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Anatomical studies of the testes of wild African catfish (*Clarias gariepinus*) in spawning and non-spawning seasons in Maiduguri, Nigeria

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Abstract
The study was aimed at documenting the gross and histology of testes of wild African catfish (*Clarias gariepinus*) during spawning and non-spawning seasons in Maiduguri. Grossly, the testes were lobulated and whitish during the spawning season; during non-spawning season the lobulations were not clear and it was pinkish in colouration. Histologically, the testes were encapsulated with connective tissue fibres and the dense interstitial connective tissue divided the testis into wide compartments with presence of spermatozoa during the spawning season. During non-spawning season, the seminiferous tubules were markedly reduced with clusters of dormant spermatogonia. The tunica albuginea covering the testis was of loose connective tissue but the underlying dense collagen fibres divided the testis into trabeculae containing seminiferous tubules when viewed at higher magnification. The epithelium of the seminiferous tubules also contained spermatogenic cells. Also during the spawning season, different shapes and sizes of seminiferous tubules containing darkly stained spermatozoa undergoing regeneration were observed.

Keywords: Anatomy, Testes, African Catfish, Maiduguri, Nigeria

1. Introduction
Fish consumption in the north eastern part of Nigeria to some extent depends partly on wild catches by local fishermen and partly on domestic fish production through aquaculture [1]. However, animal protein is generally essential for proper growth, repair and maintenance of body organs and tissues. Most estimates put an average daily protein requirement (DPR) at 64g/individual/day and it is considered optimum that fish provides at least 5% of this i.e. about 10kg/individual [1]. The productive potential of fish compared to any other farmed animal is also very high. Fish is an important source of human protein that has increasing importance as supplement to dietary requirement of human population worldwide; to meet this need, many countries have turned to fish farming [2]. Fish is a highly digestible food (>95%) because of low collagen level [3]. The African catfish have been reared for more than 20 years in Africa due to its ability to utilize atmospheric oxygen for some times when outside water as well as low dissolved oxygen tolerance in their culture environment [4]. Clariids have high consumer preference ranking [5], high fecundity rate [6], tolerance to high stocking density and environmental extremes, i.e. hardy species [7], and has efficient food conversion [8]. The African catfish are disease resistant [5]. They also accept wide range of natural food organisms and adoption of variety of feeding modes in expanded niche [9]. The increase in total fish production between 1992 and 1993 came entirely from aquaculture. In Nigeria, capture fisheries and aquaculture play leading roles in fish production, contributing an average of 84.2% of the total domestic output between 1990 and 1994 [10]. Out of the approximately 650,000 metric tons annual fish output in Nigeria, 350,000 metric tons was locally produced with inland water and aquaculture accounting for 110,000 metric tons and 18,000 metric tons, respectively [11]. In fish production, several attempts to collect milt from African catfish have not been successful [12], and the traditional method of breeding have been that the males are sacrificed in order to remove the testis and the milt (sperm) macerated for breeding purposes. However there are information on the histology of these organs in some teleosts like Corydoras aeneus [13], sharptooth African catfish from Lake Kinneret in Israel and Blyde river South Africa [14] and farmed African catfish [15]. Despite all these, there is still a dearth of information available from literature on the anatomy of the testes of the wild African catfish

from commercial aquaculture. Therefore, the data obtained from this study will be used as a source of information on the anatomy of testes of *C. gariepinus* found in Maiduguri, Nigeria and to which comparison could be made to other species.

2. Methodology

2.1 Period of study

The fish samples were procured in the months of January to February during the non-spawning season while in the spawning season it was collected in the months of July to August.

2.2 Study area

The study was conducted in Maiduguri, Nigeria, located between latitude 11º and 50º north and longitude 13º and 36º east. The annual rainfall average 320mm, rainy season begins in June and last till October and dry season begins in November and last till May. The rainfall is monsoonal, generally been heaviest in August. The annual temperature average 35.4ºC, the climate of Maiduguri can be divided into six zones: Guinea, Sudano-Guinea, sudano-sahelian, sahelo-sudanian, sudano-saharan and Saharan zones [16].

2.3 Source of fish

Ten adult male African catfish (*C. gariepinus*) were utilized for the study. Five fish each during spawning and non-spawning seasons. They weighed an average of 750 g and measured a standard body length of 45 cm in length. All fish were procured from Gamboru fish market in Maiduguri, and transported live in a plastic trough to the Veterinary Anatomy Laboratory, University of Maiduguri.

2.4 Experimental design

Each fish was euthanized using tricaine MSS anaesthetic at the dose of 8 drops/litre of water [17] after which a mid-ventral incision was made and the testes was exteriorized en mass using scalpel, scissors and tissue forceps and it was photographed using canon digital camera power shot (A470). Samples for histological examination were taken and fixed in Bouins fluid for 24 hours. The tissues were then dehydrated through grade concentrations of ethanol (70%, 95% and absolute), cleared in Xylene. Tissues were infiltrated in Xylene paraffin in the oven at 62 degree for 3 hrs and embedded in paraffin wax. The embedded tissues were sectioned at 7 micrometer thickness and stained with haematoxylin and eosin (H&E) for light microscopic examination [18]. The tissues observed under microscope were also photographed using the same camera.

3. Results

The testes were seen located deep within the abdominal cavity as a paired organ lying parallel along the long axis of the body. They are lobular and whitish during spawning season (plate1) and pinkish in colouration during non-spawning season (plate2). Histologically, the tunica albuginea was seen covering the underlying dense collagen fibres divided the testis into trabeculae containing seminiferous tubules that are markedly reduced in size during non-spawning season (plate3). At higher magnification, Sertoli cells and dormant spermatogonia were observed (plate 4). The epithelium of the seminiferous tubules is pseudo stratified cuboidal cells. The transverse section of the testes revealed a non-secretory epithelium of simple cuboidal cells supported by basement membrane in each of the regressed seminiferous tubules. During spawning season, the seminiferous tubules containing the darkly stained spermatozoa and interstitial connective tissue were visible (plate 5). At higher magnification during spawning season, the seminiferous tubules were clearly seen (plate 6), indicating the regeneration of the regressed testes have completely taken place.

Plate 1

Plate 2

Fig 1: Pictures of testes of African catfish (*C. gariepinus*) during spawning (Plate 1) and non-spawning (Plate 2) seasons.

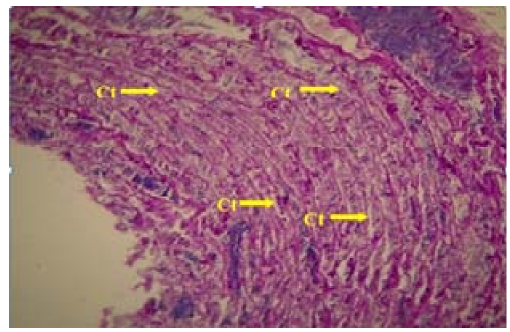

Plate 3

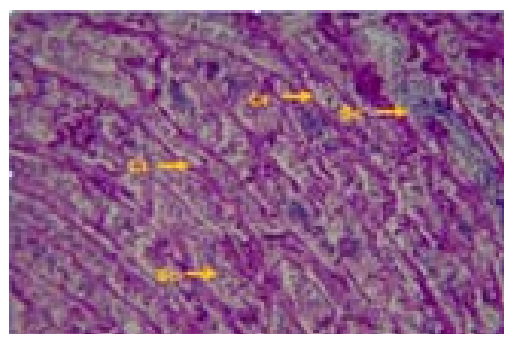

Plate 4

Fig 2: Photomicrograph of testis of *C. gariepinus* during non-spawning season, arrows showing regressed connective tissue (Ct) at low plate 3, and higher magnification at plate 4.

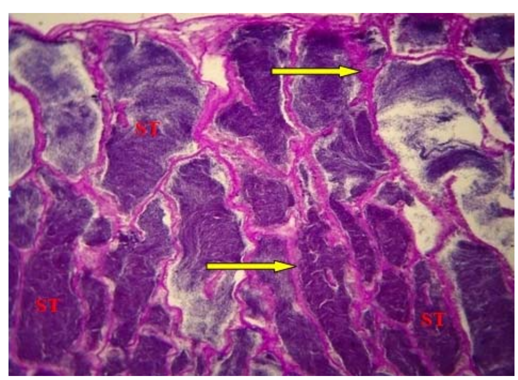

Plate 5

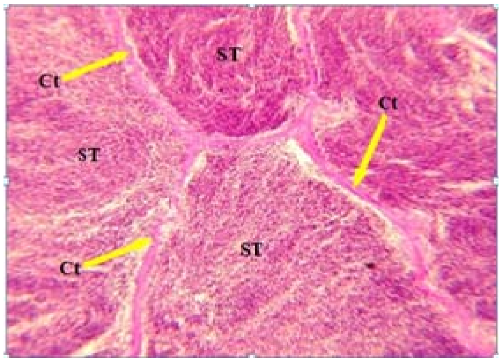

Plate 6

Fig 3. Photomicrograph of testes of African catfish (*C. gariepinus*) during spawning season showing seminiferous tubules (ST) containing spermatozoa and arrows depicting interstitial connective tissue at low (plate5) and high magnification (plate6)

4. Discussion

The testes of catfish are slender paired organ located deep in the caudal part of the abdominal cavity that are whitish and milky colour appearance during spawning period which agrees with the report of [19] that during non-spawning season the reproductive organs of fish including the testis tend to regress and probably changes colour from whitish during spawning period to pinkish in non-spawning period as also observed by [12] that the testis are located deep within the abdominal cavity that are whitish during breeding season which could be as a result of presence of milt (sperm) in the testis. It was also reported that the testis as a paired organ is located in the posterior part of the abdominal cavity [15] and has been documented in other fish like *Parasilurus aristotelis* [20]. The connective tissue of the testis; tunica albuginea is for protection of the seminiferous tubules. The germinal epithelium of the seminiferous tubules containing spermatogenic cells is the site of spermatogenesis [15]. Other workers have reported that some teleost testis may in addition to sperm cell formation serve as storage organ for mature sperm cells [21], which agrees with the findings in this study as presence of spermatozoa were found in the seminiferous tubules which could be responsible for the white colouration during spawning season but during non-spawning season the spermatozoa may shrink and later be active and motile during spawning period. Testis of the Catfish may also have secretory function [22]. The testis of African catfish, were also reported to be steroid genic [23, 24, 25] and produce a variety of steroids during breeding season [26, 27].

African catfish is one of the vertebrate that exhibit seasonal breeding. Seasonal breeding is the lack of continual reproduction throughout the year in particular species or populations inhabiting non-equatorial areas of the Earth, due to circannual fluctuations of the environmental conditions resulting from climatic seasons [28]. This is an adaptive process that ensures that the new individuals are born or hatched and grown during the season that provides the best conditions for survival. In females, ovulation is halted in a particular period of the year, whereas in males testis function is also either reduced or depleted during the non-breeding season. The regression of the testes as observed during the non-spawning season agrees with the report of [28]. According to [28], males undergo a reduction in testis size accompanied by lower testosterone production, spermatogenesis arrest, and inhibited mating behaviour. In some species, spermatogenesis is completely arrested, and the animals are unable to breed for several months. This occurs in a variety of species. A consequence of seasonal breeding is seasonal testis regression, which implies the depletion of the germinative epithelium, permeation of the blood-testis barrier, and reduced androgenic function. This process has been studied in a number of vertebrates, but the mechanisms controlling it are not fully understood [28].

In seasonal breeders, the reproductive status of individuals must change cyclically between the active and the inactive stages, implying that the function of the Hypothalamus-pituitary-gonadal (HPG) axis must also be modulated so that reproduction is activated during the spawning season and halted during the resting period or non-spawning season. The factors responsible for the seasonal variations in reproduction of African catfish and other vertebrates are not known [29]. However, environmental cues such as temperature, food availability, rainfall, water turbidity, salinity and photoperiod are known to be involved in this process [28]. Photoperiod provides valuable information about the climatic seasons that remains constant from year to year, but response to photoperiod is species-specific and depends mainly on gestation length [29].

Also in seasonal breeders, the architecture and function of the testis undergo profound circannual changes that generally imply the elimination of the germinative epithelium during the non-breeding period, a phenomenon in which processes and structures such as the blood-testes-barrier (BTB), androgenic production, apoptosis, and cell proliferation are significantly altered. Nevertheless, the mechanisms underlying the transition between the spawning and non-spawning seasons, the so-called testis regression and regeneration differ among species [30].

The different shapes and sizes of seminiferous tubules containing spermatozoa were observed during spawning season in this study. However, during non-spawning season the seminiferous tubules regressed completely to the extent that only interstitial connective tissues were seen. This could be as a result of direct consequence of the germinative epithelium depletion during testis regression and reduction in the diameter of the seminiferous tubules and the subsequent shrinkage in testis size in the males of most seasonally breeding species [31, 32]. In some species of vertebrate, the inactive testis maintains some meiotic activity, as meiosis onset is not completely interrupted and some few primary spermatocytes are still present in the regressed seminiferous tubules [31, 32]. In this study, clusters of Sertoli cells and spermatogonia were observed at higher magnification during non-spawning season. This situation appears to be quite common in many species that undergo seasonal testis regression, as observed in the *Cynops pyrrhogaster* [33], the silver fox, *Vulpes vulpes* [34], the Syrian hamster [35, 36], and the Chinese soft-shelled turtle, *Pelodiscus sinensis* [37]. However, in African catfish species and other vertebrates, the regressed seminiferous tubules contain only Sertoli and spermatogonial cells, showing complete absence of meiosis onset in the inactive period. This is true for the white-footed mouse, *Peromyscus leucopus* [38], the European starling, *Sturnus vulgaris* [39], and the large hairy armadillo, *Chaetophractus villosus* [40, 41].

During spermatogenesis, primary spermatocytes must pass through the BTB to gain access to the luminal compartment where meiosis is completed. This process implies Sertoli-Sertoli and Sertoli-germ cell interactions at the level of cell junctions, and the BTB acts as a dynamic structure that undergoes cyclical changes of 'opening' and 'closing' to facilitate germ cell migration. This event is tightly regulated and involves a complex network of signalling cascades and the rapid turnover of junction-associated molecules [43]. The BTB is also of great physiological importance, selectively permitting passage to some molecules that can enter the luminal compartment. Moreover, it is an immunological barrier that segregates late meiotic and post-meiotic germ cell antigens from the systemic circulation. It creates a unique microenvironment for germ cell development and confers cell polarity. Thus, when the BTB is dysfunctional, germ cell differentiation and development usually fail [42].

Also histological seasonal changes of African catfish testes as observed in this study have been investigated in a number of vertebrate species, including reptiles, amphibians, birds, and mammals. The generally accepted hypothesis derived from these studies states that apoptosis is the main cellular process mediating seasonal testis involution [43, 44]. Apoptosis permits the elimination of damaged, diseased, or superfluous cells from many parts of the body during tissue remodelling and differentiation [45], playing essential roles in reducing cell numbers. Spermatogenesis is characterized by high proliferation rates, and the coincidence of spermatogonial proliferation and spontaneous degeneration of spermatogenic cells seems to be normal in the vertebrate testis [46]. Although spermatogonia and spermatocytes have been described in many species as the main cell types undergoing apoptosis, this may affect all types of male germ cells, including spermatogonia, primary and secondary spermatocytes, spermatids, and sperm [47]. In the rat testis, spontaneous apoptosis of spermatogonia occurs regularly while primary and secondary spermatocytes as well as spermatids occasionally undergo apoptosis [48]. The comprehensive analysis of spermatogonial apoptosis is important in the regulation of spermatogonial population density of all seasonal breeders, as well as in the maintenance of the required homeostasis among the various germ cell types that can be supported and nursed by Sertoli cells. In addition, germ cell apoptosis seems to safeguard the genetic integrity of the male gamete and the synchronization between the spermatogonial and the spermatocyte cycles, eliminating harmful, irreparably damaged cells that are not able to pass checkpoint-monitored transitions due to improper synapsis between homologous chromosomes [49, 50].

5. Conclusion

In conclusion, Maiduguri because of its proximity to Lake Chad and Lake Alau, many people are artisan fishermen. However, capture fisheries from the wild is inadequate to meet the demand of fish and fishery products. As a result, more and more people are picking interest in fish farming. Although despite advances in the studies of aquaculture, there is still a dearth of information available from literature on the gross and histology of the testes of the wild African catfish from commercial aquaculture. The data obtained from this study will be used as a source of information on the gross and histology of testes of *C. gariepinus* found in Maiduguri, Nigeria and to which comparison could be made to other species.

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