THE BREEDING CYCLE OF MALE *LIZA DUMERILI* (TELEOSTEI: MUGILIDAE) IN THE MOUTH OF THE SWARTKOPS ESTUARY

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ABSTRACT

The breeding cycle of male *Liza dumerili* was studied in the Swartkops Estuary using a visual index, a gonosomatic index and a histological index. Histological studies were superior to any other means of establishing the breeding cycle in detail. Male fish were in the inactive or non-breeding state during the winter months. During early spring the gonads started to mature and full ripeness was attained during December and January. January and February were the main spawning months, and in addition a short spawning peak might be present during October. The seasonal appearance of spermatogonia in the testes is discussed.

INTRODUCTION

According to Wallace (1974) the spawning period of *Liza dumerili* along the east coast of South Africa (Natal and estuaries) extends from June to August but probably also includes early summer. No account exists of the breeding cycle of the species along the south-east coast of South Africa (including the Swartkops estuary where this study was performed). The climatic conditions of these two areas differ considerably and it was therefore decided to investigate the breeding cycle in the Swartkops estuary as an example of a more temperate climate. Moreover, Wallace (1974) used only seasonal changes in the macro-appearance of the gonads as an index of spawning activity. In this report we extended investigations on the breeding cycle using several criteria, particularly histological methods.

MATERIALS AND METHODS

All fish used in this investigation were captured in the mouth of the Swartkops Estuary. The estuary enters Algoa Bay which is on the south-east coast of South Africa. Figure 1 is a map of the estuary indicating the general features while Figure 2 depicts the capturing areas near the mouth.

Approximately 500 *Liza dumerili* were captured from August 1973 to October 1974 and it was possible to catch at least 10 adult males between the 10th and 15th of each month. Fish were also captured during December 1974; January, February, March, July, August, October 1975 and October 1976.

Fish were captured, transported and maintained in the laboratory as described by Van der Horst (1976). Each fish was anaethetized in 0,01% MS 222. Anaesthesia allowed easy

measuring of the standard length (ls) and mass accurate to 0,1 g. The abdomen was opened with a mid-ventral incision from the pelvic fins to the vent. The colour and macroscopic appearance of the testes were noted. Both testes were removed and weighed to the nearest 0,01 mg. At least one testis of each fish was fixed in Bouin's solution or 2,5% buffered glutaraldehyde and then processed for histological studies as described by Van der Horst (1976). At least 10 adult males (ls = 150–180 mm) per month were used for the above investigations.

The diameter of the main sperm ducts and testes lobules was measured with the aid of a microscope to the nearest micro-metre.

The criteria used to evaluate the breeding cycle of male *L. dumerili* were basically similar to those reported in the literature (Gokhale 1957; Ruby & McMillan 1970; Hiroi & Yamamoto 1970; Nayyar & Sundararaj 1970; De Silva 1973; Hyder 1969). The most important criteria

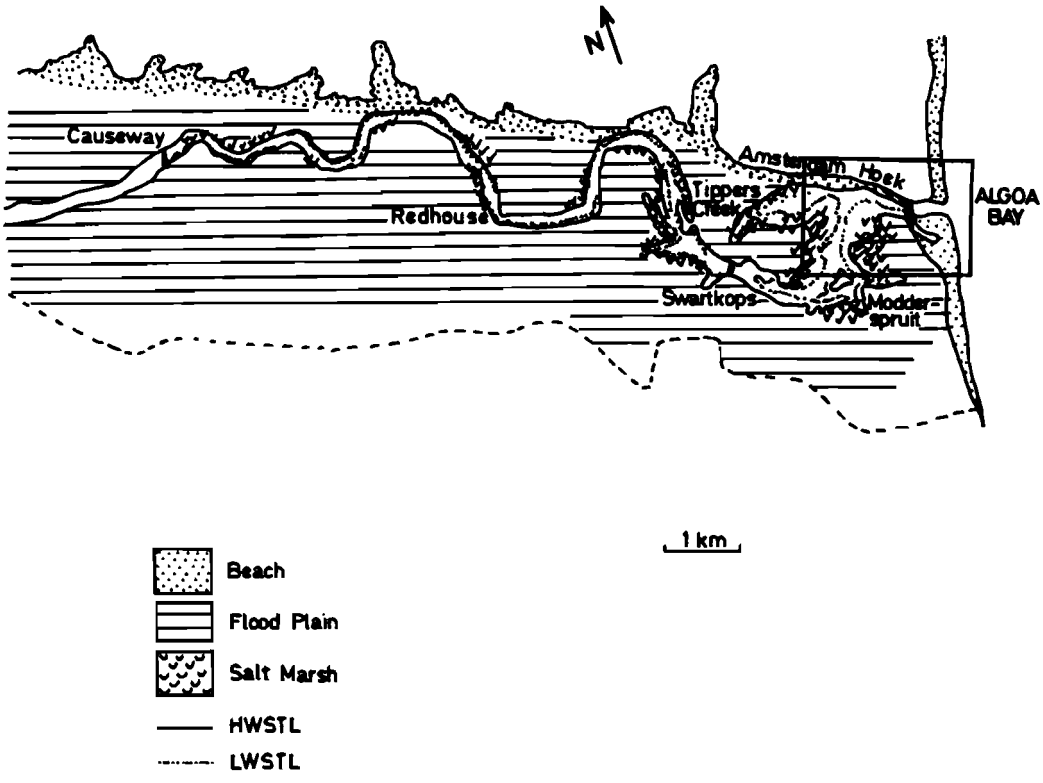


FIGURE 1.

Map of the Swartkops Estuary showing the flood plain, salt marshes, intertidal areas and main creeks. Map from McLachlan (1972).

used by these investigators were: a visual inspection of the testes; the gonosomatic index (GSI —testes mass expressed as a percentage of body mass) and a seasonal histological study of the testis. In order to quantify the results, we used the following three main criteria:

1. The visual appearance of the testes, which could be divided into six distinct stages, called the testicular visual stages (TVS), outlined in Table 2 and illustrated in Figure 3.
2. Gonosomatic index of males (GSI = testis mass expressed as a percentage of the body mass) (Table 1; Figure 3).
3. A histological study; eight histological stages (THS) (Table 2) and the average monthly THS (Figure 3).

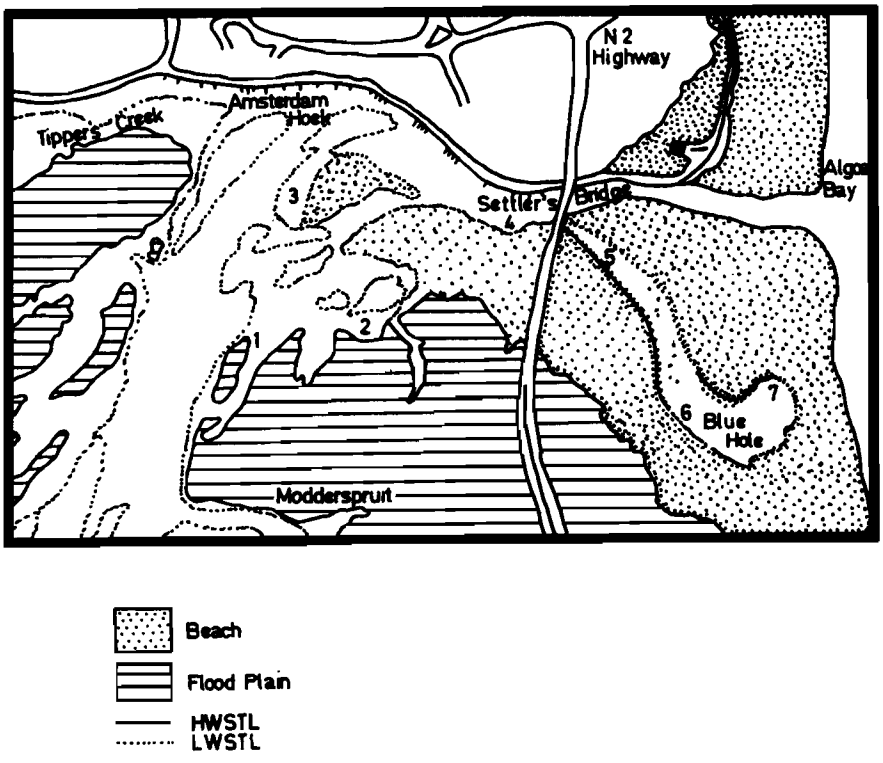


FIGURE 2.

Enlarged map of part of estuary (indicated by square in Figure 1), indicating the seven most common capturing sites (1–7).

RESULTS

From a seasonal study of the testis it was possible to distinguish six reproductive phases (Tables 1 and 2).

(a) Inactive phase
(b) Early recovery phase
(c) Active recovery phase
(d) Pre-spawning or ripe phase
(e) Spawning phase
(f) Post-spawning or spent phase

In order to compare the photographs representing the different THS (Figures 4–8), the same magnification has been used except for Figures 9 and 10; thus, testicular structures are directly comparable.

(a) *Inactive phase* (Figures 3, 4)

The testes were minute and stringlike; their appearance varied from white to translucent and the main blood vessels were just visible (TVS 1). This picture was typical from April to August (1973–1975) and in almost all fish studied during this period no variation was evident. The slightly higher GSI value for April 1974 in comparison with those for May to August 1974, was probably due to the fact that 12% of the April fish (Figure 3) were in the spent condition, when the testes contained remnants of sperm (Figure 10).

TABLE 1.

Average gonosomatic index (GSI) and month(s) for each reproductive phase.

Reproductive phase	Month	Average GSI
(a) Inactive	April and May	0,10
(b) Early recovery	June and July	0,05
(c) Active recovery	Late August and early September	0,25
	September, October and November	0,81
(d) Ripe	December and January	1,35
(e) Spawning	January, February and early March	1,25
(f) Post-spawn or spent	March	0,35

TABLE 2.

Testicular histological stage (THS) and testicular visual stage (TVS) for each reproductive phase.

Reproductive phase	THS	TVS
(a) Inactive	*Stage 1:* Few primary spermatogonia scattered between randomly dispersed connective tissue (collagenous fibres and fibroblasts; few mast cells). Few sperm from previous breeding cycle still present in main sperm duct.	*Stage 1:* Testes minute, thread-like, white to translucent. Main sperm duct not macro-anatomically visible. Crushing of testes yields few sperm from previous breeding cycle.
(b) Early recovery	*Stage 2:* Early spermatogonial proliferation. Increase in residual primary and primary spermatogonia. No clear testis lobule organization. No traces of sperm.	*Stage 1:* As above.
	Stage 3: Further increase in spermatogonia and testis lobule organization.	*Stage 1:* As above.
(c) Active recovery	*Stage 4:* Early spermatocytogenesis. Testes packed with cysts of spermatogonia. Testis lobules organized as in Stage 3. A few nests of primary and secondary spermatocytes.	*Stage 2:* Testes visibly larger than Stage 1. Colour changing from white to cream with a rosy touch. Gonoducts macro-anatomically visible.
	Stage 5: Active spermatocytogenesis, meiosis and spermiogenesis All testis lobules filled with cysts of primary and secondary spermatocytes and spermatids. Sperm content of testis and main sperm duct varying from few sperm present to packed with sperm.	*Stage 3:* Testes cream to completely white and flat. Main sperm duct clearly visible and distended with sperm. Milt with milky consistency obtainable from sperm duct. *Stage 4:* Although also included under active recovery, typical for ripe phase. See below.
(d) Prespawning or ripe	*Stage 6:* Late spermiogenesis. More than 90% of testis filled with sperm. Most cysts containing spermatids and few with primary and secondary spermatocytes. Main sperm duct filled almost to capacity with sperm.	*Stage 4:* Testes at maximum size, white and very swollen, and tending to burst open while handling. Main sperm duct very large. Milt obtainable by slight pressure on abdominal region.
(e) Spawning (ripe-running)	*Stage 7:* Spermiogenesis complete. Testis filled with sperm. No other spermatogenic cells present except residual primary spermatogonia. Movement of sperm from peripheral lobules towards sperm ducts. Main sperm duct maximally distended.	*Stage 5:* Often difficult to distinguish from Stage 4, but generally testes slightly smaller. On handling milt flows from vent. Main sperm duct maximally distended.
(f) Post-spawn or spent	*Stage 8:* Testis lobules with few sperm. Lobules broken up and infiltrated by collagenous fibres. Spermatogonia present in large numbers. Sperm within lobule walls indicating phagocytic activity.	*Stage 6:* Testes resembling Stage 2, small, cream to rosy in colour though main sperm ducts clearly visible and containing sperm. Little milt obtainable from main sperm duct.

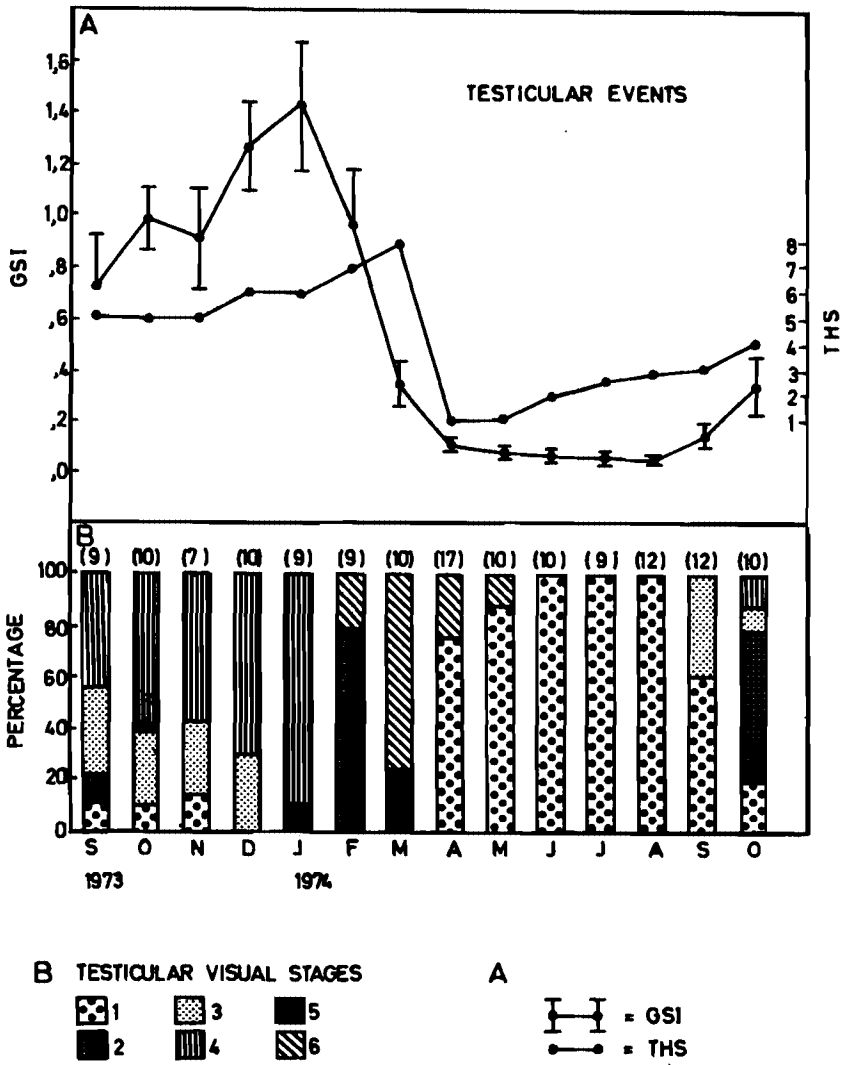
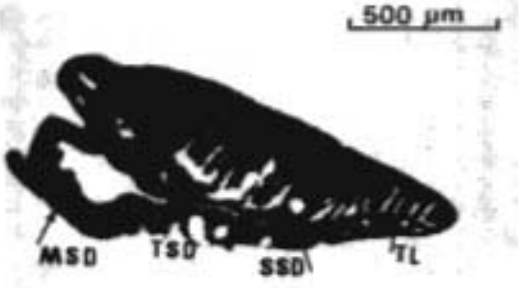


FIGURE 3.

A. Monthly average gonosomatic index (GSI) and testicular histological stage (THS) of male *L. dumerili* (1973–1974). T-bars indicate standard error of the mean.

B. Monthly composition of fish with testes in different visual stages (TVS) expressed as a percentage of total monthly number of fish (1973–1974). Numbers of fish above histograms.

During April and May the testes were typical of THS 1. They consisted mainly of connective tissue with a few residual primary spermatogonia. Randomly dispersed collagenous fibres, a small amount of elastic fibres and loose connective tissue, mainly fibroblasts and mast cells, contributed to the bulk of the inactive testes. A few sperm remnants from the previous breeding cycle were present within the main sperm ducts of some fish. Sperm remnants were noted in 25% of the fish examined during April and 12% of the fish examined during May and represented the "late spent fish" which had testes in TVS 6 (Figure 3). Matthews (1938) also noticed clusters of sperm in the inactive testes of *Fundulus*, which were apparently remnants from the previous period of sexual activity.



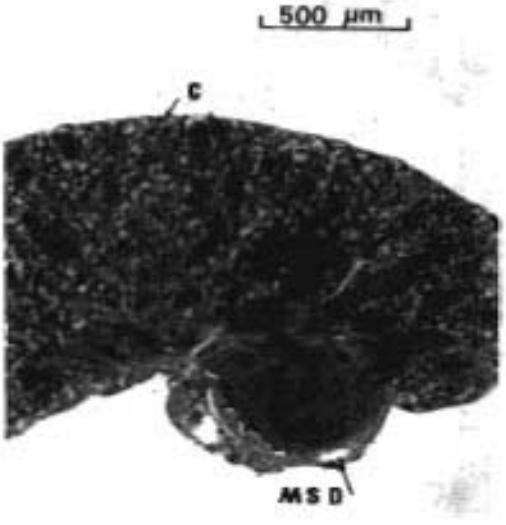


FIGURE 4 (top left).

T.S. inactive testis (THS 1). MSD, main sperm duct.

FIGURE 5 (bottom left).

T.S. early recovery testis (THS 3). MSD, main sperm duct; SSD, secondary sperm duct; TL, testicular lobules; TSD, tertiary sperm duct.

FIGURE 6 (right).

T.S. active recovery testis (THS 5). C, cyst with various spermatogenic cells; MSD, main sperm duct with sperm.

(b) *Early recovery phase* (Figures 3, 5)

The early recovery phase started in June and proceeded until August. Visually the testes were similar to those of fish in the inactive phase (TVS 1). The GSI values over this period were the lowest recorded for the reproductive cycle with a minimum value of 0,047 for July. *Per se*, this would be indicative of the most inactive period of the non-breeding part of the cycle. Histological evidence, however, cleary indicated an early recovery phase which histologically represented THS 2 and 3. The most obvious feature of the THS 2 testes was the progressive increase of spermatogonia especially evident along the peripheral parts of the testes. The testes were still disorganized, with connective tissue randomly dispersed, and this appeared to be the typical histological picture for all testes in June and some testes in July.

Testes in THS 3 showed a marked increase in spermatogonia particularly along the peripheral parts. The most dramatic change, however, was the organization of the testis lobules into longitudinal rows, running from the peripheral to the medial part of the testes (Figure 5). The organization of the testes in its constituent parts, particularly the lobules and collecting ducts, was most noticeable during this stage.

The THS 3 testes of *L. dumerili* were similar to the Stage 3 testes of the whiting (Gokhale 1957) and the Stage 2 testes of the brook stickleback (Ruby & McMillan 1970).

(c) *Active recovery phase* (Figures 3, 6)

The testes became visibly larger, with a colour change from white to cream with a rosy touch (TVS 2). The gonoducts, though small, were first observed during this stage. All the TVS 2 testes were in THS 4 and this was only evident during the early recovery phase. THS 4 is referred to as early spermatocytogenesis, since only nests or cysts of primary and secondary spermatocytes were visible. In some of these testes a few cysts of spermatids were observed, but they were uncommon.

Testes in TVS 3 were cream to homogeneous white, and flat. The main sperm ducts were usually distended with milt which was of a milky constitution (Figure 6). All testes in TVS 3 were always in THS 5. Testes in this phase of development showed active spermatocytogenesis, meiosis and spermiogenesis, and the testes were packed with cysts containing all types of spermatogenic cells (each cyst contained only one cell at a specific stage of development). Sperm content in THS 5 testes varied from a few clusters of sperm to testes approximately half-filled with sperm.

Although testes in TVS 4 were more typical for fish with testes in THS 6 (ripe phase) many fish were captured during September, October and November 1973 which had testes in TVS 4 but only in THS 5. TVS 4 is therefore also included under the active recovery phase (Table 2). Testes in TVS 4 reached their maximum size and were milky white and very swollen. Milt could be extruded from these fish by exerting slight pressure on the abdomen. During September 1973, 65 percent of fish examined were in TVS 1, 2 and 3 and as many as 44 percent were in TVS 4 although all fish in TVS 3 and 4 were in THS 5. The average GSI for September 1973 was 0,73 (Figure 3). During October 1973 the GSI increased to almost 1,0 and 60 percent of fish examined had testes in TVS 4. Although these fish were still in THS 5 their main sperm ducts were almost fully extended with milt. Since fish examined during

November had a slightly lower average GSI than in October, probaby because fewer fish had testes in TVS 4 (58%), it is suggested that some fish might have spawned during October 1973.

It was of particular interest that fish captured during September 1974 had a GSI of only 0,13 and most fish examined (60%) had testes in TVS 1 and THS 3. They were therefore in the early recovery phase as compared to September 1973 fish which were already in the active recovery phase. Fish captured during October 1974 had testes mainly in TVS 2 (60%) and THS 4. Only 10% of the fish examined during October had testes in TVS 4 and THS 5 (Figure 3).

It can therefore be noted that fish captured during September and October 1973 were in the active recovery phase while fish captured during the same period of 1974 were only in the early recovery phase. It was furthermore noted that the GSI values for September and October 1973 were statistically significantly higher than for September and October 1974 respectively ($p < 0,01$). On the basis of these facts it would appear that the breeding season commenced at least one and a half months later in 1974 than in 1973.

The diameter of the main sperm duct for the active recovery phase was 494 μm (Table 3). The average testis peripheral lobule diameter increased from 97 μm (range 58–105 μm) in September to 128 μm (range 100–190 μm) in October, to reach a diameter of 111 μm (range 95–130 μm) in November.

(d) *Prespawning or ripe phase* (Figures 3, 7)

The prespawning or ripe phase extended from approximately middle December to January. Seventy percent of fish examined during December and 80% during January had testes in TVS 4 (Figure 3). The GSI increased from a December value of 1,27 to reach a maximum value of 1,43 in January (Figure 3). The highest individual GSI (2,46) was also recorded during January 1974.

All testes examined during this period were filled to capacity with sperm. This was particularly obvious for January. In some December testes only very few cysts with primary and secondary spermatocytes were evident, although many late spermatids could still be observed. A few persistent residual primary spermatogonia could still be detected along the tunica albuginea.

The main sperm duct diameter for December and January was 661 μm. The peripheral lobule diameter increased to 142 μm in December, to reach a maximum diameter of 149 μm in January (Table 3). Fish during this period were in the fully ripe state and on the point of spawning (Figure 7).

(e) *Spawning phase* (Figures 3, 8)

The main spawning period occurred during January and February. These fish had large testes and on mere handling, large volumes of a viscous milt were ejected (TVS 5). Seventy-eight percent of fish captured during middle February 1975, had testes in the late spawning state. Although these testes generally appeared smaller than those in January, the main sperm ducts reached their maximum size (1050 μm diameter) (Table 3, Figure 8). The much

lower GSI-value of February compared to January, was probaby due to the loss of sperm.

In the early spawners a movement of sperm from the lateral towards the medial parts of the testes and main sperm duct, was clearly observed. This resulted in emptying of the distal parts of the lobules first with subsequent decrease in diameter which could be measured. The peripheral lobule diameter was 142 μm and the medial lobule diameter (close to the collecting ducts) was 197 μm (134–340 μm). Only sperm were evident in the testes (Figure 8) and no other spermatogenic cells were noted, except for a few persistent residual primary spermatogonia (THS 7).

(f) *Postspawning or spent phase* (Figures 3, 9, 10)

Fish with testes in the spent phase were evident from middle February (12%) and especially during March (76%), judged on a visual basis (Figure 3). These testes resembled the TVS 2 testes to a large extent. Small testes, dirty cream to rosy in colour, with a clearly visible main sperm duct, were the most important features. The GSI dropped to 0,33 in March and was significantly lower than the GSI for February ($p < 0,01$).

The histological picture of the spent testis (THS 8) differed drastically from that of any other stage. In the early spent testis the lobule walls became much thicker, and connective tissue started to invade the lobule lumen. Many sperm were still present (Figure 9). In the late spent testis the lobule walls started to rupture (Figure 10) and collagenous fibres invested the

TABLE 3.

Average diameter and range of main sperm duct and peripheral testicular lobules in different reproductive phases.

Reproductive phase	Average main sperm duct diameter and range (μm)	Average testicular lobule diameter and range (μm)
Inactive	254 (200–310)	
Early recovery	253 (168–290)	
Active recovery	494 (419–539)	112 (58–190)*
Ripe	661 (543–810)	146 (113–226)
Spawning	1050 (905–1333)	142 (92–288)

* Excluding THS 4 which was small and difficult to measure.

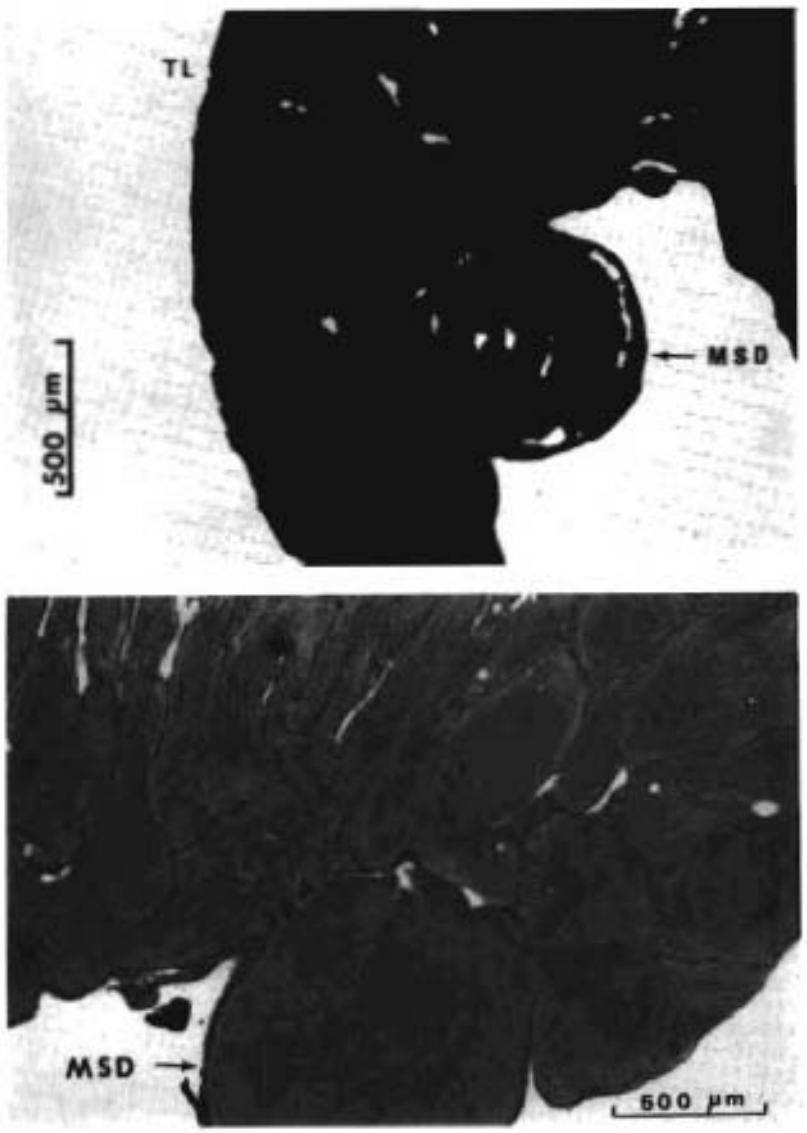


FIGURE 7 (above).

T.S. ripe testis (THS 6). Testicular lobules (TL) and main sperm duct (MSD) with sperm.

FIGURE 8 (below).

T.S. ripe testis (THS 7). Main sperm duct (MSD) maximally distended with sperm.

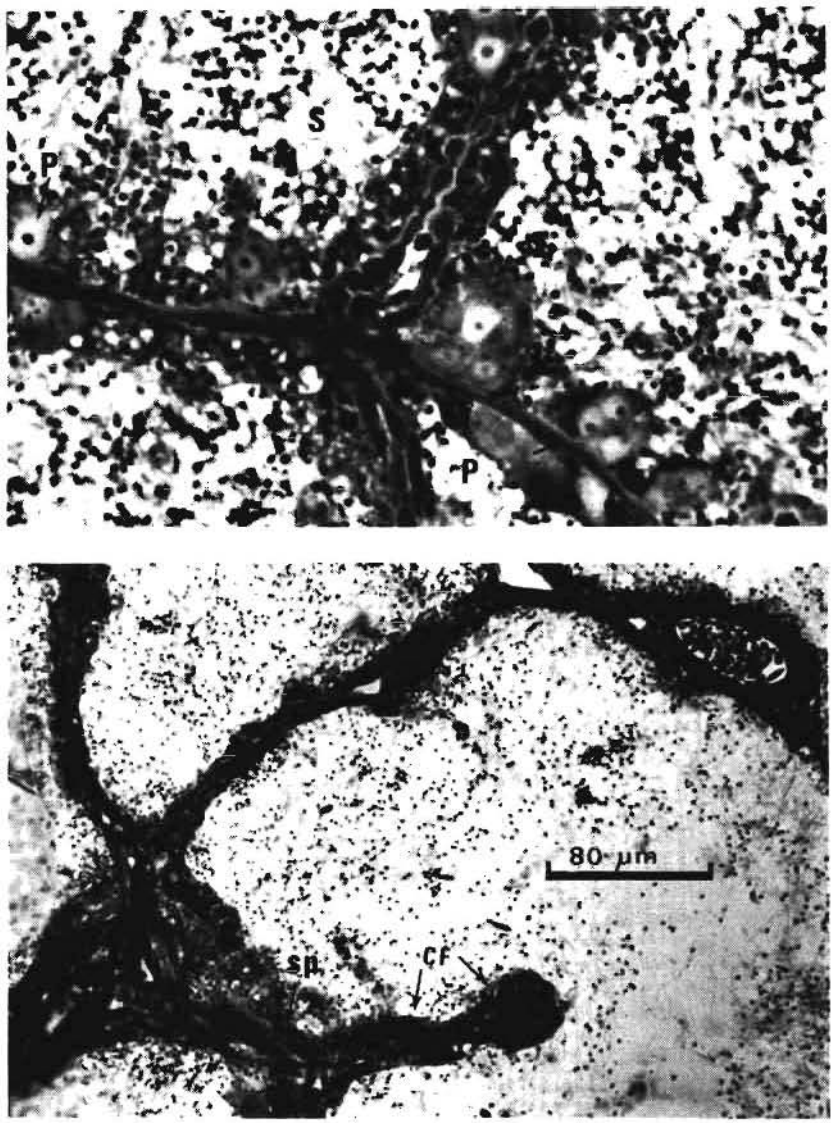


FIGURE 9 (above).

Early spent testis (THS 8) more highly magnified. Large numbers of spermatogonia (P) have appeared. Many sperm (S) still present.

FIGURE 10 (below).

T.S. late spent testis (THS 8). Lobules breaking up and collagenous fibres (CF) infiltrating lobules. Sperm phagocytosis taking place (SP).

lobule lumen to a great extent. Eventually the testis appeared disorganized and collagenous fibres with many fibroblasts were randomly dispersed. The testis began to resemble the inactive testis. Ruby & McMillan (1970) found many phagocytes in the spent testes of *Eucalia inconstans*. They also observed actual phagocytic invasion into the lobule lumen, and demonstrated with fresh testis material that the phagocytes ingested large numbers of sperm. Van der Horst (1976) also observed active phagocytosis of sperm in the testes of *L. dumerili* (after ultrastructural studies). The most interesting aspect of the THS 8 testis of *L. dumerili* was the appearance of many spermatogonia, which invaded the lobule walls (Figure 9). Great spermatogonial proliferation was therefore evident. It was thus clear that new spermatogonia for the following season had already started to form towards the end of the present breeding season.

DISCUSSION

It appeared that the breeding cycle of male *L. dumerili* started in September, full ripeness was attained during December and early January and spawning possibly took place from middle of December until the middle of March. The main spawning period appeared to be January and February. No fish showed breeding activity from April to August. The fact that the main sperm duct was considerably distended during October 1973 as compared with September and November 1973, supported the idea of a short spawning period also during October. Data obtained during 1975 and 1976 further supported this idea. During 1974, however, the breeding cycle commenced one to two months later than during 1973. Van der Horst (1976) studied this peculiarity at length and concluded that severe dilution as a result of minor flooding in the Swartkops estuary contributed towards the later onset of reproductive activity during 1974 as compared with 1973. This is supported by the fact that most species of mullet spawn in water with a salinity close to that of sea-water (35‰) (Blanc-Livni & Abraham 1969; Eckstein 1975).

The spawning time of *L. dumerili* in the mouth of the Swartkops Estuary furthermore differed greatly from that of the same species along the Natal Coast. In Natal estuaries *L. dumerili* seem to spawn mainly during the winter months and possibly early summer, whereas spawning occurred during the summer months in the mouth of the Swartkops Estuary and/or the immediate offshore marine environment. The difference in breeding time was possibly related to differences in temperature, rainfall and salinity of the spawning waters of Algoa Bay and the Natal Coast.

The appearance of spermatogonia also seemed to exhibit a seasonal cycle. It was stated that residual primary spermatogonia were present in testes of all fish throughout the year. Particularly during the late active recovery, ripe and spawning phases, a few of these cells remained dormant and could be found along the tunica albuginea most of the time. They seemed to increase mitotically just after spawning and gave rise to other residual primary spermatogonia and primary spermatogonia for the next breeding season. Van Oordt (1924) made a similar observation for the spent testis of *Gasterosteus pungitius*. It would also seem

that these cells, once established in the testes, could never be completely depleted throughout the reproductive life of the fish. The spermatogonia of *L. dumerili* seemed to show some resemblance to the A-type spermatogonia of mammals, which are, however, morphologically different.

Ruby & McMillan (1970) stated that two theories have been advanced to answer the persistent problem of the source of germ cells (spermatogonia) in the teleost testes. Turner (1919) suggested that they originate from an extratesticular source, although this could not be verified. Several investigators, however, support the idea that a persistent stock of resting spermatogonia remain in the testis (Van Oordt 1924; Henderson 1962; Weisel 1943; Ruby & McMillan (1970). It has been shown above that *Liza dumerili* has persistent spermatogonia and this might be the case for all teleosts.

Some investigators (Munro *et al.* 1973; Wallace 1974) only used "state of maturity" to evaluate the spawning season of fish. This method is basically a visual one and corresponds to our divisions (a) to (f) (Table 2). Although very useful as an estimate of breeding season, it will not give exact information on annual testicular changes and could create confusion. In *L. dumerili* for example there were not visual differences in TVS during the inactive and early recovery phases or the late ripe and spawning phases but only histological ones. Further complications arose, e.g. the early active recovery testis resembled the spent testis to a large extent. It would therefore seem that a visual evaluation of the testes should be accompanied by histological work if an accurate estimation of the different stages of the breeding cycle is to be known.

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