# SEASONAL TESTICULAR HISTOLOGY AND REPRODUCTIVE CYCLE OF THE RAINBOW LIZARD, *Agama agama agama*, L, (AGAMIDAE, REPTILIA) IN ILE-IFE, SOUTH WESTERN NIGERIA

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**ABSTRACT**

***Seasonal histological features of the testis and epididymis were studied in male A. agama agama from July l990 to June, 1992 at Ile-Ife, Nigeria. Testis weights showed no significant difference (P > 0.05) in the dry and rainy seasons, but were generally low from August to January. Whereas seminiferous tubule diameter and epithelia heights showed no seasonal variation (p > 0.05), the epididymal tubule diameter and epithelia heights varied seasonally (P < 0.001). Although males in full breeding condition were caught all through the months, such were more prevalent from April to July. Females with eggs or enlarged ovarian follicles were caught all through the study period. Cases of multiple clutches were predominant from February to July. However vitellogenic activities decreased from August to January thus coinciding with the observed decrease in spermatogenic activity in the male. We propose that individual male Agama lizards maintain peculiar breeding patterns and that reproduction in Agama seems to be influenced by food availability as well as microclimatic conditions at oviposition sites.***

**Keywords:** Tropical lizards, Agamidae, Histology, Testes, Spermatogenesis, Reproduction

## INTRODUCTION

Various studies on testicular cycles of tropical lizard species indicate that spermatogenic patterns are peculiar to each species. Many tropical male lizard species exhibit continuous spermatogenic cycle with little or no evidence of variations in the testicular or seminiferous tubule size (Wilhoft, 1963; Somma and Brooks, 1976; Simbotwe, 1980; Vial and Stewart, 1985; Wikramanayake and Dryden, 1988). Others undergo cyclic changes in their testicular mass as an indication of their reproductive readiness (Wilhoft and Reiter, 1965; Marion and Sexton, 1971; Sexton *et al*., 1971; Sherbrooke, 1975). Most of the observed changes in testicular mass were found to occur mainly during the dry season at which time spermatogenesis also continued at a more or less reduced rate.

The roles environmental factors play in regulating lizard reproduction in the tropics have been variously reported (Licht, 1971, 1973; Gorman and Licht, 1974; Vitt, 1982). Generally, environmental factors exert indirect influence on reproductive strategies of tropical lizards. In tropical regions with clear cut wet and dry seasons, the lizard species have been reported to reproduce during the wet season which is generally regarded as a period of abundant proteinous food (Marshall and Hook, 1960; Janzen and Schoener, 1968; Sexton *et al*., 1971; Janzen, 1973). Lizards occupying tropical environments with non-thermal seasonality have been reported to manifest continuous breeding patterns (Sherbrooke, 1975; Vitt, 1982; Vial and Stewart, 1985). On the other hand, the influence of low temperatures on hatching potentials of eggs have been deduced as the major cause of variations in ovarian cycles of some Anoline lizard species in Puerto Rico (Gorman and Licht, 1974).

The Rainbow lizard, *A. agama agama* is the most common and widely distributed lizard species in Nigeria. It has an enviable tolerance for considerable range of climatic conditions (Harris, 1963, 1964). Information concerning reproductive activities of male *Agama* has been very limited. Sodeinde and Kuku (1989) reported a more or less general account of the presence or absence of spermatozoa in the lumen of the testes for some months. Ejere and Adegoke (2002) linked the presence of polyploid spermatocytes to the reproductive success of male *Agama* at Ile –Ife, South western Nigeria.

The present study was aimed at utilizing data accumulated for 24 month period for males and females of *A. agama agama* to: -

(i) describe the gross morphological and histological features of the testis and epididymis of the males;
(ii) establish the seasonal pattern of their spermatogenic and vitellogenic cycles, and;
(iii) compare the observed reproductive pattern with those described for similar *Agamid* species in other localities within and beyond Nigeria.

## MATERIALS AND METHODS

**Study Area and Meteorological Data:** Data collection was carried out at Obafemi Awolowo University, Ile-Ife, Nigeria sited within the rain forest region of South Western, Nigeria (Harris, 1964). The campus is located at latitude $07^{o}$ $28^{1}$N and longitude

$04^{o}$ $33^{1}$E with an altitude of 800 m above mean sea level (MSL).

The meteorological data collation and interpretation are in accordance with that reported earlier for the study area in respect of rainfall, relative humidity and temperature from July 1990 to June 1992 (Ejere and Adegoke, 2002; 2003). Two seasons were determined for the study area. The rainy season began in April and ended in October, while the dry season began from November and ended in March of the following year. The study area also showed little or no monthly temperature fluctuations. This regimen of fluctuating rainfall and lack of thermal seasonality is consistent with the definition of most tropical environments (Sexton *et al.*, 1971; Sherbrooke, 1975; Vitt, 1982; Vial and Stewart, 1985).

**Field and Laboratory Methods:** Male and female specimens of *A. agama agama* used were caught randomly on a biweekly basis for the 24-month period of the study. The animals used were manually trapped from the walls of houses, in gutters, and on grass lawns which minimized injury to the lizards. They were collected both during the day and night periods. The live lizards were taken to the laboratory within 24 hours of capture and anaesthetized with chloroform.

The snout-vent length (SVL) and intact tail length were obtained to the nearest millimeter using a meter rule. Anaesthetized lizards were then autopsied. For males, the left testis weight was obtained using a Mettler balance. Observations made on the condition of the testes and epididymides were recorded. The left testis and epididymis were then fixed in Bouin's fluid. Standard histological procedures including dehydration in series of graded ethanol, clearing in xylene, embedding in fresh molten paraffin wax, sectioning at $8\mu$, staining with Ehrlich's haematoxylin and counter-staining with Eosin (Humason, 1979) were employed.

Histological interpretation of the testis and epididymis, as well as the spermatogenic stages were in accordance with the technique of Mayhew and Wright (1970). Diameters of the seminiferous and epididymal tubules as well as their epithelial heights were read off from a microscope fitted with an ocular micrometer. These measurements enabled the determination of probable seasonal changes in the testis and epididymis. Simultaneously, the females were examined for the presence of oviductal eggs; yolking enlarged ovarian follicles, as well as evidence for multiple clutches (Vitt, 1977).

**Data Analysis:** Pairwise comparisons were made using the T-test while the relationship of variables was determined by linear regression analysis (Sokal and Rohlf, 1981). Results of all statistical tests were considered significant at $P < 0.05$ and highly significant at $P < 0.001$.

## RESULTS

A total of 130 male *Agama* lizard species were sampled for the 24 months. All male *Agama* measuring 115.0 mm SVL and above had spermatozoa in the lumen of the seminiferous tubule and were considered as adults.

**Testicular Weight Cycle:** The mean left testis weights in *Agama* were 200.92 $\pm$ 113.879 mg. Whereas the left testis weights showed no significant difference ($p > 0.05$) between the dry and rainy seasons, some form of monthly variations were evident. The testis weights showed maximal increase from about the end of the dry season in March (298 $\pm$ 113.879 mg) through the beginning of the rainy season in April (340 $\pm$ 113.879 mg). The weights were lowest from about the end of the rainy season in October (52 $\pm$ 113.879 mg) through the beginning of the dry season in November (53 $\pm$ 113.879 mg). For the rest of the months, the testis weights exhibited more or less intermediate values. Secondly, the testis weights in *Agama* showed no correlation with the SVL ($P > 0.05$, $r = 0.038$, $n=72$).

**Spermatogenic Cycle:** Examination of the testicular histo-sections revealed that spermatogenic activity in adult male *Agama* lizards followed the same general pattern developed for lizard species (Mayhew and Wright, 1970). The proportions of male *Agama* exhibiting these various spermatogenic stages per month are illustrated in table 1. There was the absence of stage 2 tubules in adult male *Agama.* Whereas males with fully developed testes (stage 6) were obtained in all the months of the year, some kind of monthly variations occurred in the proportions. The proportion of male *Agama* possessing testes in full breeding condition was generally low from about the late rainy season (August to October) through the dry season (November to March). There was an increase in the proportion of males in full breeding condition from onset of the rainy season, April (92 %) such that by June and July, all the males caught had stage 6 testes.

There was no statistical difference in the seminiferous tubule diameter and epithelial heights between seasons in this lizard species ($p > 0.05$). However, the seminiferous tubule diameters correlated positively with the testis weights ($p < 0.05$, $r = 0.672$, $n = 72$). Similarly, no correlation was observed between the seminiferous tubule diameter and the SVL ($p > 0.05$, $r = 0.003$, $n = 72$).

**Epididymal Cycle:** The histological appearance of the epididymis in male *Agama* is in accordance with the descriptions of Mayhew and Wright (1970). They consisted mainly of an external basement membrane with a single row of cuboidal to columnar epithelial cells lining the lumen of the tubule.

**Table 1: Monthly percentage of adult male *A. agama agama* exhibiting various spermatogenic stages**

| Organism | Stage | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sept | Oct | Nov | Dec | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | | | | | | | | 14 | | 31 | 37 | 9 | |
| | **2** | | | | | | | | | | | | | |
| | **3** | | | | | | | | | | 8 | 13 | 9 | |
| **Agama** | **4** | | | 7 | | | | | 14 | | | | | |
| | **5** | 82 | 75 | 73 | | | | | 43 | 33 | 8 | 37 | 46 | |
| | **6** | 18 | 25 | 20 | 92 | 75 | 100 | 100 | 29 | 33 | 8 | 13 | 36 | |
| | **7** | | | | 8 | 25 | | | | 33 | 14 | | | |
| | **8** | | | | | | | | | | 31 | | | |
| **Number** | | 11 | 12 | 15 | 12 | 8 | 7 | 6 | 7 | 9 | 13 | 8 | 11 | 119 |

**Table 2: Snout-vent lengths, clutch size, number of enlarged follicles and multiple clutches in female *A. agama agama***

| Month | Snout-vent lengths (mm) | | Oviductal eggs | | Enlarged follicles | | Proportion of females with evidence of multiple clutches |
|---|---|---|---|---|---|---|---|
| | Mean SVL | Range | Mean clutch size | Range | Mean | Range | |
| **January** | 106.6 | 98-116 | 4.5 (2) | 4-5 | 10.6 (5) | 7-22 | 0 |
| **February** | 112.9 | 102-120 | 6.9 (7) | 6-8 | 0 | 12(1) | 5/8 |
| **March** | 108.6 | 99.5-122 | 7.1 (8) | 6.9 | 0 | 0 | 4/8 |
| **April** | 110.3 | 100.5-123 | 6.0(7) | 4-7 | 0 | 0 | 3/7 |
| **May** | 112.5 | 102-118 | 5.5(4) | 4-7 | 0 | 0 | ¼ |
| **June** | 109.7 | 105-115 | 6.5(2) | 6-7 | 0 | 22.0(1) | 1/3 |
| **July** | 114.0 | 110-118 | 3.7(3-4) | 3-4 | 0 | 0 | 2/3 |
| **August** | 118.5 | 118-119 | 0 | 4.0(1) | 0 | 10.0(1) | 0 |
| **September** | 115.0 | 112-121 | 5.8(4) | 4-7 | 0 | 9.0(1) | 3/5 |
| **October** | 110.3 | 103-121 | 4.7(3) | 4-5 | 6.3 (3) | 5-7 | 0 |
| **November** | 0 | 116.0 | 0 | 0 | 0 | 9.0(1) | 0 |
| **December** | 116.0 | 110-125 | 0 | 0 | 12.5(4) | 4-24 | 0 |

*Sample sizes are in parentheses.*

The epididymes of testes in stages 3, 4 and 5, had a somewhat mixture of columnar and pseudostratified epithelium. In the full breeding condition (stage 6) the epididymis had a columnar epithelium usually with prominent basophilic granules. The epididymides of testes in stage 7 were observed to possess purely pseudostratified cuboidal epithelium.

There was a steady increase in the epididymal tubule diameter during the breeding stage over that observed in the non-breeding stages. The mean tubule diameter of the epididymis which testes was in stages 3, 5 and 6 caught were 67.80 $\pm$ 26.82$\mu$, 88.40 $\pm$ 26.82$\mu$ and 117.80 $\pm$ 26.82$\mu$ respectively. The epididymal epithelia heights also showed variations which were largely associated with the developmental stage of the testis in individual males. The mean epididymal epithelia heights of testes in stages 3, 5 and 6 were 11.80 $\pm$ 6.66$\mu$, 19.30 $\pm$ 6.66$\mu$ and 23.90 $\pm$ 6.66$\mu$ respectively.

Morphologically normal spermatozoa were observed in the lumen of the epididymes irrespective of the stage of development of the testes. However the functional capability of the spermatozoa observed in the epididymes of non-breeding testes was not verified. Furthermore, a highly seasonal statistical significant difference, $p < 0.001$ was observed in the mean epididymal tubule diameters and epithelial heights. The mean epididymal tubule diameter (107.11 $\pm$ 26.992$\mu$) and epithelia heights (21.98 $\pm$ 6.654$\mu$) were highest during the rainy season.

**Ovarian Cycle:** Female *Agama* with oviductal eggs was caught throughout the year except in the months of November and December (Table 2). The percentage of such females with oviductal eggs was much more in February (87.50 %), March (100 %) and April (100 %). Generally, the percentage of females with oviductal eggs was higher in the rainy season (58.50 %) as against 41.50 % obtained in the dry season. Clutch size varied from 3 to 9 eggs with a mean clutch size of 6 eggs. A relatively high percentage of female *Agama* species exhibiting multiple clutches were observed in February, March, April, July and September. Females with enlarged follicles were observed monthly except in March, April, May and July. No correlation was observed between the SVL and clutch size ($p > 0.01$, $r = 0.166$, $n = 41$).

## DISCUSSION

Reports on agamid reproduction in the rainforest region of Nigeria include those of Harris (1964); Ekundayo and Otusanya (1969); Sodeinde and Kuku (1989) and Sodeinde 1992) among others. None of these past studies had dealt in details with the seasonal changes in the morphology and histology of the male reproductive system as well as the state of the ovary in the females. The current study provides a comprehensive data on the gross morphological and histological features of the male reproductive system as well as the reproductive readiness of the female *Agama* lizard.

Spermatogenic activity in adult male *Agama* here-in reported, showed close similarities with those obtained for other adult male lizards (Mayhew and Wright, 1970) with minor variation. Our bi-weekly data showed that the transition from primary to secondary spermatocytes in male *Agama* was very rapid. The consistency of this phenomenon tends to suggest that it is adaptive and actually underscores the reproductive urgency in the adult male of this lizard species. Furthermore, the lack of seasonal variations in the testicular parameters fully indicates that males of this lizard were capable of reproductive activity in both seasons of the year. However, it seems plausible that each adult male is capable of exhibiting distinct spermatogenic pattern. This is more so because males in full breeding condition were observed in those months of the year when the *Agama* population experiences a reduction in reproductive activity (Table 1). This trend is consistent with the assumption that in adult tropical lizards exhibiting continuous spermatogenesis, a reduction in testis weight or size is an indication of reduced sperm production (Daniel, 1960; Sexton *et al*., 1971; Sherbrooke, 1975).

A base line always utilized in any attempt to fully explain reproductive strategies of Adult male lizards have remained the secretory activities of the epididymis and the associated ducts (Hahn, 1964; Wilhoft and Reiter, 1965). Presently, the most distinct feature perceivable in the testicular cycle of this lizard species remains our data on the secretory activities of the epididymis. The changes observed in its morphology were associated considerably with the stage of the breeding condition of individual animals. This constitutes an important indicator of the timing of their breeding activities (Ejere, 1997). Thus, it appears conceivable that much of sperm production, maturation and copulation do occur during the rainy season in male *Agama (Ejere* and Adegoke, 2002). This assertion is also consistent with our data on the proportion of males in full breeding condition (stage 6) (Table 1). Approximately, 64 % of male *Agama* caught during the rainy season was in full breeding condition as against the 23 % of such males sampled in the dry season. Nevertheless both sexes of the *Agama* species experience maximal breeding activity from February to July depicting an overlap between the dry and rainy seasons. This overlap ruled out an overall seasonal component in the breeding behaviour of the Rainbow lizard at Ile-Ife (Ejere and Adegoke, 2002). Daniel (1960) obtained similar results for the *africana* race of *Agama* species at Liberia. This synchrony is very interesting and suggests that the two races might be sub-species of same organism occupying more or less similar ecological setting which is quite distinct from that occupied by the *lionotus* race in Kenya which breeds only at the onset of the rainy season (Marshall and Hook, 1960).

Whatever life history pattern evolved by any lizard species at any point in time should be such which tends to maximise the sum of "present" reproductive success in addition to the probable "future" reproductive success (Stearns, 1976). Hence it is of utmost importance that the ultimate factors which condition the reproductive behaviour of *Agama* species at Ile-Ife, must be fully understood. Our data tend to support the notion that reproduction in *Agama* species is not directly associated with rainfall, but with food availability, soil texture and success of incubating eggs. Food availability for females and hatchlings is of utmost importance in the reproductive behaviour of lizard species (Abts, 1988; Wikramanayake and Dryden, 1988). Though the *Agama* lizard is catholic in its feeding behaviour, the bulk of its nutrition at Ile-Ife is insects (Ejere, 1997). Cases of cannibalism among this lizard species, as well as its subsistence on various food materials such as leaves, grasses, bread crumbs, biscuits etc. during the months of least rainfall (November-January) in the rain forest zone of Nigeria have also been elucidated (Cloudsley-Thompson, 1981). During such dry months, insect abundance is grossly inadequate than the situation during the early first rains and the rainy season when insect foods are abundant in tropical areas (Marshall and Hook, 1960; Janzen and Schoener, 1968; Janzen, 1973). There is a complete implication of dependence on resource availability judging from the fact that the *Agama* population at Ile-Ife experiences an increase in reproductive activities from the end of the dry season (February-March) through the early rainy season (April –July). This opinion is further strengthened by past reports that the oviposited eggs of this lizard species take approximately two months to hatch; 58 days (Sodeinde and Kuku, 1989); 60 days (Sodeinde, 1992). Our data showed an increase in oviposition of eggs by female *Agama* species from February to May (Table 2). Such eggs will hatch at the beginning of the increased rains (April-July) when small insect foods are usually abundant in the environment at Ile-Ife (Ejere, 1997). Females that lay eggs towards the end of the rainy season would have their offspring emerging during the very driest of the year (November-January) when animal protein will be grossly inadequate in the environment (Cloudsley-Thompson, 1981). Hence, it is conceivable that an important consideration in the evolution of life history traits of insectivorous lizard species in the tropics remains the rate of survival of hatchlings (Sherbrooke, 1975; Wikramanayake and Dryden, 1988). Secondly, the preponderance of females exhibiting multiple clutches from February to July, further suggests that the females of this lizard species in South Western Nigeria, have a short period between clutches. As such an individual female *Agama* lizard probably lays at least two or three clutches per year.

Past reports on oviposition by female *Agama* species in the rainforest region of Nigeria, occurred at those periods of the year when sunshine hours are high, resulting in soil temperatures being above the mean, as well as at sites with soft soil texture. Oviposition have been observed in January and March at Ijebu-Ode (Sodeinde, 1992); June at Port Harcourt (Romer, 1953); and February to October at Ibadan (Harris, 1964). That we obtained female *Agama* lizards with oviductal eggs from January to October is

therefore not surprising. Our data therefore, further attest to the fact that oviposition by female *Agama* lizards generally occur within these months in the rainforest zone of Nigeria. This is more so because the soil conditions during these months (January-October) in the rainforest zone have been reported to be favourable to the incubation process of this lizard's eggs unlike the case in the Sudan Savannah region of Nigeria (Harris, 1964; Sodeinde, 1992). Presently, our data seem to suggest that the reproductive behaviour of this lizard may have evolved under predictable environmental conditions. The extent to which this hypothesis holds true will only be unravelled by studies on more widely separated populations of the *Agama* lizard inhabiting varying ecological regions within Nigeria.

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