ASPECTS OF SEX DEVELOPMENT IN NILE PERCH (*LATES NILOTICUS* (L.); PISCES: CENTROPOMIDAE): IMPLICATIONS ON AQUACULTURE

BY

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DECLARATION

I, Basiita Komugisha Rose, declare that, this is my original work and that it has never been submitted for the award of a degree in any University.

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DEDICATION

I dedicate this research to my family.

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ABBREVIATIONS/ACRONYMS

APEP: Agricultural Productivity Enhancement Program of USAID

Dept.: Department

CNO: Chromatin Nucleolar Oocytes

EAFFRO: East African Freshwater Fisheries Research Organization

Ev. : Eviscerated weight i.e. wt of fish minus weight of viscera

FAO: Food and Agricultural Organization

GSI: Gonadal Somatic Index

H&E: Heamatoxylin and Eosin

IUCN: International Union for Conservation of Nature

MAAIF: Ministry of Agriculture Animal Industry and Fisheries

NEMA: National Environmental Management Authority

MT: Masson's Trichrome

NFP: National Fisheries Policy

PNO: Perinucleolar oocyte

SL: Standard Length

SPC: Spermatocytes

SPG: Spermatogonia

SPZ: Spermatozoa

TL: Total Length

T wt: Total weight

USAID: United State Agency for International Development

V wt.: weight of viscera (e.g. intestines, liver, fat)

WoG: weight of gonad

WoL: Weight of Liver

GLOSSARY OF TERMS

Gonochoristic: Species that develop and possess purely ovarian or testicular tissues

Protogynous: Hermaphroditic species that can initially mature as females and then change to males

Protandrous: Hermaphroditic species that can initially mature as males then change to females

ABSTRACT

The histology of the gonad of Nile perch, *Lates niloticus* was examined in order to study the early gonadal development and sexual maturation in a natural population from the Ugandan waters of Lake Victoria. The study describes the anatomical and histological morphology of *L. niloticus* gonad as was observed in the males and females. The samples examined were collected over a period of four months (October 2005 – January 2006). All the fish individuals (178) analyzed with histological, microscopic and macroscopic techniques were found to be either undifferentiated, males with testis or female with ovaries. It was found that the Nile perch gonad did not exhibit any form of intersexuality within the size range sampled (4 cm -110 cm, SL) and period of the year when the study was undertaken. The gonadal development pattern concluded was differentiated gonochoristic where individuals develop from an ovarian or testicular juvenile pathway. The size at which the first indication of sex differentiation for females and males was defined at 9.7±3.3cm and 11.13±2.7 cm, SL respectively. The oocyte development followed through six distinct stages from oogonia (9.8 ± 3.0μm), chromatin nucleolar oocyte (16.2 ± 3.3 μm), perinucleolar oocyte (47.86± 18.3 μm), primary yolk vesicle oocyte (122.6 ± 38.8 μm), secondary oocyte (260.9 ± 61.2μm) and the tertiary oocyte stage (475.5 ± 70.7μm). Broodstock collection from the wild for induced spawning in aquaculture should therefore aim at females with egg size of 475.5 ± 70.7μm. In the males the type of spermatogenesis was cystic and the testicular structure was lobular. Ripe males exuded copious amounts of sperm and for induced spawning it would not be necessary to sacrifice the males in order to obtain milt as is done in other species under culture like the African catfish, *Clarias gariepinus* and the American channel catfish, *Ictarulus punctatus*. Macroscopically the male had only the anal and urogenital openings just anterior to the anal fin, whereas the female had a genital orifice separate from the urinary opening. The sexual dimorphism exhibited by the Nile perch is key in broodstock management.

1.0 INTRODUCTION

1.1 Background

A range of gonadal differentiation patterns have been described for fish, including gonochoristic species possessing purely ovarian or testicular tissues, as well as hermaphroditic species that can initially mature either as males (protandrous) or females (protogynous). Sex determination in fish has been reported to be a very flexible process with respect to evolutionary patterns observed among genera and families, and within individuals, and is subject to modification by external factors (Devlin & Nagahama, 2002). The study of sex determination in fish is important for several reasons;

- There are more than 24,000 species of fish (Nelson, 1994) inhabiting a wide range of aquatic habitats worldwide, providing a rich source of material for academic and applied studies on vertebrate sex determination.

- Because fish are amenable to artificial culture and experimental investigations in many cases, they also provide unique opportunities to investigate and test theoretical concepts of sex determination ranging from evolutionary mechanisms to biochemical processes.

From a practical point of view food from aquatic systems is an increasingly important component of the human supply and finfish comprise the majority of biomass harvested from the aquatic systems. Fish catches from the wild have plateaued in the last decade (Devlin and Nagahama, 2002), forcing demand for aquatic protein to be met through aquaculture production systems. It is imperative that we understand the reproductive biology of harvested species in the wild to allow effective management and prediction of potential impacts. Aspects of sex change for instance in reef dwelling species has been highlighted as a basis to assist formulate policies for harvesting rates as reviewed by Shapiro, (1984).

Similarly, in aquaculture systems, understanding and controlling reproduction is central to the efficient propagation of organisms due to differential growth rates of the sexes and a need for synchronous and reliable maturation. The manipulation of a fish's reproductive system under culture conditions requires an understanding of other influential factors like

sex change in addition to natural spawning patterns.

Information on sex development has also been applied in fish culture to either initiate sex change or to improve growth of particular sex (Yamazaki, 1983; Nakamura *et al.*, 1989). Sex change has been reported in the genus *Lates*, for example the Asian sea bass (Baramundi), *Lates calcarifer* is protandrous (Williams *et al.*, 2004). Knowledge of the reproductive biology such as the structure of gametes can be used to accurately assess maturation and sperm preservation. Administration of sex steroids has shown they can play a direct role in sex reversal of protandrous fish (Devlin and Nagahama, 2002). These manipulations are all geared to increase fish production through aquaculture or through restocking of culturally propagated juveniles. Limited data exists on the sex development of the species, *L. niloticus* (the Nile Perch). A comprehensive knowledge of sexuality would be beneficial and this research will focus on understanding sex development in the *L. niloticus* and document the sex pattern followed in this species, and undertake histological validation of possible juvenile intersexuality or protandry as has been speculated since such occurrences would have consequences to the management of natural stocks and successful domestication of the species.

1.2 Problem statement

Aquaculture has been cited as the fastest growing food producing sector accounting for about 50% of the world food fish with the greatest potential of meeting the growing demand of aquatic food (FAO, 2006). Diversification of the currently cultured species in Uganda to include the *L. niloticus* (a high value export commodity for Uganda, Kenya and Tanzania), has been considered as a strategy to boost aquaculture production in Uganda (NFP, 2004). However, there is limited information on the culture of this species and yet understanding the biological processes both structural and functional is a prerequisite for successful production of the species. Understanding the development of the gametes involved in the propagation of the species will be vital for sustainable culture (farming) of *L. niloticus* under captive conditions. Unfortunately the reproductive biology of the *L. niloticus* is not well known (Ogutu-Ohwayo, 1988) The available information on the work done by other researchers (Worthington, 1929; Keinchngton, 1939; Holden, 1963; Okedi, 1970; and Ogutu- Ohwayo, 1988) on the reproductive biology of *L.*

niloticus lacks any documentation on sex development patterns which is investigated in the present study. This research has contributed to the scientific understanding of sex differentiation of L. niloticus and laid background in the development of aquaculture technologies for propagation of *L. niloticus*.

1.3. Objectives

1.3.1. Over all objective

To investigate the pattern and sequence of sex development in the Nile perch, *Lates niloticus*, in Lake Victoria.

1.3.2. Specific Objectives

1. Establish occurrence of juvenile intersexuality in *L. niloticus*
2. Describe the macro and microscopic structure of the testis and ovary of the *L. niloticus*

1.5 Significance of the study

L. niloticus has a very high economic value and is highly marketable world wide and thus a foreign exchange earner to the country. *L. niloticus* is Uganda's highest fish export to the European Market. The fish flesh is white and competes favorably with other high value species like cord on the international market (www.ugandainvest.com/fishing). It is of concern that the requirements of the export oriented *L. niloticus* fishery exceeds the yields from the lakes and as a result fish processing plants have been reported to operate at below 50% of installed capacity (Balirwa, 2007). The high price for which Nile perch is sold internationally makes it a scarce commodity on the local market and the local people can only afford the remaining axial skeleton after filleting. It is not surprising that options including culturing *L. niloticus* have been advanced, however the breeding technology of the species needs to be in place. This study investigates the sex development of *L. niloticus*, and discusses the use of generated information in areas of induced spawning, sex reversal and hand sexing for the successful culture of the species.

The increasing average population growth rate (estimated at 3.2% for 1991-2002 population census from 2.5% of the earlier inter-censual period 1980-1991) of Uganda (UBOS, 2002) is likely to increase the gap between the supply and demand of fish.

predicted by the year 2010. There is thus need for the rapid growth of the aquaculture sector in order to meet this fish production deficit. Species diversification especially *L. niloticus* should be considered as a strategy since this fish has already established regional and international markets. It is believed that Aquaculture has the potential to meet the growing fish demand and the two key development trends of the sector being intensification and diversification through use of new species and systems and practices (FAO, 2006). In regard to *L. niloticus*, which is a new aquaculture species in Uganda, the biological aspects pertaining to gonadal development cannot be overlooked since it may have relevancy in the culture o f the species.

There have been various attempts to culture *L. niloticus* in polyculture system with Nile tilapia and *Tilapia zilli* in ponds(Gregory and Orukan, 2004; Hamblyn, 1965), monoculture system trials were also done in tanks, and cages suspended in ponds (Gregory and Orukan, 2004), in both cases the source of seed was from the wild. Unfortunately no success has been achieved in closing the cycle through induced spawning for production of *L. niloticus* seed in captivity. Information on culture of a species in the same genus, *Lates calcarifer* in Australia indicates that the culture relied on eggs initially from the wild but later through research; they were able to breed the fish in captivity (McNee and Grieve, 1993). This was found more profitable, as it required less labor and the fish bred and raised under captive conditions (in a flow through tank system) was found to be having less deformities. As a result, most operations retained captive brood stock for breeding (McNee and Grieve, 1993).

1.6. Scope of study

The histology of the gonad of the of *L. niloticus* was examined in order to study the early gonadal development, sexual maturation and sex ratio in a natural population from Lake Victoria. The samples examined were collected over a period of four months (October -January) from Ugandan waters of Lake Victoria. The sites explored were (Lutembe beach; 32^0 35'E 00^0 10'N, Liddo beach; 32^0 28'E 00^0 03'N, Bwerenga at Bugiri landing site; 32^0 34'E 00^0 09'N and Mawala landing site; $32^0$18 E 00^0 32'S. The occurrence of the different sexes against fish size (Length and Weight) supported by histological evidence was used to assess the sexual pattern followed *L. niloticus*.

evidence was used to assess the sexual pattern followed *L. niloticus*.

2.0 LITERATURE REVIEW

2.1 Classification

The Nile perch belongs to the Family Centropomidae (Linnaeus, 1758; http://en.wikipedia.org/wiki/Nile_perc), of order Perciformes, genus Lates and the species is *Lates niloticus* (Common name: Nile perch- English, *Mputa* – Luganda; Uganda, *Mbuta*- Luo; Kenya and Tanzania). The fish is characterized by a deep body and somewhat compressed, with small ctenoid scales. The pre- orbital and pre-opercula bones are armed with spines, a large spine on the free edge of the operculum. The dorsal fin of the *L. niloticus* is almost completely divided into two parts by a deep notch (Greenwood, 1966). The fish also has a visible lateral line that gets even clearer as the mucus runs off. The *L. niloticus* is silver in color with a blue tinge, a distinctive dark black eye and a bright yellow outer ring (http://en.wikipedia.org/wiki/Nile_perc).

2.2 Distribution

L. niloticus is a large voracious predatory fresh water fish (Ogutu-Ohwayo, 1988) that is widely distributed throughout the Ethiopian Region of Africa, occurring commonly in all major river basins including the Nile, Chad, Niger, Senegal and Volta (Hopson, 1972; Lipton, 2003). The species thrives under lacustrine as well as riverine conditions and important populations exist in lakes of tropical Africa; Chad, Albert, Rudolf and some of the Ethiopian lakes (Hopson, 1972; Greenwood, 1976). Until recently the range did not extend up the Victoria Nile above Murchison falls (Hopson,1972) but the species is now established in lakes Kyoga and Victoria following its introduction in these lakes in 1950's and 1960's (Gee,1969; Ogutu-Ohwayo, 1988)

2.3 Population Trends of Fish in Lake Victoria

The Ichthyofauna diversity in Lake Victoria are declining steadily, this has been largely attributed to the *L. niloticus* introductions in the early 1950s and 1960s (Ogutu-Ohwayo, 1988; Gee, 1969; Anderson, 1961). The introduced *L. niloticus* at first led to enormous increases in the total quantity of fish landed (Hughes, 1983; Oguto-Ohwayo, 1984;

Okaronon *et al.*, 1984; Ogutu-Ohwayo, 1988). The species established itself in the new habitat and became the major fish landed and exported as fillets from Uganda, Kenya and Tanzania. However it is noted that lake systems that are highly eutrophic and oligotrophic and dominated by alien species, deliver production in bursts for a few years but must be reconfigured through intense manipulation (Kaufman, 1993). It is noted that *L. niloticus* being alien to Lake Victoria and a predator, cannot sustain high yields for a long time, and its catches were likely to decline. The total production of the formally multi species fisheries of Lake Victoria that was 100, 000 tones increased to 400, 000 tones per year, a four to five fold increase in the 1980s (Muhoozi, 2002) with the catch being dominated by the introduced *L. niloticus* and *Oreochromis niloticus.* However evidence of over fishing the Nile perch has resulted into down ward trends in Nile perch catch levels, declining mean sizes of fish caught and decreasing catch rates per unit effort (Abila, 1998). Further observations of the fisheries trends of lake Victoria indicated that the total contribution to the annual catches of *L. niloticuss* have continued to decline despite the increased fishing effort (Muhoozi, 2002) with contribution to the lake's biomass from *O. niloticus* and *R. argentea* increasing (Munyaho, 2004).

2.4 Sex Determination Systems

The gonads of fish generally are very labile with respect to sex determination (Warner, 1978; Devlin and Nagahama, 2002), but once a particular development profile has been selected by intrinsic controls, or directed by exogenous factors such as hormones, the state of gonadal differentiation may then be stably perpetuated through out subsequent development (Devlin and Nagahama, 2002). The stability of sex differentiation in gonochoristic species implies that sex-determination events primarily function during early development. In gonochorists, individuals develop as males or females and do not reverse sex at any time during their lifespan, this is unlike hermaphrodites where fish can produce mature male and female gametes at some time in their lives (Devlin and Nagahama, 2002).

It is not clear whether sex reversal represents stimulation of development of previously quiescent, but already determined, germ cells of the second sex or whether new primary germ cell determination is occurring (Sadovy and Shapiro, 1987). In their analysis of the

situation, Devlin and Nagahama, (2002) thought it possible that hermaphroditism and sex reversal is regulated at the gonadal level, with individuals possessing mixtures of male and female determined germ cells that are stimulated to proliferate and differentiate under differing conditions.

The sexual pattern that has been reported in members of the genus *Lates* has been protandry, for example, in the *L. calcarifer* (Baramundi). The Baramundi are protandrous hermaphrodites that undergo sex inversion during their life cycle. Females are generally absent in the smaller length classes, but dominate larger length classes. Most Barramundi mature first as males and function as males for one or more spawning seasons, before undergoing sex inversion. A few females will develop directly from immature fish. Similarly, some males may never undergo sex inversion (www.edaff.gov.au/nfpd/atlas/37310006.cfm-21k). The time at which sex inversion occurs in this fish varies in different locations for instance in the Northern Territory of Australia it occurs between 4 and 8 years at 84-93cm total length. In *L. calcarifer* populations from the gulf of Carpenaria, sex inversion is reported to take place earlier when they are between 3 and 7 years of age and 68-90 cm total length (Shaklee *et al.*, 1990)

A similar pattern of protandry has been speculated and thus used to explain the size dimorphism of larger females and smaller males in *L. niloticus* (Hughes, 1992) but this required histological validation.

In their study carried out on the *L. calcarifer* reared in cages in French Polynesia, Guiguen, *et al.*, (1993) found that sex inversion begins at the end of the reproductive period (October- February) in post-spawning males. The theory underlying this sex change could be sex cell reserves in fish that are continuously replenished from the germinal epithelium (Shapiro, 1987) and that a pool of biopotential germ cells remaining quiescent during the initial development of sex and then differentiate into appropriate germ cells at the time of sex change. In *L. calcarifer* the main histological features of this process of sex change were: degeneration of testicular tissue, appearance of peripheral female germinal cells, and centripetal proliferation of ovarian tissue.

Completion of sex inversion required profound morphological changes in the gonads because of the strong dimorphism that exists between testis and ovary (Guiguen, *et al.*, 1993).

In addition it has been documented that in the order Perciformes (Devlin and Nagahama, 2002) to which the *L. niloticus* belongs, there are complex variables and mechanisms used in sex determination processes. The same authors envisage a rich array of biological material providing significant opportunity for future sex determination and recommends research about control of gonadal differentiation at the cellular, organismal, population and species levels.

2.4.1 Gonadal sex differentiation and Reversal in Fishes

Gonadal sex differentiation broadly relates to all occurrences during development that lead to the expression of genetic sex via the appropriate phenotype and encompass all the events that take place in the primordial gonad, including the migration of primordial germ cells (PGCs), the establishment of the gonadal ridges and the differentiation of the gonads into testes or ovaries (Hendry *et al.*, 2002). However this process can be influenced by exogenous hormones and environment which may override the genetically determined sex (GSD) and thus modifying the sex during differentiation (D'cotta *et al.*, 2001; Baroiller *et al*, 1999). Exogenous steroids given during the gonadal development period can control the phenotype overriding the expression of the genotypically determined sex (Phelps & Popma, 2000).

Sex reversal using exogenous agents has been successful where the fish in question have undifferentiated gonads (Yamamoto, 1969), however the results of treatment of guppy *Poecilia reticulata,* (whose ovarian differentiation occurs during the embryonic life by 14 days after the proceeding parturition) with androgen showed complete masculinisation of their female offspring even if it was done after the gonads of the embryos had begun to differentiate into ovaries (Takahashi, 1975).

2.5 Reproductive Potential of *L. niloticus*

A dramatic increase in *L. niloticus* has been associated with its high reproductive potential in favorable conditions as shown by studies in Lake Kyoga and the Nyanza Gulf

of Lake Victoria (Ogutu-Ohwayo, 1988). The fecundity of *L. niloticus* has been estimated at 140 eggs per gram (Ogutu Ohwayo, 1984, Schindler *et al.*, 1998) and at 162 eggs per gram (Dadebo *et al.*, 2005) of body mass in the adult females. The average number of eggs per gram of ovary ranged from 6,200 to 9, 300 with a mean number of 8100 eggs per gram of

ovary. Mature eggs of *L. niloticus* are pelagic, measure about 0.53mm in diameter and are shed freely (Ogutu-Owhayo, 1988). Hopson, (1972), reported that the eggs of *L. niloticus* in Lake Chad measure about 0.67-0.92mm in diameter and are buoyed by a large oil globule of 0.39-0.5mm in diameter. The mature eggs of another fish in the same genus, *L. calcarifer* (sea bass) are pelagic with an average 0.7 mm in diameter (MacKinnon, 1987). The fish is also highly fecund producing up to 46 million eggs (Williams *et al.*, 2004) for an adult fish of 124cm total length. According to Ogutu-Ohwayo, (1988) there were significantly more males than females among fishes less than 110cm total length and more females than males among those more than 120cm total length. The same study analyzed the relationship between size and fecundity and it was found that in *L. niloticus* fecundity (F) increases with length (L) in cm according to the equation: $F= 4.436 \times 10^{-6} L^{2.92}$

It is speculated that the high fecundity in *L. niloticus* is an adaptation to harsh conditions. *L. niloticus* has been found to have a prolonged breeding season for example in Lakes around the equator where there is little change in temperature, breeding persists for the whole year (Hamblyn, 1961). Rabour *et al*, (2003) observed that natural spawning of *L. niloticus* occurs throughout the year with two peaks during the long rainy season (March – May) and short rainy season (September – November). This is unlike in reverine environment where the breeding season is limited and may be associated with migratory movements of the adult fish (Hamblyn, 1961).

2.6. Captive Propagation

Centropomids (including the snooks; *centropomus spp.*, the Japanese giant perch; *L. japonica* and the Barramundi; *L. calcarifer*) are candidates for propagation for both culture and conservation purposes. However, propagation has been found to be the only option in some instances for conservation. The international Union for Conservation of nature (IUCN) recognizes the principle of captive propagation and considers it part of the global strategy to conserve biodiversity (Ribbink, 1986). Propagation of any species can only be possible if the reproductive biology of the species in question is well understood. Unfortunately the reproductive biology of *L. niloticus* which is exclusively indeginous to the African continent is not well known (Ogutu-Ohwayo, 1988). According to Okedi, (1970), "the breeding of Nile perch has yet to be unraveled and its life history remains largely unknown". This constraint can only be over come through scientific research on the

reproductive and other aspects of the biology of the *L. niloticus*. In the present study the sex development of the species from the smallest juvenile accessible from Lake Victoria where the species has fully established itself and competes favorably against other fish species was investigated.

2.7 Sex Ratios

A smaller proportion of females have been observed in the small size *L. niloticus* populations as opposed to the larger ones where the sex ratio is biased towards the females (Hughes, 1992). Two contradictory theories have been advanced for this female bias among adult *L. niloticus*, Hopson, (1972) attributes this to sampling bias among the juveniles and fishing mortality in combination with natural mortality to account for the relatively low male numbers in the adult *L. niloticus*. However, the second theory that has been documented to explain the female sex bias among adult *L. niloticus* is protandry, it's been speculated that males change sex to females (Hughes, 1992; Gregory and Orukan, 2004). This has been based on the sexual pattern followed in the life cycle of the closely related species, *L. calcarifer*. In other studies, however, a one to one sex ratio was reported in the *L. niloticus* from the Blue Nile (Kenchington, 1939) and from trawl catches on Lakes Victoria and Turkana (Okedi 1970, Hopson 1972). It was observed that the number of males and females offshore may be the same, Hopson (1972).

The importance of knowing the actual sex ratios in the different size classes cannot be underestimated, Vicentini & Araujo, (2002) pointed out that sex ratio and size structure constitute basic information in assessing reproductive potential and estimating stock size in fish populations. Whereas macroscopic sexing of fish during sampling can provide a clue about sex ratios in a population no inference can be made without histological validation.

3.0 MATERIALS AND METHODS

3.1 Study area and Sample Collection

L. niloticus of varying sizes were randomly collected over a period of four months from Ugandan waters of Lake Victoria both inshore and offshore (Fig. 1) where *L. niloticus* has fully established itself. The sites explored were (Lutembe beach; 32^0 35'E 00^0 10'N, Liddo beach; 32^0 28'E 00^0 03'N, Bwerenga at Bugiri landing site; 32^0 34'E 00^0 09'N and Mawala landing site; $32^0$18 E 00^0 32'S. A number of fishing gears were set so as to capture a wide variation of fish sizes both inshore (for the juveniles) and offshore (for the adults), these included the 5mm lampara net (commonly known as the mukene net) to capture the very small juvenile *L. niloticus* (fingerlings), a method adapted from the mukene fishermen who always get *L. niloticus* fingerlings as a by catch with mukene (Personal communication with one of them; Mr. Tom Mbwoya at Bugiri landing site). It is known that small juveniles of the *L. niloticus* are restricted to the shallow near-shore environments (Fish Base, 2004; Hamblyn, 1961; Musenero, 2000). It is also noted that all records of *L. niloticus* juveniles and fry were made in reference to shallow in shore water where the depth was not greater than twenty feet, and for this reason, the shallow inshore habitat were fished for the required specimens. The juvenile fish were caught from mainly sandy bottoms and shallow waters of not greater than four meters. Other gears used were the beach seine (400m long, 3m deep, with a rope of 400m,mesh size of 1"wings and 3/4 inch at the cod ends), gillnets and long lines. The long lines were used to capture large *L. niloticus* individuals in the very deep waters (>50m deep).

A total of 183 samples was obtained from the lake; 63 fingerlings ranging from 4cm – 10.5 cm (standard length)were captured using lampara nets from shallow near shore habitats particularly at Bwerenga (32^0 34'E 00^0 09'N), Lutembe beach (32^0 35'E 00^0 10'N), and Liddo beach (32^0 28'E 00^0 03'N). The beach seines captured fish ranging from 11cm to 45 cm, gill nets of 41/2" were used to capture fish ranging from 30 cm to 60cm. In the deep waters at Mawala Island landing site ($32^0$18 E 00^0 32'S) 5- 6" gill nets and long lines with hooks captured the large sized fish (4cm to 110 cm, SL).

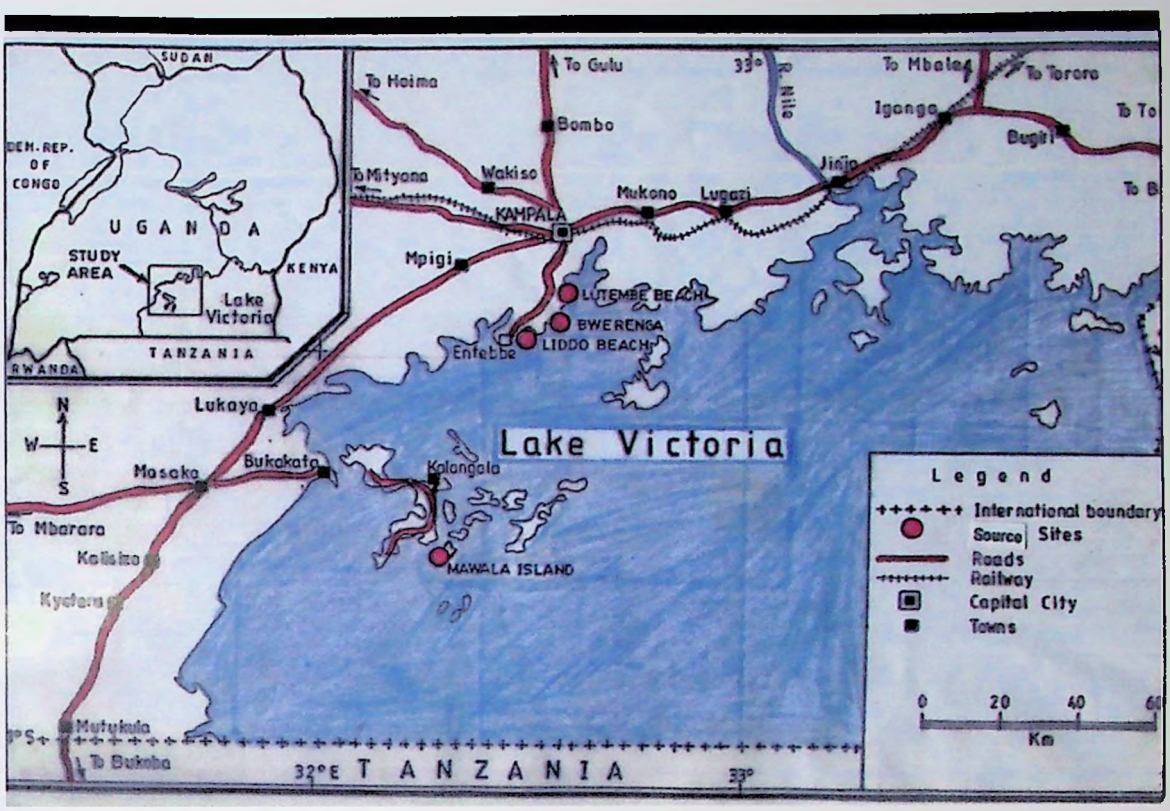


Figure 1: Source of samples from Ugandan waters of Lake Victoria

3.2. Length-weight measurements

For every sample SL and TL measurements were done to the nearest 0.1mm with the aid of a measuring board, for individuals less than 100cm and by use of a tape measure for individuals greater than 100cm.

3.3 Sexual maturity

After measurement of body length and weight, the fish was cut open ventrally to expose the gonads and the viscera, gonadal and eviscerated weight (Ev.) were then measured. The gonads were macroscopically identified as testes, ovary or undifferentiated gonad and later validated histologically. Using a subjective scale (modified from Hopson, 1972) the gonads were assigned maturity stages. The stages were I and II (for immature), III (transitional/maturing) and IV and above (mature).

3.4 Processing histological Samples

Sections of the gonad from the rostral, middle and caudal parts were cut and fixed in Bouin's solution for not less than 72 hours. The ratio of fish to fixative volume was 1:10 for effective fixation of the tissues protecting them from autolysis. Fixed samples were processed in the histology laboratory at the Faculty of Veterinary Medicine, Makerere University in Uganda.

In the Laboratory histological samples were trimmed to pieces of approximately $1cm^3$ before loading in cassettes. The sample-loaded cassettes were placed into an automatic tissue processor (model no. 25500, Lipshaw manufacturing cooperation. Detroit, MICH 48215) where dehydration of the tissues in ascending grades of alcohol (70%, 80%, 90%, absolute I and absolute II) was done for two hours in each concentration. The samples were cleared shortly in two changes of Xylene and impregnated in two changes of molten wax. The tissues were then removed from the automatic processor and embedded in pelleted molten paraffin wax at 60°C melting point. The gonad tissues were embedded in wax and attached to wooden blocks, latter frozen to cool the tissue to allow for sectioning with a well-sharpened knife. Sections 5µm were made and floated onto glass slides in a warm water bath, oven dried and stained.

3.6.1 Staining and microscopic observations

The Mayer's Heamatoxylin and Eosin (H&E) stain was used. In addition the Masson's Trichrome (MT) stain was used to emphasize the connective tissue. The stained sections were covered with a drop of DPX and a cover slip placed over them, pressing gently to spread the DPX and expel the air bubbles. The slides were then oven dried (45-50°C) for two days before microscopic examination.

Slide observations were done under a light microscope (Carl Zeiss, Axiostar plus). The different cells and their nuclei were identified and measured using a fitted eye piece graticule calibrated for every objective lens with a stage micrometer or graduated slide.

4.0 RESULTS

4.1 Morphological determination of sex of Nile perch

The *L. niloticus* were found to be sexually dimorphic in the larger size classes (above 30 cm) and especially in the ripe individuals. When ripe, males were oozing out milt at slight pressure on the abdomen and it was easy to differentiate the males from females macroscopically in this state (plate1).

a) b)

Plate1a): Macroscopic appearance of a ripe running *L. niloticus* male with copious amounts of milt oozing out of the vent on application of slight pressure on the abdomen. b) Male (left) Nile perch with two openings and female (right) with three openings.

Macroscopically the male had only the anal and urogenital openings just anterior to the anal fin, whereas the female had a genital orifice separate from the urinary opening (plate 1b). Macroscopic sex observations were followed by histological examination of the tissue samples.

4.1 Morphological Description of the Gonads

The gonads of *L. niloticus* were macroscopically visible from the smallest fish caught (4.3 cm, SL) as small, transparent, indistinguishable tissue strands covered by a peritononeum and lying dorso-laterally along the length of the swim bladder. The gonads were paired and attached to the abdominal cavity by mesenteric tissues.

4.1.1 The Ovary

The ovaries of *L. niloticus* were paired structures located in the peritoneal cavity (plate 2a) and suspended on either side of the mid line. They were enclosed in a capsule; the tunica albuginea. The shape, size and appearance of the ovaries varied with the stage of development. In the immature and spent fish, the ovaries appeared as thin straight and translucent thread-like structures but as oogenesis progressed, the organs enlarged to become lobed, saclike, creamish yellow organs (Plate 2b) filling the abdominal cavity. The mature ovaries were cream yellow in color, well vascularised and the blood vessels visible macroscopically (Plate 2b). In the transverse section the ovaries were oval shaped with numerous ovigerous lamellae from the tunica albuginea extending towards the centre of the ovary (plate 5b).

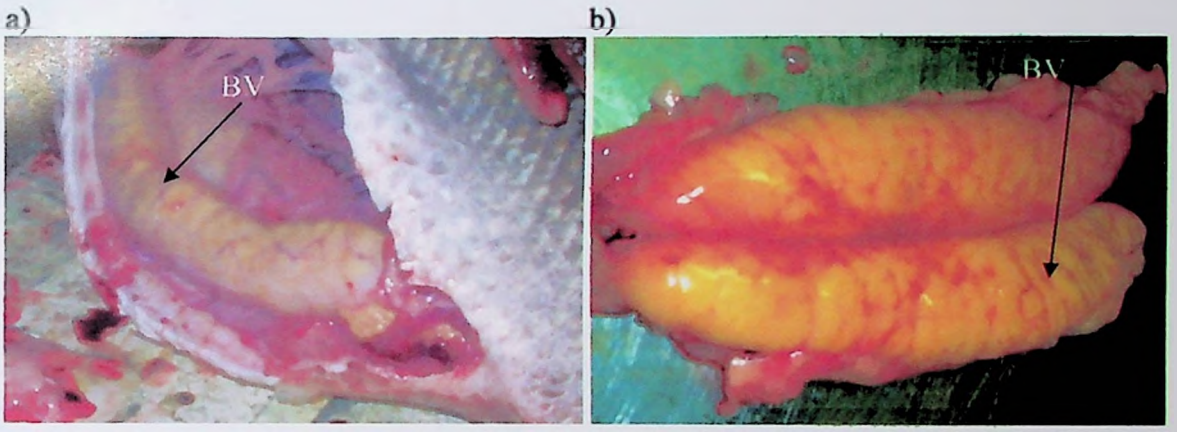


Plate 2a: Showing the location of the left ovary in the peritoneal cavity. b) Pair of ripe ovaries with distinct lobes and well vascularised ,arrows pointing at blood vessels (BV).

The oocyte development was asynchronous, this was clearly observed in the mature ovaries where different oocyte stages characterized the ovary. The mature ovaries had a few homogeneous oocytes that were yolked with an average diameter of 475.5 ± 70.7µm (table 4.4) co existing with other oocyte stages like the cortical alveolar oocytes as well as basophilic oocytes.

Gonadal somatic index (GSI)

Juveniles had very low GSI (Less than 0.3%) and the mature fish>50cm standard length with mature oocytes had a higher GSI of 0.5 – 3.65%. There is a strong correlation between

GSI and standard length with a regression equation of GSI= $0.0002SL^2$ -0.0007SL + 0.0685 (R^2 = 0.725) as illustrated in the graph (figure 2).

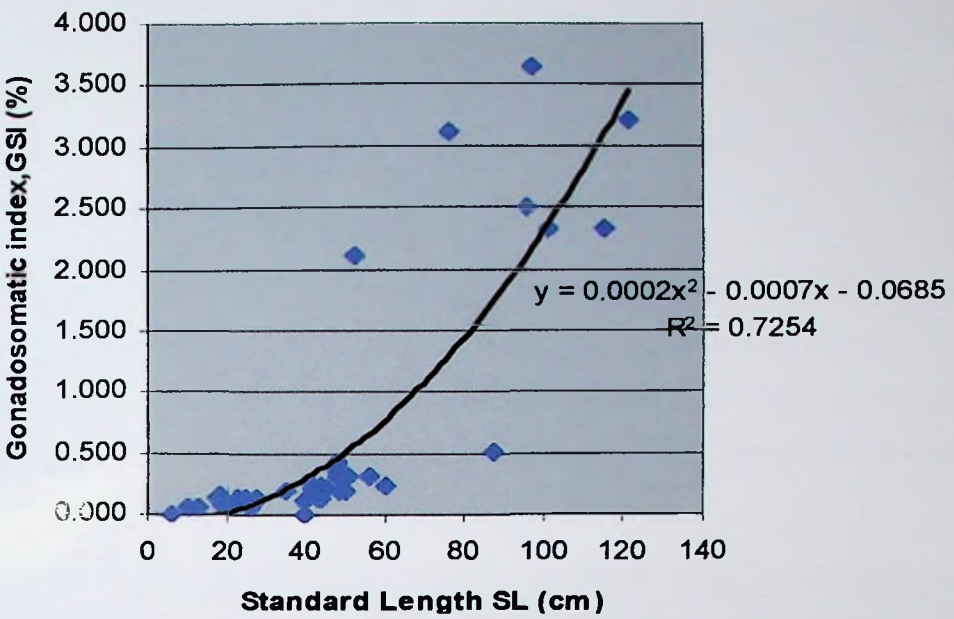


Figure 2: Relationship between gonadosomatic index and total length (TL) for *Lates niloticus*

4.1.2 The Testis

The testes of *L. niloticus* were two elongated structures situated in the dorsal region of the abdominal cavity. In sexually immature individuals the testis were seen as threadlike transparent paired structures running longitudinally along the dorsal wall of the body cavity joining together at the caudal end.

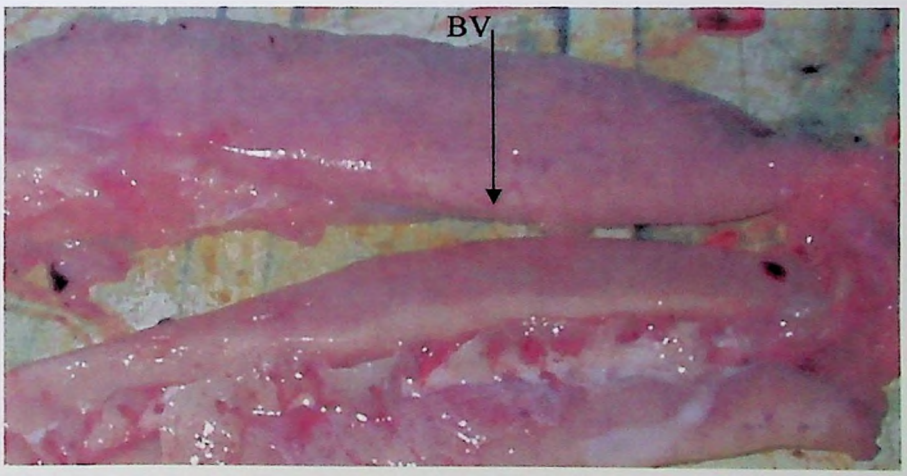


Plate 3: Mature and well vascularised testis of *L. niloticus*, arrow pointing at a blood vessel (BV)

Macroscopically, sexual maturation was characterized by enlargement and convolution of the testes becoming grayish white, then whitish or pinkish with clear vascularisation (Plate 3) and somewhat flattened at the edges and finally to ivory white in color with well rounded edges in the ripe running individuals.

4.2 Histological description of gonadal development

Stage I: Undifferentiated primary germ cell stage

Primordial germ cells were visible in the undifferentiated primordial gonads as large conspicuous cells (12.34±1.79µm) with large spheroid nucleus (7.28±1.38µm) containing a single nucleolus. The cell distribution was sparse along the length of the gonad (Plate 4a) interspersed with somatic cells. The somatic cells were spindle shaped while some were ovoid.

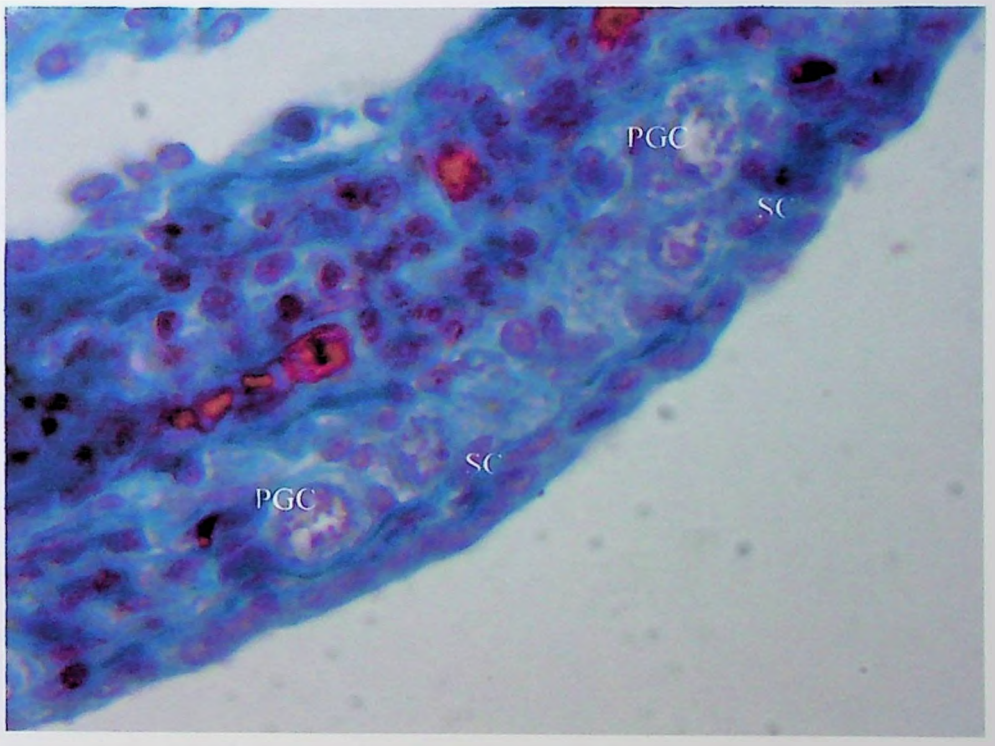


Plate 4a Primordial germ cells (PGC) with large nucleus interspersed with somatic cells (SC) as seen in an undifferentiated gonad.MT X 1000

The Primordial germ cells were surrounded by connective tissue. These cells remained

quiescent with no evidence of mitotic division. At this stage histological examination did not reveal distinction of male and female gonadal cell types.

Stage II: Mitotic division of Primary germ cells

The primordial germ cells gave rise to smaller primary germ cells (gonocytes), 9.8± 3.1µm, that were very clearly seen with MT staining (plate 4b). The gonocytes appeared in groups with a dark staining nuclear wall but light staining nucleus and cytoplasm with a single nucleolus (plate 4b). With proliferation of these primary germ cells, the gonads differentiated into male or female structures. Further cell division was clearly observed in specimens that were to develop into females, here the cells continued to divide and appeared in groups within cysts (Plate 4c) . For the males the somatic cells surrounding the primary germ cells differentiate into lobules and supporting connective tissue (plate 4d)

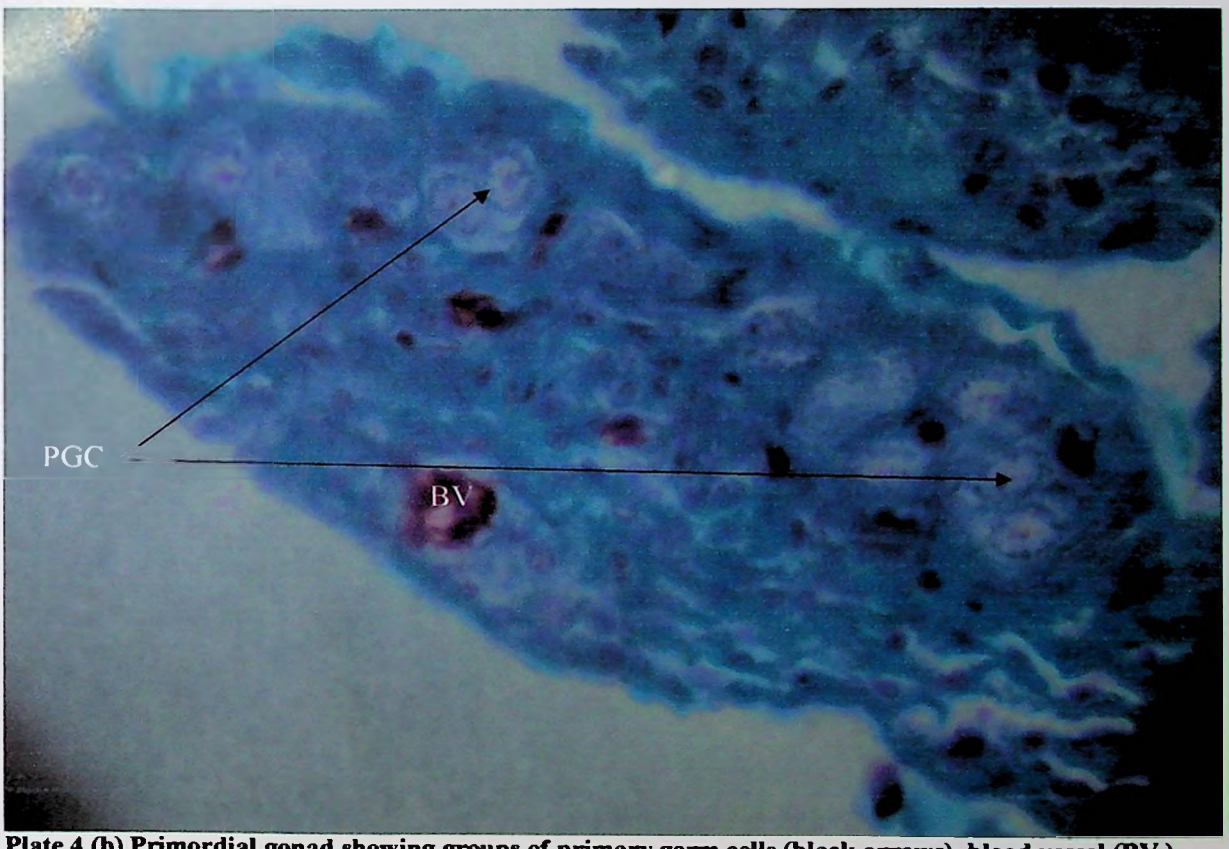


Plate 4 (b) Primordial gonad showing groups of primary germ cells (black arrows), blood vessel (BV) H & E x 1000

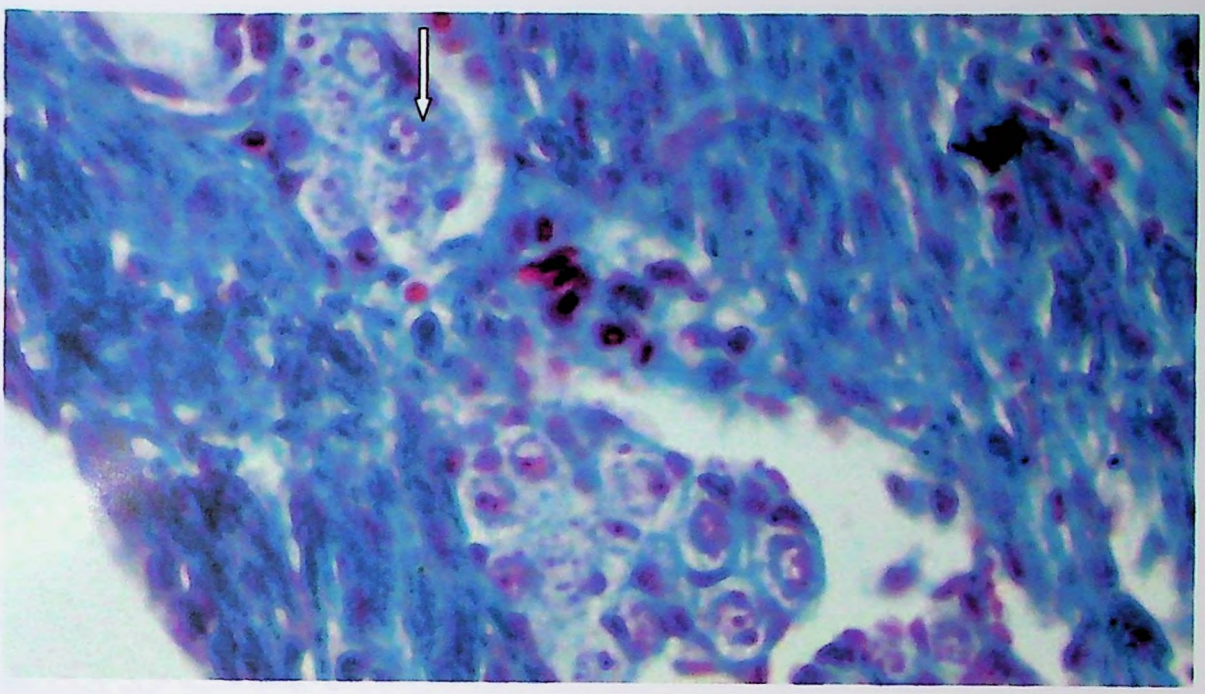


Plate 4c: Differentiating female with mitotic cells within cysts (white arrow pointing at a group of cells in a cyst). MTX 1000

Stage III: Differentiated stage

In this stage the gonads were clearly discernable as either male (plate 5a) or female (plate 5b). Results indicated that females differentiated at an earlier size of 9.7±3.3 cm than males 11.13±2.7 cm. Development continued in either direction from spermatogonia till Spermatozoa in males or from oogonia till tertiary oocytes for the females.

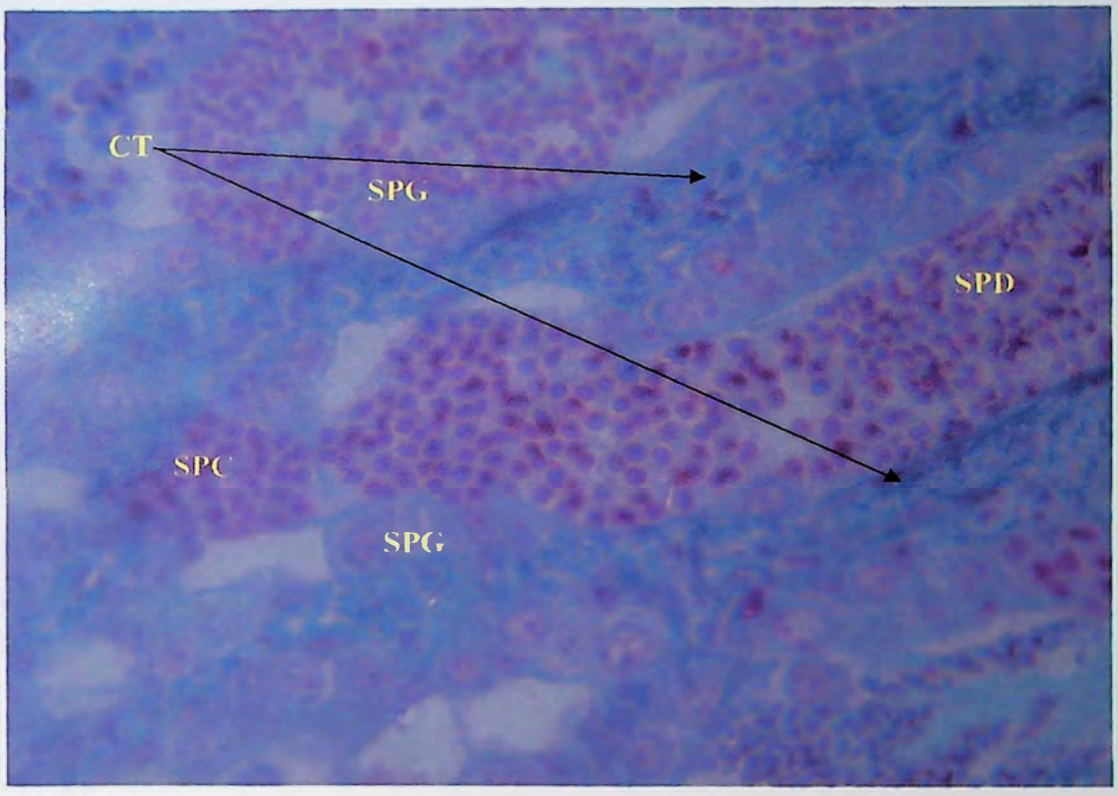


Plate 5a): Testis of a fully differentiated male with spermatogonia (cells with the large nucleus and nucleolus), spermatocytes as well as the darkly stained spermatids (SPD). Connective tissue (CT) strands from the tunica albuginea sub dividing the testis into lobules MT X1000.

In the females the ovarian capsule (tunica albuginea) projected it vascularised connective strand into the developing ovary. The oogonia lay close to the ovigerous lamellae and there was proliferation and differentiation of early oocytes, the chromatin nucleolar oocytes and later the perinucleolar oocytes (5b & 6a). The differentiated ovary was characterized by a clear ovarian lumen and distinct basophilic oocytes (plate 5b).

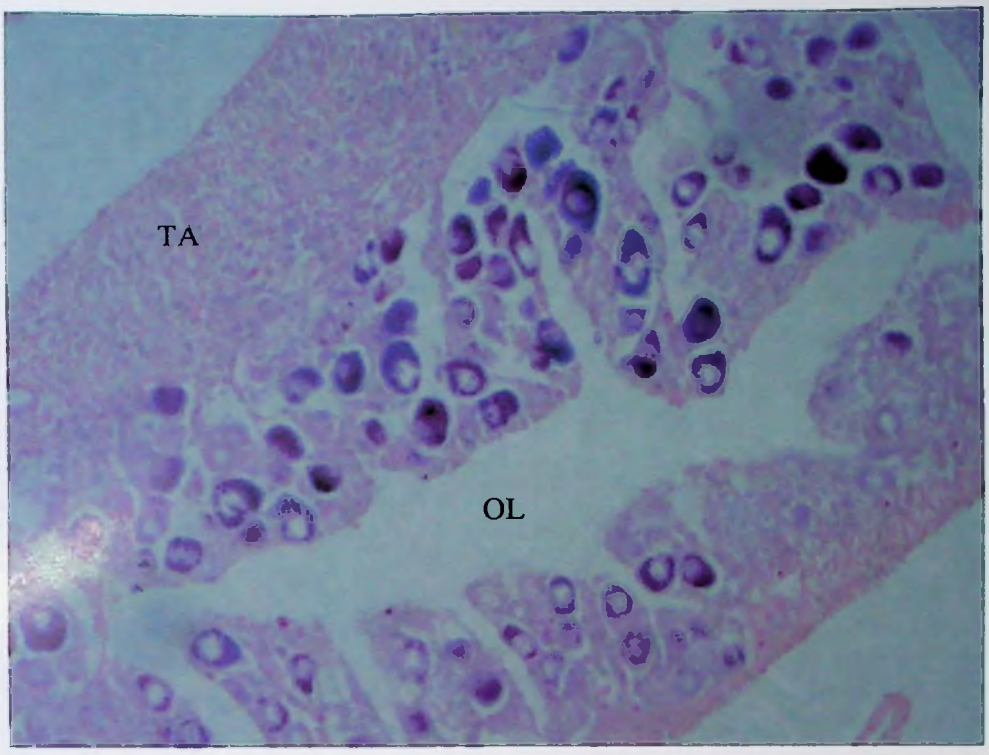


Plate 5b): Ovary of a differentiated female with a characteristic ovarian lumen (OL), and distinct basophilic oocytes. Ovary is enclosed in a tunica albuginea (TA). H&E X400

4.3 Detailed description of oogenesis and spermatogenesis in the *L. niloticus*

4.3.1 Oogenesis

The process of oogenesis in the females was characterized by the different oocyte stages based on their size (table 4.4), staining properties of the nucleus and cytoplasm, number of nucleoli and deposition of yolk and oil globules. The cells developed in the following stages; oogonia (OOG), chromatin nucleolar oocyte (CNO), perinucleolar oocyte (PNO), Late perinucleolar oocyte (LPNO), primary yolk vesicle (1^0YVO), secondary yolk vesicle oocyte (2^0YVO) and tertiary oocyte stage (3^0YVO).

Table 4.4: Mean Cell Diameters of Various Oocyte stages from Fixed Ovaries of *L. niloticus*

Stage	Cell Size/Diameter (μ)	Number of cells (n)
Primordial germ cells (PGC)	12.3± 1.8	38
Oogonia (OOG)	9.8 ± 3.0	44
Chromatin Nucleolar Oocyte (CNO)	16.2 ± 3.3	47
Perinucleolar Oocyte (PNO)	33.8 ± 12.1	27
Late Perinucleolar Oocyte (LPNO)	74.8 ± 16.5	44
Primary yolk vesicle ($(1^0$YV)	122.6 ± 38.8	34
Secondary Yolk Vesicles (2^0YV)	260.9 ± 61.2	38
Tertiary Yolk Vesicles (3^0YV)	475.5 ± 70.7	37

In the immature juveniles, the ovary consisted of mainly oogonia and chromatin nucleolar oocytes (CNO) as well as early perinucleolar oocytes (Plate 6a).

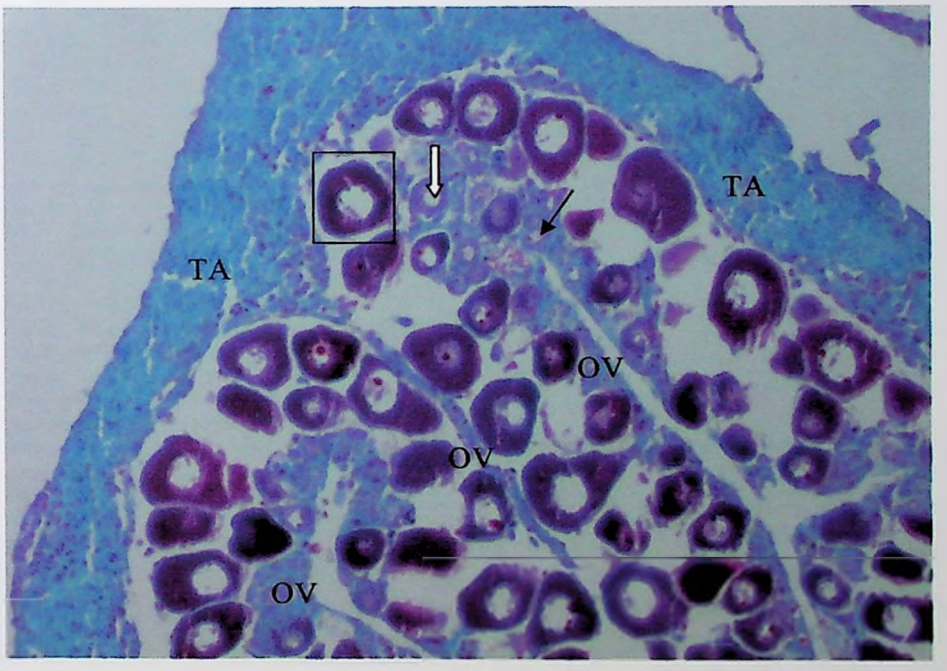


Plate 6a) Micrograph showing Ovary enclosed in a fibrous capsule, the tunica albuginea (TA) that invaginates inwards to give the ovigerous lamellae (OV). It also illustrates oogenesis with various cell stages; oogonia (black arrow) lying close to the ovigerous lamellae, CNO (white arrow) and PNO (enclosed in a box). MT X 400

The oogonia (with an average diameter of 9.8± 3μm) stained lightly in H&E and MT and were located close to the ovigerous lamellae (plate 6a). The oogonia gave rise to chromatin nucleolar oocytes that had a proportionally large and centrally located chromatin nucleus wall and light basophilic cytoplasm. The chromatin nucleolar oocytes measured 16.2±3.3 μm in diameter. As the oocytes develop, the nucleo-cytoplasmic ratio decreases, and the nucleoli increase in number and arrange around the inner part of the germinal vesicle, defining another oocyte stage, the perinucleolar oocyte stage. The perinucleolar oocytes had a nucleus with nucleoplasm containing numerous nucleoli of varying sizes.

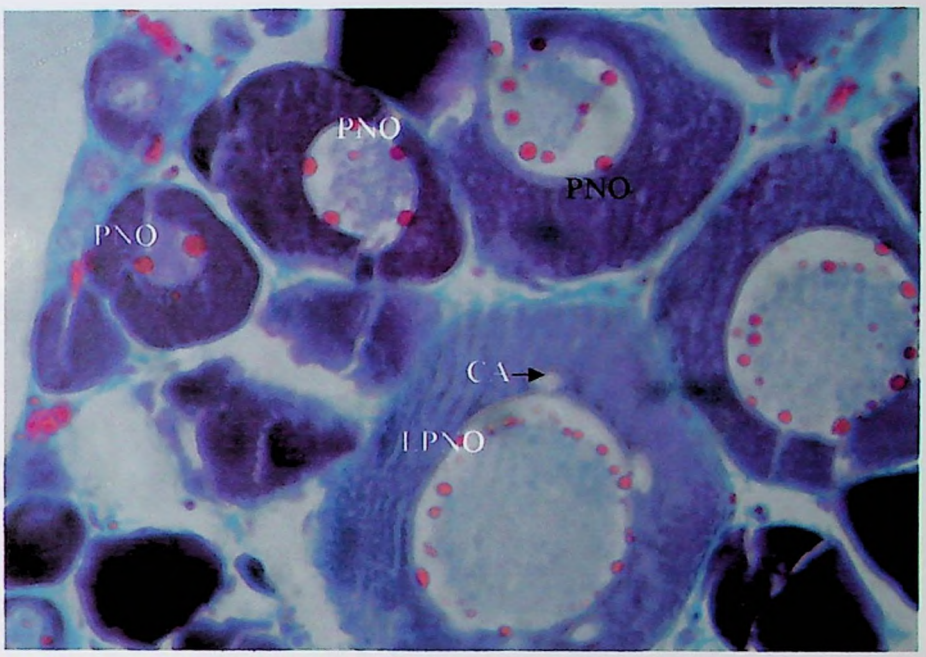


Plate 6b: Micrograph showing Perinuclear oocytes (PNO) with nucleus containing several nucleoli neatly aligning the periphery of the nuleoplasm, cortical alveolar begin to appear at this stage. MT X 1000

The polygonally shaped perinucleolar oocytes (with a mean cell diameter of 33.8 ±12.1 μm) became larger and more ovoid as development progressed to the late perinucleolar stage (plate 6b) with oocyte diameter measuring 74.8±16.5 μm (table 4.4). Cortical alveoli started to appear in the late perinucleolar oocytes marking the end of the primary growth phase (plate 6b).

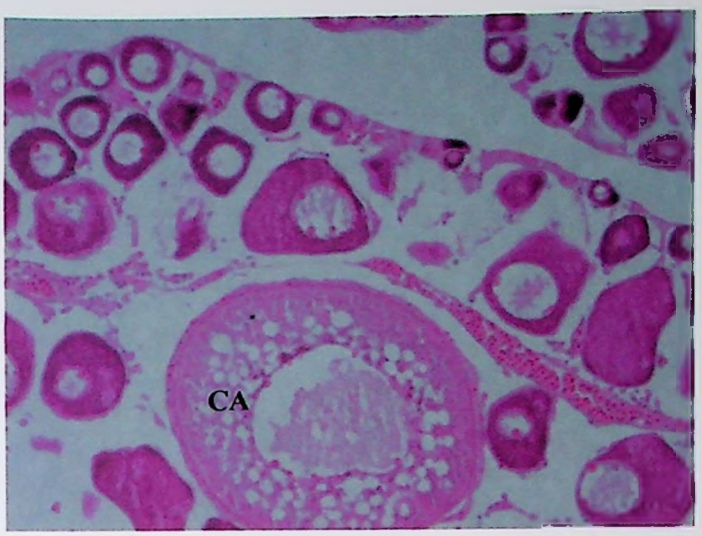


Plate6c: Micrograph showing primary yolk vesicle characterized by presence cortical alveoli (CA) X 400

The beginning of the secondary growth phase that was characterized by the appearance of primary yolk vesicles (plate 6c).The primary yolk vesicles contained numerous cortical alveoli in the cytoplasm (plate 6c and d). The nucleoli in this stage arranged on the nuclear wall like tiny beads (plate 6c and d).

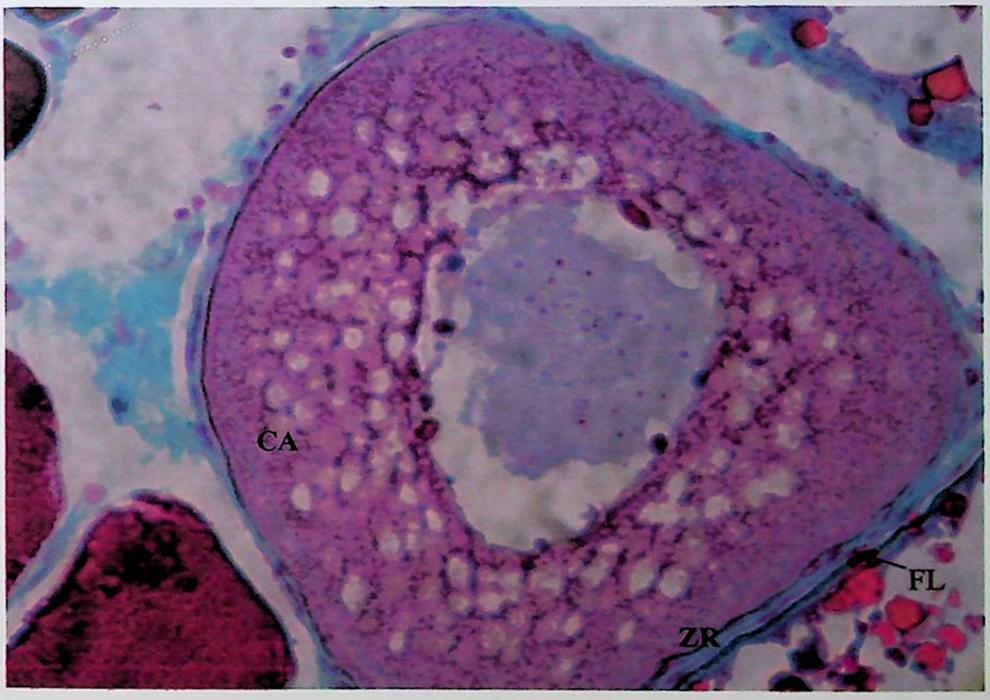


Plate 6d: Primary yolk vesicle with cortical alveoli (CA) with thick Zona radiata (ZR), follicular layer (FL) and changing cytoplasm from basophilic to acidophilic. MT X 1000

The primary yolk vesicle oocytes measured on average 122.6 ±38.8μm in diameter (table 4.4). The primary yolk vesicle oocytes(1^0YVO) had a cellular zona radiata and follicular layers; theca and grannulosa. The oocytes had thicker follicular layers than the late perinucleolar oocytes and the cytoplasm changed from basophilic (plate 6b) to acidophilic (Plate 6c and d). Development proceeded to secondary yolk vesicle oocyte stage, the oocytes in this stage were characterized by an acidophilic cytoplasm staining red in H&E and they had a mean cell diameter of 260 ±61.2μm (table 4.4).

The tertiary oocytes (plate 7a & b) were the largest cells (with an average cell diameter of 475.5 ± 70.7 μm) observed in this study for the gonads that developed into females. The tertiary oocytes were yolked and had characteristic oil globules, the yolk stained red with H&E, but the oil globules were colorless (Plate 7 b).

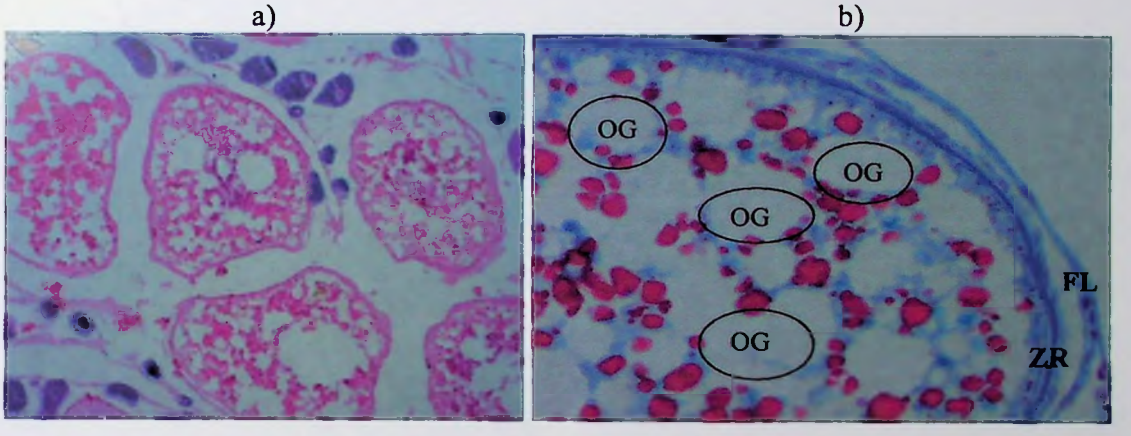
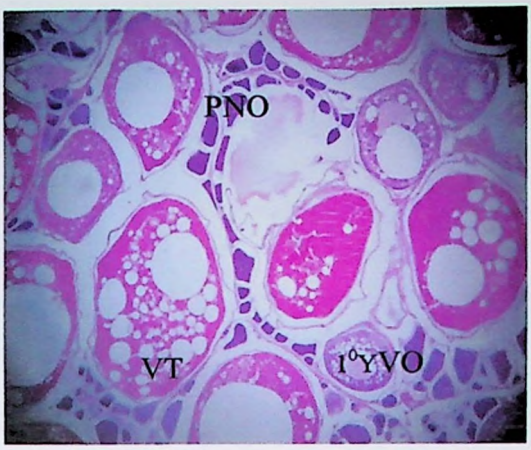
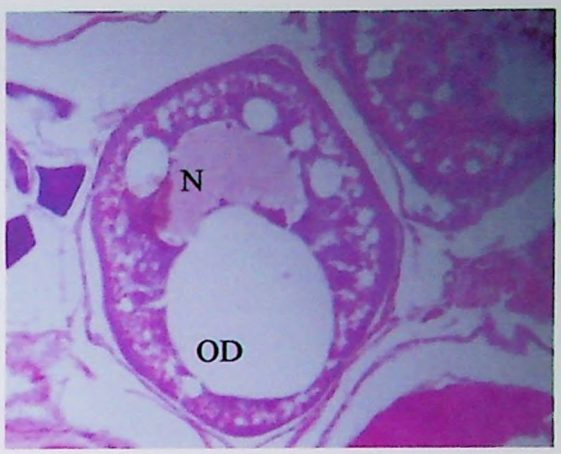


Plate 7 a) section of Ovary with yolked oocytes from a mature female, notice the yolk deposition in the oocyte stains red in H&E, X100. (b) Tertiary oocyte with many oil globules (OG), oocyte is enclosed in Zona radiata (ZR) and follicular layer (FL), MT X400

The nucleus migrates to the animal pole and oil globules begin to fuse to form a large oil droplet that fills the entire ooplasm (plate 8b). Oocyte development exhibited in the Nile perch ovary was asynchronous with various stages of oocytes observed in the mature ovaries (plate 8a).

a) b)





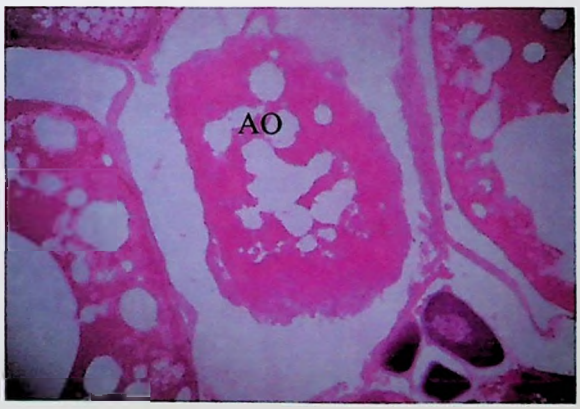
H&E X 100 **H&E X 400**

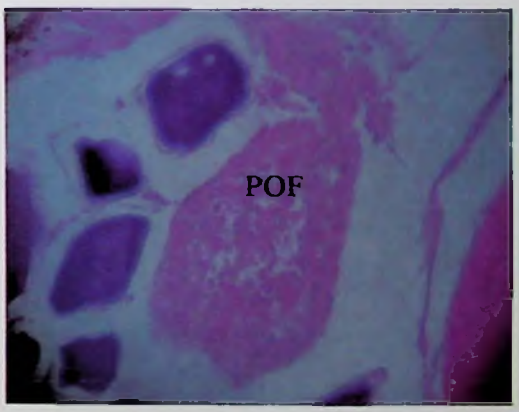
(Plate 8a)Transverse section through an ovary showing asynchronous oocyte growth, ovary with oocytes at various stages; PNO-Perinucleolar oocytes, (1^{o}YVO)- primary yolk vesicle, Vitellogenic oocytes oil globules and yolk staining. b) Micrograph showing an oocyte with a large oil droplet (OD) formed as a result of fusion of small oil globubes, notice the position of nucleus (N), moving to wards the animal pole

Atresia

Oocyte atresia was observed in the vitellogenic oocytes and was characterized by the fragmentation of the zonna radiata and the dissolution of the cytoplasimic contents (plate 8c). This type of atresia was observed in only secondary and tertiary oocytes (in the vitellogenic oocytes). Another type of atresia was observed in the spawned ovaries in the old post ovulatory follicles (POFs)

c) d)





H&E X 100 H&|E X400

Plate 8c) Micrograph showing an atretic oocyte (AO) in which macrophages are starting to dissolve the cytoplasmic contents in the vitellogenic oocyte. d) An old post ovulatory follicle (POF) undergoing atresia

Table 4.5a: Maturity stage classifications for *L. niloticus* females as modified from Hopson, (1972)

Maturity stage	Macroscopic description	Histological description
1-immatue	Ovaries were seen as a pair of thin transparent strands running longitudinally along the dorsal wall of the body cavity. The sexes were indistinguishable macroscopically	Primary germ cells indistinguishable and located randomly along the entire length of the gonad. In some females a few chromatin nucleolar oocytes were evident though there were clear compartments and ovigerous lamellae
2-Early maturing	Ovary reddish pink, pear shaped in section tissue was well vascularised	Irregularly shaped or polygonally shaped Peri-nucleolar oocytes (PNO) with multiple nucleoli (2-16) dominating, a few Chromatin nucleolar oocytes and Oogonia were still present at this stage
3-Late maturing	Ovary with small opaque yolky ova visible through the capsule. Considerable amounts of fat deposits around the fatty body and mesenteries	Late PNO with a more ovoid shape and large primary yolk vesicle oocytes (1^0YVO) with numerous cortical alveoli in the cytoplasm. A few secondary oocytes were also present.
4-Ripe	Ovary is cream yellow in color, fully obovate in section and the dorsal lobes only distinctly defined. There was less mesenteric fat deposits	Ovary dominated by vitelloginic oocytes and few primary yolk vesicle oocytes as well as primary oocytes. The tertiary oocytes have yolk deposits that appear red in H&E and numerous oil globules that appear transparent.
5-Ripe running	The ovary is yellow orange in color and well-defined dorsal lobules and clearly obovate. The blood vessels are sharply defined and the organ is soft on touching. When pressure is exerted on the belly some few ova are expelled.	Ovary dominated by tertiary oocytes and a few secondary oocytes as well as cortical alveolar oocytes and primary oocytes. The oocytes have large oil droplet that was formed by fusion of numerous oil globules. In some oocytes the nucleus was seen migrating to the animal pole.
6-Spent	Ovary flabby and loose containing torn follicular tissue rich in blood with scattered ova.	Ovary characterized by presence of fresh post ovulatory follicles (POF) among other vitellogenic oocytes and primary oocytes. Atresia seen in some of the vitellogenic oocytes

4.3.2 Spermatogenesis

The testis were enclosed in tunica albuginea (TA), consisting of irregular connective tissue with fibroblasts and smooth muscle fibres (plate 9a). The tunica albuginea inviginated into the testicular mass and subdivided to form the lobular compartments (plate 9a)

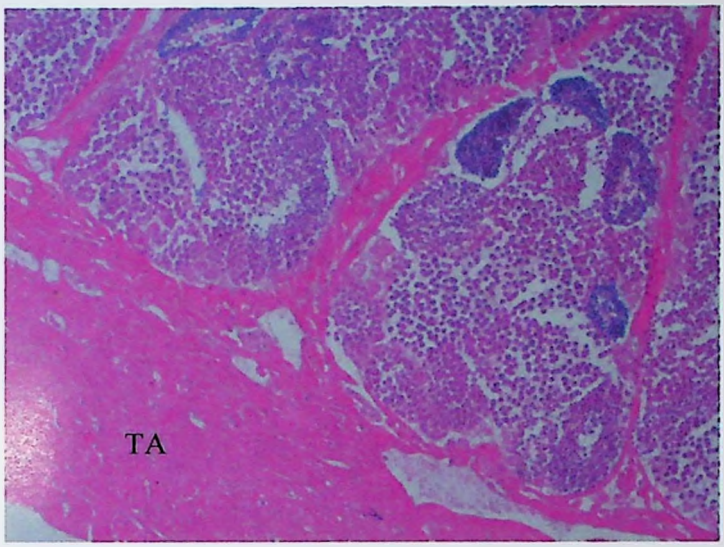


Plate 9 a) Lobular testis of *L. niloticus* with a tunica albuginea (TA) with fibroblasts and smooth muscle fibres H&E X400

Four main cellular stages in the testis were recognized and these were; spermatogonia (type A and B), spermatocytes, spermatids and spermatozoa. Spermatogonia were characterized by one to two rounded to oval shaped nucleolii with heterochromatin granules spread along the nuclear envelope (plate 9b).

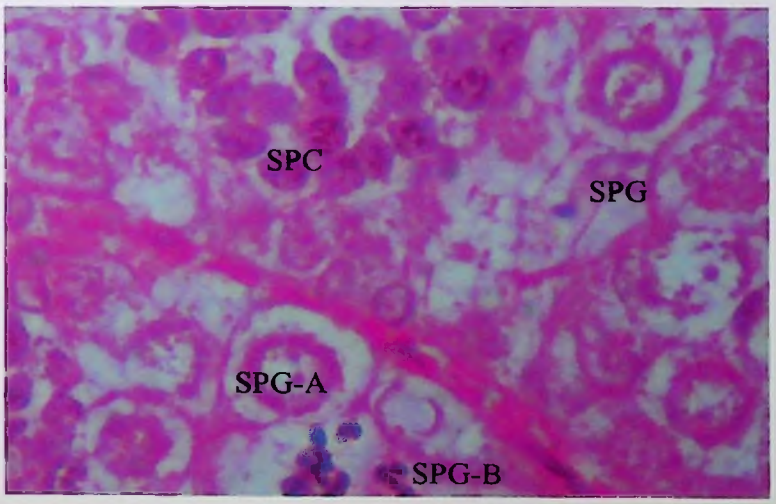


Plate 9b) showing the heterochromatin granules spread along the nuclear envelope in a spermatogonial cell. H&E X1000

Spermatogonia type A (average diameter of 15±1.84 µm) had a clearly defined ovoid central nucleus with hetrochromatin granules around the nuclear wall. Two distinct nucleoli were discernable in the nucleoplasm. Type B (average diameter of 12.7±1.43 µm) had an eccentric nucleus and a single nucleolus

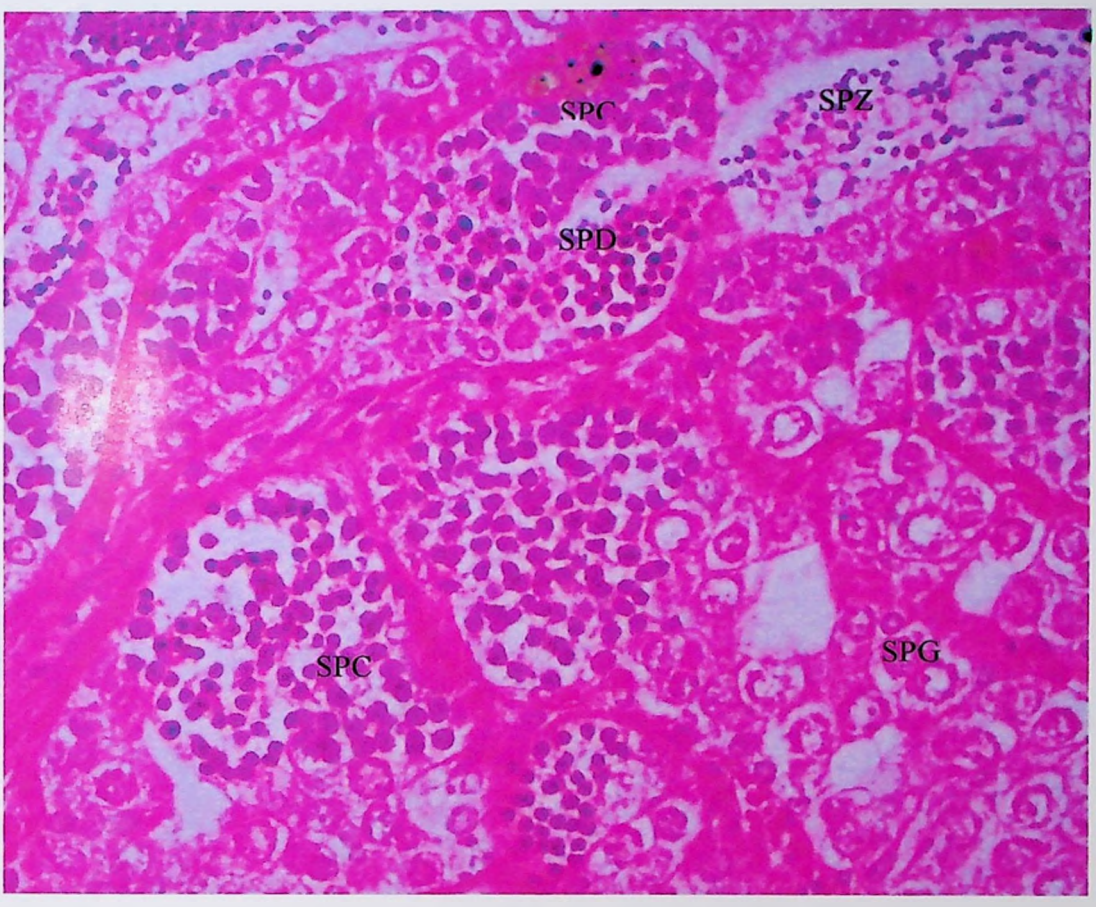


Plate 9c) Transverse section through the testis of *L. niloticus* showing cystic spermatogenesis in which the spermatids (SPD) in cysts rupture to give the spermatozoa (SPZ) into the lumen of the lobules. The spermatogonia(SPG) were the youngest and largest cells (diameter of 15±1.84 µm). Spermatocytes (SPC) were smaller than the spermatogonia but with a dense chromatin nucleus X 1000 H & E.

The spermatocytes (5.54±1.84 µm) were smaller than the type B spermatogonia (diameter of 12.71±1.46 µm) with dense basophilic chromatin strands from the nuclear wall filled the condensed nucleus (Plate 9b). The spermatids were smaller than the spermatocytes. The spermatids developed in cysts and ruptured to give the spermatozoa that were constantly shed into the lumen of the lobules (plate 9c). The spermatozoa from the lobules were then collected in sperm ducts into the main sperm duct (plate 9d).

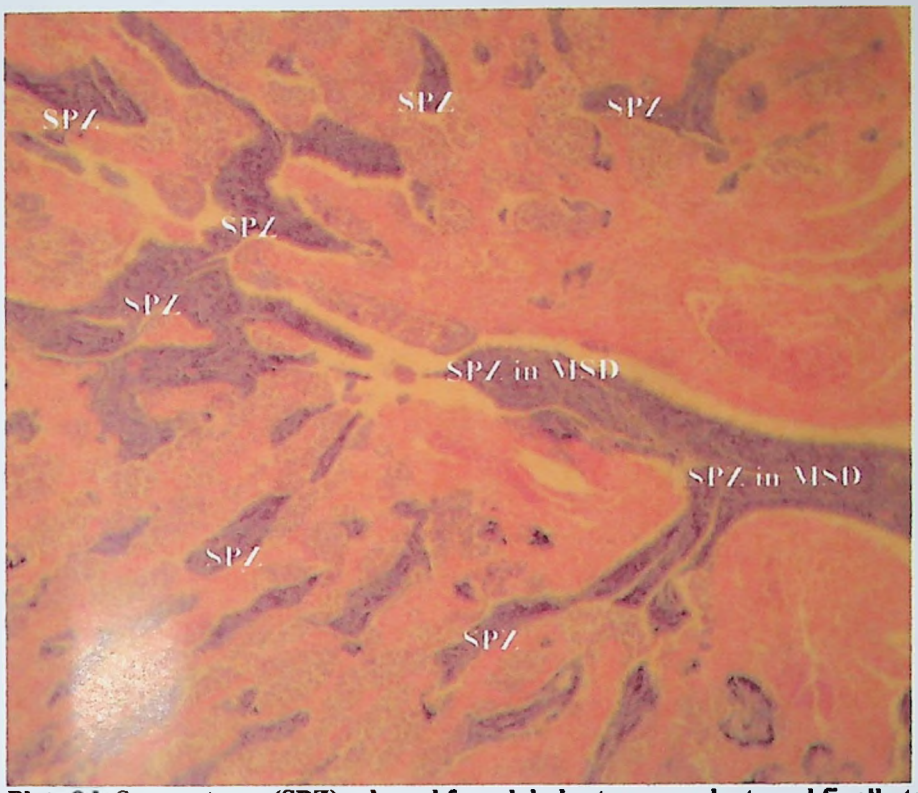


Plate 9d: Spermatozoa (SPZ) released from lobules to sperm ducts and finally to Main sperm duct (MSD) X 400 H & E

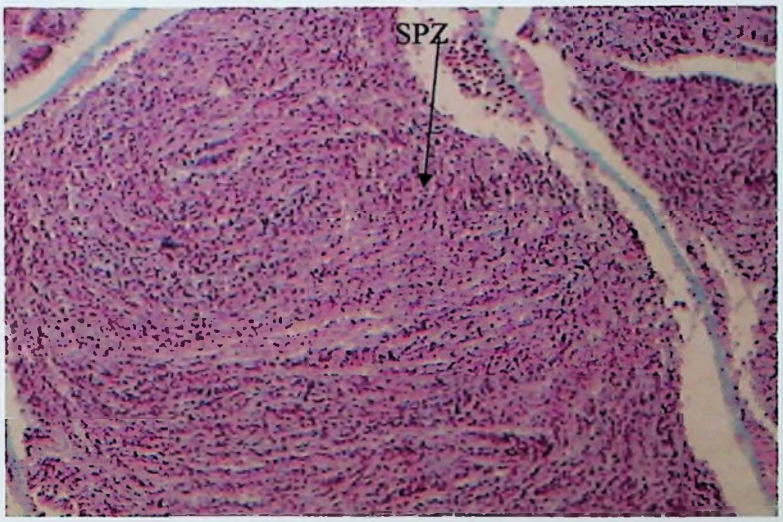


Plate 9e: transverse section through testis of ripe running male showing a lobule fully filled with spermatozoa X400 MT

The spermatozoa (SPZ) were the smallest germ cells in the *L. niloticus* testis and were shed into the lumen of the lobules (plate 9 a, c & d). In the ripe and running individuals the spermatozoa filled the entire lumen of the lobule (plate 9 e) as well as the ducts. In the

spent males, the old spermatozoa (OSPZ) were clearly visible in the lumen of the lobules and small numbers of spermatogonia were lining the periphery of the lobules (plate 9f). As the fish started recovering the OSPZ were steadily replaced by spermatogonia, the later now appeared in large numbers (as shown in Plate 9g)

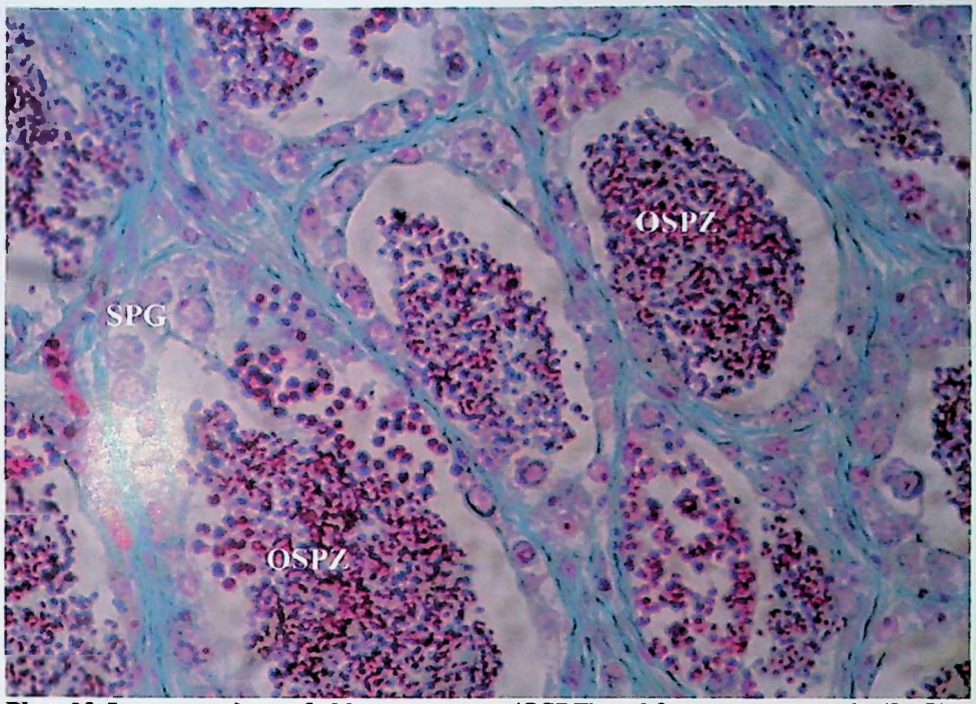


Plate 9f: Large numbers of old spermatozoa (OSPZ) and few spermatogonia (SPG) at the periphery of the lobules MT X 1000

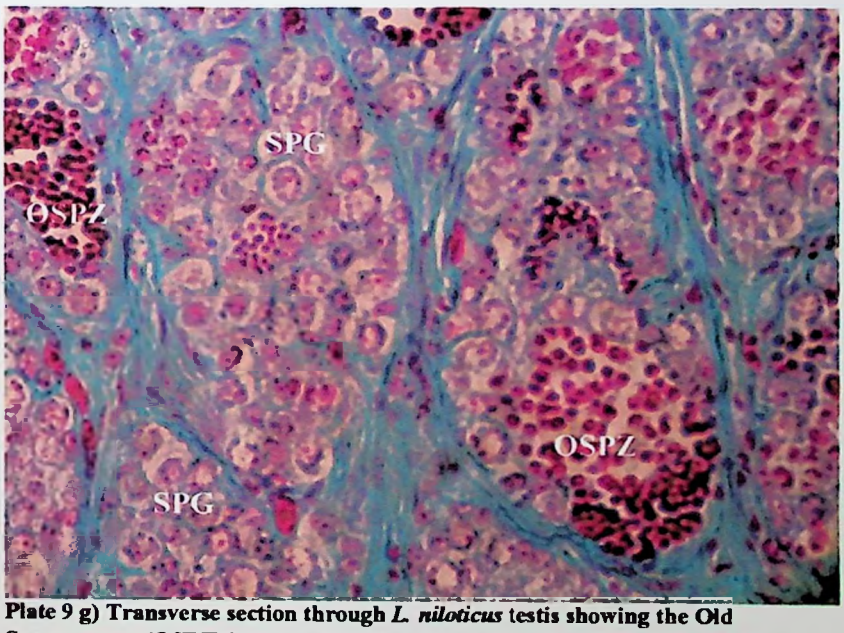


Plate 9 g) Transverse section through *L. niloticus* testis showing the Old Spermatozoa (OSPZ) being replaced by Spermatogonia (SPG) MT X1000

Somatic cells were evident in the spent testis surrounding sermatogonia. The sertoli cells lay close to the spermatogonia (plate 9h) while the interstitial cells of leydig were lying close to the blood vessels.

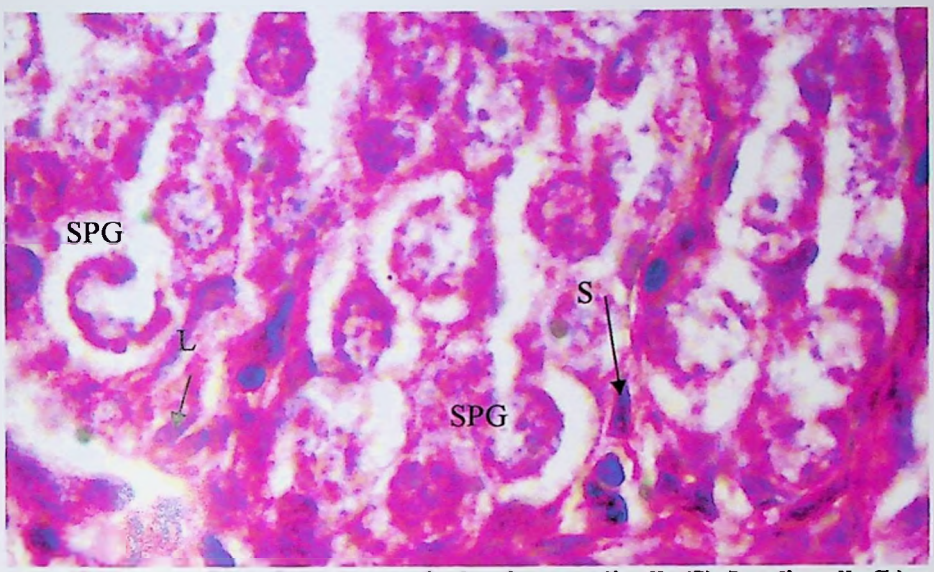


Plate 9h: Section through a spent testis showing sertoli cells (S), Leydig cells (L) and spermatogonia (SPG) X1000 H&E

A summary of the major maturity stages of male *L. niloticus* as observed macroscopically and microscopically have been tabulated (table 4.5b). The macroscopic staging has been greatly modified from Hopson, (1972) and histological correspondence described.

Table 4.5b: Maturity stage classifications for *L. niloticus* males as modified from Hopson, (1972)

Maturity stage	Macroscopic description	Histological description
I-Immature	Paired thin transparent strands running along the dorsal wall of the cavity. The sexes were indistinguishable macroscopically	Indistinguishable sections with Large ovoid cells. Some specimens especially the very small sized fish 10.6 ± 4.2 cm were characterized by presence of large primordial germ cells ovoid shape with a central nucleus. These cells were lightly colored with a darkly stained nucleolus (plate 4a & b)
2-Developing	Testis were semi transparent, grayish white and sometimes pinkish. Fat strand in the early developing was as large or lager than the testis in transverse section	Dominated by spermatogonia (both Type A and B) and a few spermatocytes (spherical in shape). The lobules were beginning to form at this stage
3-Maturing	Testis were semi transparent, grayish white and sometimes pinkish. The testes were vascularised, flattened in transverse section. At this stage there was no milt	Dominated by spermatogonia together with the other germ cells; spermatocytes, spermatids and very few spermatozoa within the lobules of the testis (plate 5a and 9c)
4-Mature/ripe	The testis was opaque, ivory white and soft. Triangular in section with rounded edges. when cut the testis exuded copious amounts of milt	The main sperm duct filled with spermatozoa. High proportion of spermatozoa in the lobules as well (plate 9d). The spermatogonia were greatly reduced in number. Spermatocytes and spermatids were present too.
5-Ripe/ running	Appearance the same as in stage 4 but milt was running freely from the vent on very slight or no pressure applied to it. (plate1)	All sperm ducts and lobules filled with spermatozoa. (plate 9e).
6- spent	Gonads thin strands and Red in colour	Old Spermatozoa in center of the lobules. Later on the spermatogonia from the periphery of the lobules started to replace the old spermatozoa

5.0 DICUSSION

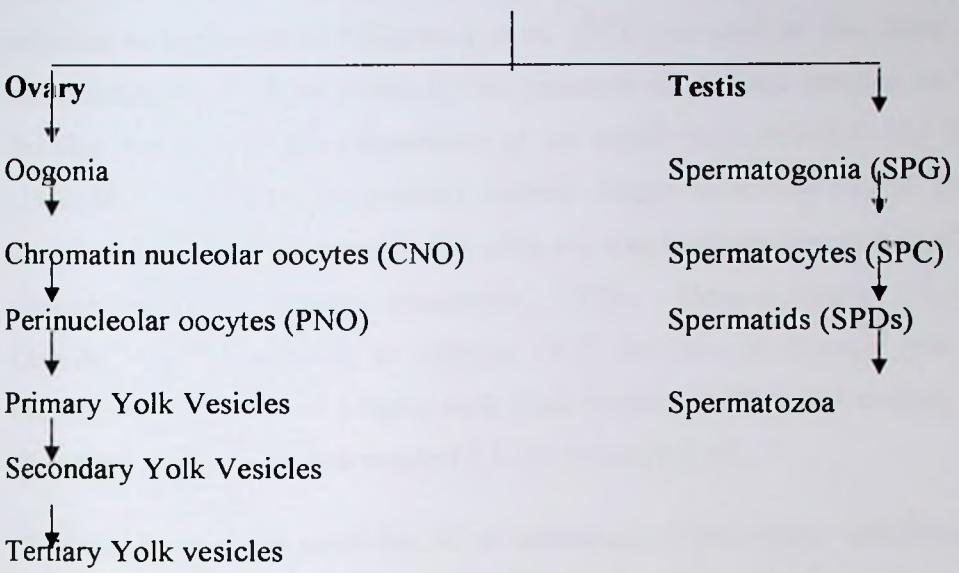
5.1 Sex development pattern in the *L. niloticus*

The absence of any intersex at any one particular size group analyzed in this study indicated *L. niloticus* is primarily a gonochorist exhibiting gonad development of the differentiated type in which the gonads differentiate directly into female or male gonad (Yamamoto, 1969) similar to that observed in the Atlantic halibut *Hippoglossus hippoglossus* (Hendry et al, 2002), *Onchorynchus kistuch* (Piferrer & Donaldson, 1989) and *Cyprinus carpio* (Komen *et al.*, 1992). This type of gonochorisim differs from the undifferentiated type with juvenile intersexuality as was observed in the cyprinid, *Labeo victorianus* also inhabiting L. Victoria (Rutaisire, 2003).

Sadovy and Shapiro (1987) reviewed the criteria for sexual categorization of fishes as gonochorists or hermaphrodites and suggested that a species can only be considered hermaphroditic if a substantial proportion of individuals in a population function as both sexes, either sequentially or simultaneously which was not the case for the *L. niloticus* in this study.

Results in study revealed the presence of gametes of a single sex per gonad that develops to contain spermatozoa in males and ova (tertiary oocytes) for the females. The female gonad differentiated into ovary and seven major oocyte development stages confirmed with histological examination; oogonia, chromatin nucleolar oocyte, perinucleolar oocyte, late perinucleolar stage, primary yolk vesicle, secondary yolk vesicle and tertiary oocyte stage. In the males, the primordial gonad differentiated through four distinct stages; spermatogonia, spermatocyte, spermatid to spermatozoa. No fish examined had both ovarian and testicular tissue and thus ruled out the possibility of hermaphroditism (intersexuality) or sex reversal with in *L. niloticus* as hypothesized by Hughes, (1992). No histological investigations on *L. niloticus* have been documented and yet macroscopic staging criteria should always be validated histologically if errors in determination of maturation patterns and reproductive seasonality are to be reduced (West 1990). It has been further suggested that a histological validation of macroscopic staging criteria descriptions of gametogenesis enable past and future reproductive studies to be compared (Booth & Hecht, 1997)

Figure 5.1: Diagrammatic Representation of Sexual Pattern in *L. niloticus*



From the results, intervention in sex change through the use of exogenous substances would be possible and appropriate in the size range of 0-9cm where 96 percent of the juveniles examined were undifferentiated. Nakamura *et al.*, (1998) noted that it is generally accepted that environmental factors mainly affect the differentiation of germ cells in the gonads at the undifferentiated and differentiating stages. For accurate and complete sex reversal in *L. niloticus* would be ideal before the fish are 9.7± 3.3 cm standard length, and should be after which sexual differentiation occurs

Sex reversal has been accomplished in several species of fishes using exogenous steroids to override natural steroid levels (Schreck, 1974). In order for the process to be effective, steroid hormones must be administered at a sufficient dosage while the gonad is undifferentiated and must continue to be administered throughout gonadal sex differentiation (Yamamoto, 1969) though some other studies have illustrated that treatment throughout the gonadal sex differentiation is not necessary (Piferrer & Donaldson, 1989).

The timing of sex differentiation as highlighted above is crucial and the study thus revealed with histological evidence to be in the size range of 6-19cm SL (beyond 20cm, 70% of fish

revealed with histological evidence to be in the size range of 6-19cm SL (beyond 20cm, 70% of fish were differentiated, figure 1) for both male and female *L. niloticus*. It is generally accepted that the criterion for initial ovarian differentiation in teleost fish is the occurrence of meiotic germ cells for oogenesis (Nakamura *et al.*, 1998). The same criterion as suggested by Nakamura *et al*, 1998, was used in this study where females were distinguished from males by the presence of meiotic oocytes in the ovary. The females were found to differentiate at an earlier size (9.7±3.3 cm) than the males (11.13±2.7 cm, SL). This meiotic nuclear change occurring earlier in the ovary for females and later in the testis for the male has also been documented in other species like *Oreochromis mossambicus* (Nakamura, 1975), *Oryzias latipes* (Yamamoto, 1958; Onitake, 1972). According to Onitake, 1972, the mitosis of germ cells in the genetic female medaka (*Oryzias latipes)* took place before hatching and mitosis did not start in the genetic males until fish reached 6.5 cm in total length.

It should however be noted that some individuals might remain undifferentiated even up to 30cm, in this study 10 % of the *L. niloticus* were confirmed undifferentiated histologically in the size range of 30-39.9 cm, SL. The delayed differentiation of gonads in the *L. niloticus* of some individuals has been observed in other perciformes, for instance Carrillo *et al.*, (1995) observed that the gonads in the European sea bass, *Dicentrarchus labrax* L., remain sexually undifferentiated during most the first year of life and sex ratios remain skewed in favor of males. The exact age at sex differentiation in *L. niloticus* could not be established in this study. The factors that may surround the time and size at which sex differentiation occurs in *L. niloticus* could be influenced by both genetic and environmental factors. These factors may need to be investigated in cultured populations

5.3 Gonadal Morphology of the *L. niloticus* testis and ovary

5.3.1 The Ovary

The results from this study suggest that the *L. niloticus* is a batch spawner with asynchronous follicular growth. The asynchronous follicular growth was concluded based on the slides observed for the mature ovaries, consisting of oocytes at different stages of

development; the tertiary oocytes, secondary oocytes, primary yolk vesicle oocytes, perinucleolar oocytes and post ovulatory follicles. The tertiary oocytes dominated the ovary and there were primary oocytes especially of the perinucleolar stage. Being a batch spawner, the *L. niloticus* in the natural environment would thus spawn almost all year round with peaks in the rainy seasons (Rabour *et al*, 2003) ,the long rainy season (March – May) and short rainy season (September – November). Hamblyn, (1961) reports that the breeding season of L. niloticus is limited in the reverine and may be associated with migratory movements of the adult fish.

To the culturist, the accurate size of tertiary oocytes is essential for induced spawning of fish. Females are canulated and if the eggs are at the tertiary oocyte stage the fish is rendered ready for hormone induction to initiate ovulation. According to Gilles Le Moullac *et al.*, (2003), *L. calcarifer* females are canulated to obtain samples of oocytes allowing to select them according to average oocyte size (>400µm). Females showing the most homogeneous batches of oocytes superior to 400 µm are chosen for hormonal injection at 15 to 20µg/kg of body weight of luteinizing hormone-releasing hormone analogue. Based on procedures followed for induced spawning of *L. calcarifer* and consideration of findings from the current study on size of tertiary oocytes and type of spawning in *L. niloticus,* females showing homogeneous batches of oocytes superior to 475.5 ± 70.7 µm should be selected for hormone induction in order to breed the fish. The presence of cohorts (plate 8a) suggests that *L. niloticus* is a batch spawner.

The oocyte size and stage has a bearing on the GSI, in this study the highest recorded GSI was relatively low (3.65%) though higher than that recorded in the same fish by Ogutu-Owhayo, (1988). The slightly higher GSI of 3.65% in the present study may have resulted from the method used to calculate the GSI based on eviscerated weight (Ev.) and not the total weight in order to minimize variations due to feeding.

The low GSI among the mature *L. niloticus* females of between 2-3.65% (maximum of 2% observed in the study by Ogutu-Ohwayo, 1988 and 3.65 % in the present study) is characteristic for batch spawners and greatly differs from that observed in total spawners that are usually characterized by relatively high GSI. Tyler & Sumpter, (1996) in their review of growth patterns, rates and dynamics of oocyte growth in teleost fishes

concluded that in total spawners the weight of the gonad may represent as much as 40% of the overall body weight of the fish. Other total spawners like *Labeo Victorianus*, high GSIs have been recorded by Rutaisire, (2003) of 2-30% (for *L. victorianus* from River Sio) and 10.44 - 10.64% (for *L. victorianus* from River Kagera). It must be noted that maturation and ovulation were not macroscopically or microscopically noted in the duration of this study and may thus explain the relatively low GSI recorded in this study. Fish induced to spawn under captivity is easier monitored through all stages to maturation and ovulation.

The GSI increased with increase in fish length but also divergences from this trend were observed in just spawned individuals who were characterized by low GSI and relatively flabby ovaries. Fresh post ovulatory follicles (POFs) were evident at this stage.

5.3.2 The Testis

The testis in *L. niloticus* was comprised of germinal compartments that extend to the periphery and terminate blindly into lobules a pattern that conforms to lobular description (Parenti and Grier, 2004). According to these workers, the testis type in lobular form can be further divided into unrestricted lobular and restricted lobular types based on the distribution and arrangement of the spermatogonia. Based on this classification, the lobules in the *L. niloticus* are of the unrestricted type where the spermatogonia are distributed along the whole length of the lobule, this is opposed to the restricted type where the spermatogonia are found at the distal end of the testis lobules. The type of testis in the *L. niloticus* is similar to the other perciformes (in family Centropomidae; *Centropomus undecinalis* (Taylor *et al.*, 1998), family percidae; *Perca flavescens* (Grier *et al.*, 1980).

The type of spermatogenesis in *L. niloticus* is of cystic nature where the cells develop in cysts that finally rupture to release the spermatozoa, a type similar to that reported in other teleost fish species like *L. victorianus* (Rutaisire 2003). The cells in a given cyst were found to be at the same development stage. The information on testis structure and spermatogenesis in *L. niloticus* generated during this study can be used for accurate assessment of maturation and sperm preservation.

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The study illustrated the primary development of a testis (males) and ovary (female) from an undifferentiated primordial gonad. Gonadal development pattern in *L. niloticus* was found to be gonochoristic where individuals develop from an ovarian or testicular juvenile pathway. All the fish individuals (178) analyzed with histological, microscopic and macroscopic techniques were found to be either undifferentiated, males with testis or female with ovaries.

Results of this study are important in the production of monosex populations of *L. niloticus* since the size at which the first indication of sex differentiation was evident and defined at 9.7±3.3cm for females and 11.13±2.7 cm, SL for males. Following the constraints of Yamamoto, (1969) for effective sex reversal, in this species sex influencing hormones are likely to be effective at the size less than 6cm, SL.

Mature *L. niloticus* were found to be sexually dimorphic with the males having only the anal and urogenital openings just anterior to the anal fin, where as the females had a genital orifice separate from the urinary opening. However, careful observation is crucial as it was not very easy to differentiate the fish in non ripe condition and macroscopic errors were common requiring histological validation. In the ripe conditions mature males can be identified application of slight pressure on the abdomen which results in exudation of milt. Clear sexual dimorphism (male-2 openings and females -3 openings) will be a key instrument in broodstock management for successful spawning.

The testis in *L. niloticus* was if the unrestricted lobular type, with spermatogonia scattered along the whole length of the lobule and was found to undergo the cystic type of spermatogenesis where the cells developed and matured in cysts that rupture to release spermatozoa into the lumen of the lobules. The knowledge of structure of gametes can be used for accurate assessment of maturation and sperm cryopreservation.

The testes of *L. niloticus* were large and ripe males exuded copious amounts of sperm and hence would not be sacrificed as is the case in other fish species like the channel catfish, *Ictalurus* punctatus and the African catfish, *Clarias gariepinus*

6.2 Recommendations

For induced spawning in aquaculture practices it would be unnecessary to sacrifice the males since ripe *L. niloticus* males have large testis and amounts of sperm that on application of slight pressure on the belly males release sufficient amounts for artificial fertilization.

Borrowing the procedures followed for induced spawning of *L. calcarifer* and based on findings from the current study on size of tertiary oocytes and type of spawning in *L. niloticus,* females showing homogeneous batches of oocytes superior to 475.5 ± 70.7 μm should be selected for hormone induction in order to breed the fish.

Further studies focusing on specimens beyond the 110 cm SL can be done to establish the possibility of sex inversion or change as it can be triggered by a number of factors.

Further studies should be conducted with large sample sizes and continuous sampling for a protracted temporal period of at least twelve months and samples subjected to histological analysis. Variations of GSI all year round can be captured to determine peak spawning seasons.

Hormonal profiles should be determined and related to gonadal development and cycling

Further studies should be conducted correlating sex development with age of the fish this will be possible and practical if the fish is bred and grown in captivity.

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Appendix 1

Table 1: Number of fish with undifferentiated gonad, ovary, bisexual gonad or testis in different groups.

Age	SL (M+ SD)	Gonad				Total	Binomial
		Undif.Gonad	Ovary	Bisexual gonad	Testis		

Table 2: The Change in Sex Ratio with Standard length of *L niloticus*

The change in sex ratio with standard length of *Lates niloticus*				
Length class cm (SL)	No. of females	No. of Males	Intersex	Sex ratio

Table 3: Specimen label

Fish No.	
Weight(g)	
SL(cm)	
TL(cm)	
Condition	
Gonadal stage	

Table 4: Maturity stage classification for males and females modified from Hopson, (1972)

x	Maturity stage	Macroscopic description	Histological description
ale	I-Immature		
	2-Early developing		
	3-Late developing		
	4-Mature/resting		
	5-Mature/ripe		
	6-Ripe/ running		
emales	1-immatue		
	2-Resting		
	3-Early maturing		
	4- Late maturing		
	5-Ripe		
	6-Running		
	7-Spent		

Raw Data sheet: Msc. Basiita

o.
ate
te/location
ake/source of fish
mples

pe m	Hist Code	Tag. No.	S L	TL	T. Wt.(g)	Ev. Wt	wt.o G	L.o G	Egg count	Liver wt.	Wt.of V	Fat wt.	remarks