia* it does not necessarily imply that this number represents the true maximum. The chance of cutting through all the secondary spermatogonia per cyst in one section seems highly unlikely. The true number of cells per cyst can only be estimated on the serial sectioning of a number of cysts, and the reconstruction thereof afterwards. It seems then that many more secondary spermatogonia might be present per cyst as in *L. dumerili*. Finally it would appear that the secondary spermatogonia originate from the residual primary spermatogonium by a number of successive mitotic divisions. This idea is also more in line with Billard's (1969) observation on *Poecilia reticulata* as pointed out above.

The primary spermatocytes are 6,1 μm in mean diameter (5,7–6,5 μm) with a distinct spherical nucleus 4,3 μm in diameter. The earliest of these cells show some resemblance to the secondary spermatogonia, particularly in the pale-staining cytoplasm, with its very vague outline.

These cells show some resemblance to the preleptotene primary spermatocytes of mammals, which often seem to be confused with the spermatogonia from which they arise (Courot *et al.* 1970). The primary spermatocytes most often described in fish are those entering the prophase of their first meiotic division. Most investigators, however, do not distinguish between the different cell types of prophase. In *L. dumerili* it was possible to distinguish three phases of nuclear change in the primary spermatocytes, which seem to be similar to those reported for man by Bloom & Fawcett (1968 quoting Clermont 1963) and were therefore provisionally named Leptotene, Zygotene and Pachytene stages (Figures 4–6: *L, P, Z*).

The secondary spermatocytes were approximatey 4,9 μm in mean diameter (4,2–5,4 μm),

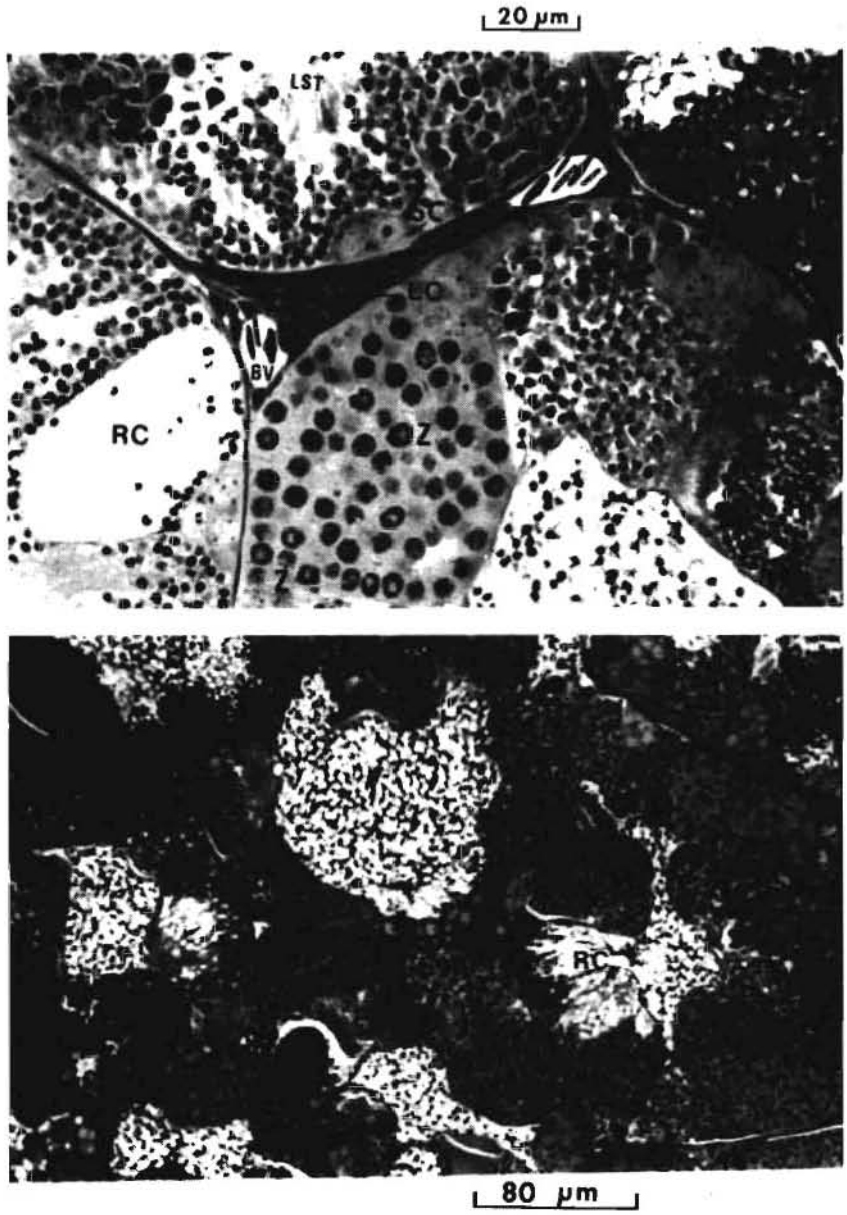


FIGURE 6 (above).
Section through testis showing a cluster of Leydig cells (LC) in close association with a blood vessel (BV), zygotene primary spermatocytes (Z), late spermatids (LST), a ruptured cyst (RC), sperm within lobule lumen, and somatic cell (SC).

FIGURE 7 (below).
Section through testes showing lobules with cysts (C) containing spermatogenic cells in all stages of development, and ruptured cyst (RC) releasing sperm into testicular lobule lumen.

with very dark-staining nuclei (*ca.* 2,9 μm in diameter), often with a lighter area towards the centre of the nucleus (Figures 4–6: SS).

The spermatids measure 4,3 μm in mean diameter with a nucleus of 2,3 μm which in most cases stained less densely with toluidine blue than the secondary spermatocytes (Figures 4, 6: ST). A cyst may start to rupture during spermiogenesis, but usually sperm maturation takes place within a cyst. In many cases mature sperm with their heads closely associated with the lobule wall were noted within a cyst (Figure 7). Eventually mature sperm are released from the cysts into the testicular lobule lumen (Figure 7).

The sperm nuclei stain intensely with toluidine blue and haematoxylin, and under high magnification the nucleus of each sperm cell appears bean-shaped (Figure 4: S). There is a long flagellum. In all teleosts studied so far, it has been shown that all spermatogenic cells undergo their maturation and divisions within a cyst (Forbes 1961; Lofts 1968). Within a particular cyst, all the cells will be in the same stage of development, which indicates a very high degree of synchronization (Figures 4–7). The only other cells present within the cyst are large and irregular in form and have an elliptical nucleus (Figure 6: SC). These cells show a striking similarity to the somatic cells in the testes of the Masu salmon, *Oncorhynchus masou* (Hiroi & Yamamoto 1970). Very few of these somatic cells could be located in *L. dumerili* and it is therefore difficult to speculate on their nature. They might be a substitute for the Sertoli cells.

According to Marshall & Lofts (1956) two distinct types of endocrine cells occur in the testes of fishes. The first and most obvious, the Leydig cell, occurs in the testes of *Gasterosteus aculeatus* (Craig-Bennet 1931), the whiting *Gadus merlangus* (Gokhale 1957), the Masu salmon *Oncorhynchus masou* (Hiroi & Yamamoto 1970), the sprat *Sprattus clupea* and *Tilapia spp.* (Marshall & Lofts 1956). This type of cell also occurs in some cartilaginous fish, as well as in *Latimeria* (Marshall & Lofts 1956). The second and less obvious type of endocrine cell occurs in the lobule walls of the testes in fish like the pike, *Esox lucious*, and the char, *Salvelinus willughbi*, and was named "lobule boundary cells" by Marshall & Lofts (1956). These latter investigators believe that the "lobule boundary cells" are derived from fibroblasts, which essentially form part of the walls of the seminiferous tubules of tetrapods. Furthermore, the "lobule boundary cells" seem to be lipoidal and cholesterol-positive and might therefore be homologous to true tetrapod interstitial cells or Leydig cells (Marshall & Lofts 1956). In *L. dumerili* tetrapod Leydig cells are present between the lobules, particularly in those areas where the walls of three lobules approach each other. The Leydig cells are always present in close proximity to small blood vessels (Figure 6: LC). They have an irregular outline, *ca.* 5 × 7 μm in diameter, and the nucleus is spherical to oval in form, *ca.* 4,9 μm in diameter. Cytoplasmic inclusions sometimes stain intensely with toluidine blue and give the cytoplasmic part of the Leydig cells a dotted appearance. Of particular interest is the relatively large number of fibroblasts present, not only within the areas between the Leydig cells, but also along the testicular lobule walls. Some of the cells along the lobule walls are unlike typical fibroblasts and seem to be suggestive of "lobule boundary cells" (Figure 4: LBC). The testis of *L. dumerili* may therefore represent a condition where both Leydig cells and lobule boundary cells are present. Hyder (1969) made a similar observation for *Tilapia.*

In conclusion it appears that the histological structure of the testes of *L. dumerili* basically resembles that of other teleosts. The only peculiarity is that the main sperm duct of *L. dumerili* runs along the whole length of the testis and is a specialized evagination of the tunica albuginea devoid of a distinct epithelium. In other teleosts the main sperm duct is short, extends from the posterior part of the testis and possesses a well defined columnar epithelium. The spermatogonia of *L. dumerili* seem to undergo several mitotic divisions before they give rise to the earliest primary spermatocytes which resemble those of higher vertebrates. Spermiogenesis is completed in the cyst before sperm are released in the testicular lobule lumen. Tetrapod-type Leydig cells are present as in many other teleosts and possibly also "lobule boundary cells" as in *Tilapia leucosticta.*

ACKNOWLEDGEMENT

I received financial support for the project from the University of Port Elizabeth, the Council for Scientific and Industrial Research and the South African National Committee for Oceanographic Research, and express my thanks to them as well as to Prof. T Erasmus for suggestions and constructive criticism. The University of Port Elizabeth also provided financial aid towards publication.

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