Reproductive cycle in the male African fruit bat
(\textit{Epomops franqueti})

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Abstract
\textit{Epomops franqueti} is common in Sub-Saharan Africa. Unlike micro chiropteran, information on the reproductive activity of this bat is scanty. The morpho-physiology of the testis and epididymis of \textit{E. franqueti} was investigated to determine the seasonal reproductive activity. Sixty adult male \textit{E. franqueti} were captured at the University of Ibadan, Nigeria. Histomorphometry of the testis and epididymis was investigated. Serum hormonal assay were carried out using ELISA. Concentrations of testosterone, FSH and LH were least (0.25, 5.80, and 7.01 IU/L, respectively) and highest (3.80, 12.03 and 14.04 IU/L, respectively) during late dry and late wet seasons, respectively, being significantly different. The early and late wet seasons had the highest gonadosomatic (0.20%) and epididymal mass (0.08%) indices, respectively. During the early dry season, the least values were recorded for both gonadosomatic (0.15%) and epididymal mass (0.06%) indices. \textit{Epomops franqueti} may be a seasonal breeder.

Keywords: African fruit bat, \textit{Epomops franqueti}, seasonal breeder, gonadosomatic index, histomorphometry

Introduction
The reproductive biology in bat species varies widely. This wide range of difference depends on factors such as temperature, rain, optimal humidity and food availability. These factors vary in degree across bat habitats \cite{1}. Seasonal environmental factors and availability of food determines production of sex hormones which affects the reproductive cycle \cite{3}. Adequate food and energy enhance regulation of reproductive cycle \cite{3}. Changes in reproductive behaviour is related to changes in gonadal activity. There are seasonal differences in the testicular and epididymal parameters reported in common vampire bat \cite{4}. Wide species-specific differences in respect to duration of gonadal activities have been reported in non-fruit eating species of bat. Non-fruit eating bat such as \textit{Myotis nigricans} in the neotropical region have been reported to show two periods of low reproductive activities, which matches the variation in the secretion of sex hormone \cite{5}.

Reproductive cycle with regards to seasons have been reported mostly in insectivorous bats \cite{6,7,8}. Also, there are reports on neotropical fruit eating bat, \textit{Artibeus lituratus} and other micro chiropteran whose reproductive cycle varies regionally with seasonal factors \cite{9,10,11,12}. However, there is little or no information on the seasonal changes in the reproductive cycle of adult male \textit{Epomops franqueti}, a megachiropteran and pteropodid \cite{13}, widely distributed in West African region, including Nigeria \cite{14}. This species of African fruit bat (AFB) has been implicated in viral and bacterial zoonotic diseases \cite{15,16}. AFB is hunted for its nutritious meat in the rural areas. AFB helps in seed dispersal through its flight zones therefore helping to balance the ecosystem by regenerating and conserving the vegetations \cite{17,18}.

In order to link up the information gap between the insectivorous and African fruit bats regarding their reproductive cycles, this study was carried out during the wet and dry seasons in Ibadan, Nigeria. The knowledge of the reproductive cycle will be useful for the conservation of this fruit bat and its population control. Also, providing basic data for further research work. \textit{E. franqueti} is crucial because this fruit bat has been mentioned in recent times to be a symptomless vector of deadly viruses such as Ebola and Corona viruses which may have predilection sites in the fruit bat reproductive tracts \cite{19,20,21}. 
Hence, reproductive activities of the testis and epididymis of *E. franqueti* in wet (early and late rain) and dry (early and late dry) seasons were investigated from the histological and hormonal profile approach.

**Materials and Methods**

**Experimental Animals**

Sixty adults male *E franqueti* (five every month of the year) were captured and used for this work. Mist net was used to trap the bats as described by Ekeolu *et al.* [22]. The average body parameters were all measured using a Draper® 115mm vernier caliper and metric tape. The body weight of the bats was measured with the aid of Microvar® weighing balance. They were anaesthetized with ketamine HCl at 25mg/kg body weight intramuscularly, through the thigh muscle. The testes and epididymides were harvested. Their weights measured with the aid of Digital Microvar® weighing balance. Anatomical nomenclature used in this work is in accordance to the report of Fard and Ghassemi [22, 23].

| GSI or EMI | Weight of organs (g) | Weight of animal (g) |
|------------|----------------------|---------------------|

Samples were fixed in Bouin’s fluid, embedded in paraffin blocks and sectioned to 4mm thickness, staining with Haematoxylin and Eosin [24] for light microscopy. Testicular and epididymal parameters were measured using T5view software.

**Hormonal assay**

In accordance to the procedure described by Olukole et al. [25], the blood sample from each of the bat was collected in universal bottle and allowed to coagulate at 25 °C. It was then centrifuged for 15minutes at a speed of 5thousand revolution and the serum stored in Eppendorf tube at a temperature of 4°C. The serum hormonal profile for testosterone (T), follicle stimulating hormone (FSH) and luteinizing hormones (LH) were assayed for each of the five animals for each month of the year using specific Kits and well labelled ELISA microplate immunoassay and microplate reader for the three hormones. Commercial ELISA kits were used to quantify the serum hormones abiding strictly to the manufacturer’s guides.

**Statistical Analysis:** The data obtained on a monthly basis were expressed as means with standard error of mean using the Graph Pad Prism version 5.00 for Windows, GraphPad Software (GraphPad Prism, 2003). Analysis of variance was performed using two-way ANOVA and significance was reported at *P* < 0.05.

**Results and Discussion**

Testicular colour was milky white irrespective of season (Fig. 1). The difference in the seasonal body weight obtained in the wet and dry seasons was significant (*P* < 0.05). The average weight of the African fruit bat used in this study falls within the range reported by Nowak and Walker [26] on bats from temperate region but larger than the average body weight recorded for the common vampire bat, *Demondus rotundus* [27]. The values of the average body weight of the bat were higher in wet season than the values obtained in dry season (table 1). Also, the average weights obtained for the testis across the seasons vary significantly at *P* < 0.05 (table 1). The mean GSI for in the early raining and early dry seasons were 0.201% and 0.154% respectively (table 1). The seasonal GSI varied significantly (*P* < 0.05). During the long period of rain, the highest average testicular weight of 0.172 ± 0.001g was recorded (table 1). The gonadal tissue investment had the highest value during the wet season. However, this value was relatively low compared to that which was reported for *Desmondus rotundus* [27], suggesting that this bat may be monogamous in nature with a lower GSI (0.2%) compared to the GSI (0.46%) of *Molossus molossus*, that is harem in nature [28]. The GSI (0.20%) recorded during the wet season was also close to the GSI (0.27%) of *Sturnira lilium* [29] higher than the GSI (0.13%) of a bull [30], supporting the hypothesis that small-sized mammals invest more in spermatogenesis than large ones [31].

There was significant difference in the seminiferous tubular and luminal diameters. The germinal epithelial heights of the testis during wet and dry seasons showed significant variations at *P* < 0.05 (table 2). During wet season, the germinal epithelial height increased with numerous spermatocytes, spermatids and fewer spermatozoa (in late wet season), anchoring on the Sertoli cells as the spermatogonia replenishes the differentiating spermatocytes but low reproductive activities during the dry season, with few spermatocytes, presence of spermatogonia and Sertoli cells. Remnant of spermatozoa are seen within the lumen of the seminiferous tubules. The Leydig cells were intact and their nuclei appear vesicular and foamy, with prominent blood vessels in the interstitium during the wet season as against its appearance in dry season (Fig. 2 A, B, C & D). The spermatogonial stem cell undergoes division to replenish itself and the other undergo mitotic division, then meiotic division and differentiation. This complex process was observed in the germinal epithelium of *Epomops franqueti*, similar to the report of Beguelini *et al.* in *Myotis spp* [12] and *Sturnira lilium* [29]. During the meiotic phase, the spermatocyte gradually migrates towards the adluminal compartment corresponding with the reports of Fard and Ghassemi [22] and Morais *et al.* [27]. Spermatogenesis peaked when there was maximum rainfall, unlike in the cave bat, *Myotis velifer*, documented by Krutzsch [32]. The epithelial height of epididymis during dry season decreased. The spermatozoa in the lumen were more in the long period of wet season but scanty in dry season. The value of the relative epididymal mass index (EMI) was higher during the wet season. The parameters measured for the epididymis showed significant difference during wet and dry seasons (table 2, Fig 4&5). This is similar to reports in *Taphozous longimanus* [33] and vampire bat [34] in their breeding seasons. The scanty spermatozoa observed in the lumen of the epididymis during dry season suggests that the bat may be a seasonal breeder unlike in sheath tailed bat [35]. The morphological, morphometrical and histological characteristics of the testis and epididymis of *Epomops franqueti* suggest seasonal variation given by the differences in the weight of the testis and epididymis. The least values of the testicular and epididymal parameters were recorded during the late dry season conform to the previous study on the seasonal breeding bat, like the Tree-roosting bat [8]. Extreme temperature affects reproductive activities negatively and this occurs between the months of January and February when also fruits were not available, in Ibadan. An increase in daylight with moderate temperature is excellent for reproductive activity [36]. The principal cell which was the predominant cell in the epididymis of *Epomops franqueti*, exhibited a vesicular lightly stained oval nucleus during the wet season indicating high reproductive activity.
Fig 1: The pelvic and lower abdominal cavities of adult male E. franqueti at the dorso-ventral view showing the relationship of the ileum (IL); colon (CL) with the reproductive organs: the right (RT) and left (LT) testes, the right (RV) and left (LV) vesicular glands, urinary bladder (UB) and the penis (PN) Note: the milky colour of the testis. There was no change in testicular colour through the wet and dry seasons.

Fig 2: Photomicrograph of the testis of E. franqueti A&B: early and late rain seasons respectively. Note the full germinal epithelium of the seminiferous tubule (ST), lumen (L) with spermatozoa (SP). Insets show: Sertoli cell (SC), spermatogonia (SG), spermatocytes (SCT) and spermatids (SP). C&D, early and late dry seasons. Note the reduced germinal epithelium of the seminiferous tubules (ST), lumen (L) with scanty spermatozoa (SP). Insets shows: Sertoli cells, a few spermatogonia and few spermatocytes and abundant debris of spermatozoa in D. Inset: Note the foamy appearance of the Leydig cell nucleus in wet season with intact interstitium and its disruption in late dry season.
Fig 3: Photomicrograph of the cauda epididymis of *E. franqueti* in A and B, early and late rainy seasons showing lumen (L) filled with spermatozoa. In C and D, early and late dry seasons showing the lumen (L) filled with very few spermatozoa. Note that the lumen of the epididymis contain more spermatozoa in the period of wetness than in dryness with period of late rain having the most abundant. H&E

**Table 1**: Seasonal variation in Reproductive parameters of the testis of *E. franqueti*

| Seasons | WOA (g)       | WOT (g)       | WOE (g)       | GSI  | EMI   |
|---------|---------------|---------------|---------------|------|-------|
| ER      | 86.89 ± 3.15  | 0.168 ± 0.001 | 0.068 ± 0.002 | 0.201| 0.0690|
| LR      | 97.64 ± 5.90  | 0.172 ± 0.001 | 0.070 ± 0.002 | 0.176| 0.0797|
| WET     | 92.67 ± 4.52  | 0.170 ± 0.001 | 0.069 ± 0.001 | 0.189| 0.0743|
| ED      | 87.19 ± 4.76  | 0.127 ± 0.003 | 0.053 ± 0.003 | 0.154| 0.0614|
| LD      | 78.76 ± 5.73  | 0.123 ± 0.006 | 0.055 ± 0.008 | 0.155| 0.0741|
| DRY     | 82.98 ± 5.25  | 0.125 ± 0.005 | 0.054 ± 0.005 | 0.155| 0.0678|

*Means with dissimilar superscripts within columns varied significantly (P< 0.05), WOA: Weight of Animal, WOT: Weight of Testes (combined), GSI: Gonadosomatic Index, WOE: Weight of Epididymis (combined), EMI: Epididymal mass index, ER: Early rain season, LR: Late rain season, ED: Early dry season, LD: Late dry season*
Fig 4: Chart illustrating seasonal variation in the animal weight (A), testicular weight (B), epididymal duct weight (C), gonadal somatic index (D) and epididymal mass index (E) of *Epomops franqueti* during the wet (early, late rainy) and dry (early and late dry) seasons of the year as seen in table 1. Bars with different alphabets are shown significantly different at *P* < 0.05

Table 2: Seasonal variation in Reproductive parameters of the testis of *E. franqueti*

| Season | STD (µm) | SLD (µm) | GH (µm) | EDD (µm) | ELD (µm) | EH (µm) |
|--------|----------|----------|---------|----------|----------|---------|
| ER     | 238.3±9.09 | 99.8±15.32 | 135.4±12.82 | 322.2±17.18 | 212.6±34.02 | 109.3±19.31 |
| LR     | 297.7±7.40  | 97.8±11.13 | 198.8±20.32 | 365.2±32.27 | 278.8±20.13 | 85.1±12.20  |
| WET    | 268.0±8.23 *  | 98.1±16.18 *  | 166.7±18.53 *  | 343.7±24.73 *  | 247.6±17.23 *  | 92.6±14.05 *  |
| ED     | 186.1±6.19  | 122.4±11.62 | 63.7±13.69 | 249.5±15.23 | 197.5±20.03 | 48.1±10.32  |
| LD     | 173.8±6.0   | 125.1±13.36 | 48.6±15.13 | 222.2±22.06 | 167.3±16.3  | 54.4±11.06  |
| DRY    | 179.9±6.09 b | 123.8±15.04 b | 56.1±16.07 b | 235.9±18.65 b | 189.3±13.07 b | 49.1±16.21 b |

*Means with different superscripts within columns are significantly different (*P* < 0.001). STD: Seminiferous tubular diameter, SLD: Seminiferous tubule luminal diameter, GH: Germinal epithelial height, EDD: Epididymal Ductal diameter, ELD: Epididymis luminal diameter, EH: Epithelial height, ER: Early rain season, LR: Late rain season, ED: Early dry season, LD: Late dry season.

Fig 5: Chart illustrating seasonal variation in the seminiferous tubular diameter (A), the diameter of seminiferous tubules lumen (B), germinal height (C), the diameter of epididymis tubules (D), diameter of epididymis lumen (E) and epithelial height of the epididymis of *E. franqueti* during the wet (early, late rainy) and dry (early and late) seasons of the year as seen in table 2. Bars with different alphabets varied significantly at *P* < 0.001.
Sex Hormonal Profiles of the African Fruit Bat (Epomops Franqueti) during the Months (seasons) of the year

The mean serum testosterone concentration of E. franqueti increased steadily up to March after and declined in May. From May, testosterone increased steadily to reach its peak in July. Then declined continuously up to October. From October to December, the testosterone concentration formed a plateau (Fig 6 A). E. franqueti shows seasonal variation in sex hormone production which is pivotal to the morphophysiology of the reproductive organs, being influenced by the availability of reproductive energy as demonstrated in Molossus molossus. [15]. In this work, it was observed that the sex hormone profile followed a seasonal variation pattern. The level of serum testosterone (T) concentration in this fruit bat decline to the minimum level during dry season in February with a low degree of sexual activities. Therefore, suggesting a low level of steroidogenesis and spermatogenesis in congruence to the report of Barros et al. [36]. The mean serum concentration of luteinizing hormones of E. franqueti declined briefly from January to February with the least luteinizing hormones recorded in February. It then increased steadily from the month of February to reach its peak in August. Thereafter, it declined in October. It however increased up till December (Fig 6 B). The mean serum concentration of follicle stimulating hormone of E. franqueti had the least value in the month of February as it declined briefly from the month of January. Thereafter it increased slightly in February to form a plateau up till May. From May it increased sharply to reach its peak July. Thereafter it declined in October then it increased, forming a plateau between November and December (figure 6 C). The serum concentration of luteinizing (LH) and follicle-stimulating hormones (FSH) reached their peak in the wet season when there was maximum rainfall and abundant food. This implies high reproductive activity. This trend is similar to the report of Kawamoto et al. [37] in Rhinolophus ferrumequinum. The peak production of testosterone during the period of maximum wetness corresponds to the reports of Kawamoto et al.

Conclusion

This research work has been able to describe and show that: The late wet season had the highest gonadosomatic (0.20%) and epididymal mass (0.08%) indices, indicating that the testis is almost three times the size of the epididymis in the animal. Concentrations of testosterone, FSH and LH were least (0.25, 5.80, and 7.01 IU/L) and highest (3.80, 12.03 and 14.04 IU/L) during late dry and late wet seasons, respectively. The late wet season was therefore established as the peak period of reproductive activities in the adult male Epomops franqueti. The reproductive biology of the Epomops franqueti is suggestive of a seasonal breeder.

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