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Research Article

Effect of Natural Environmental Conditions on the Reproductive Histomorphometric Dynamics in the Male Yellow House Bats (*Scotophilus heathii*)

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Abstract

The greater Asiatic yellow house bat (*Scotophilus heathii*) is one of the commonest and widely distributed bat species in Pakistan. Little is known about the reproductive biology of this bat. Both sexes roost separately except during January to March. The breeding season is however not well defined. This study was designed to investigate different morphological and histological changes in different months of the year in order to determine the actual period when male bats were reproductively active, along with seasonal changes in the spermatogenic activity of this bat. For this purpose, a group of 9 bat species of *S. heathii* were captured from different areas of Faisalabad in different months of the year. Morphometric measurements of each bat were made by digital Vernier caliper in order to confirm about the exact specie. Tests were taken and their different morphological parameters were taken. Histology of the testes was studied to explore various stages of spermatogenesis using different histological techniques like Fixation, Dehydration, Sectioning, Staining, and preservation. Histomorphometric measurements were also made by image J analysis. All parameter values were taken as Mean±SE. By using one way ANOVA and Fischer LSD the obtained data was analyzed, which showed highly significant values of all parameters, $P<0.005$. In this study, the morphological parameters and histological parameters were observed, which showed lowest values of all parameters except lumen area, in February and March, which was non spermatogenic period. In September, parameters showed low spermatogenic activity. In November all parameters were at their maximum value, which indicating the peak spermatogenic period for this bat. These results suggest the occurrence of an annual testicular cycle defined by three distinct reproductive periods.

Keywords: *Chiroptera; spermatogenic activity; Scotophilus heathii*

Introduction

There are more than one thousand known species of bats (Chiroptera). Chiroptera divided into two suborders, the Mega chiroptera, also called fruit bats, world over have 167 species. The Michrochiropteran bats have a great diversity. They occupy almost all the niches and consume a wide variety of food resources. They feed on vertebrates (e.g. fishes, amphibians) and invertebrates (e.g. arthropods). They also feed on blood of other vertebrates and even consume plant matter. Bats are important bio indicators of global warming which is a giant dilemma in the world [1].

Bats mostly studied on their particular characters, such as echolocation, phylogenic tree, foraging habits and distinctive reproduction [1]. Yet, it is reported that bats are also natural sources of the emerging and re-emerging infectious diseases. These infections cause serious disorders in humans and also in some animals. Bats also act as pest control agent, because each year they consume many thousands harmful pest of agriculture. At night, bats eats june bugs, ear worm moth of corn, bollworm moth of cotton, budworm moths of tobacco, all of these insects are nocturnal [2]. Bats are also seed dispersers and pollinators of many commercially important plants used for food, construction, medicines and dyes.

Bats feces are also used as fertilizer. Chiroptera bats spend almost half of their lives in roosting environment. In the biology and ecology of bats, roost sites play an important and large role. Many bat species choose such roost sites which are hidden or covered. For example caves, mines, cavities in rocks, cervices in trees, under foliage, and some time also in modified human made structure.

Male and female of *Pipistrells nathusii* form their mating groups in late autumn in Britain and during autumn in Continental Europe [1]. Sperm storage means preservation of spermatozoa for a long time, with in reproductive tract either female or male. [3]. Basically it is found in those animals, in which time of mating does not coordinate with the time of ovulation. The process of sperm storage in female is studied in insects reptiles birds and mammals. Sperm storage is mostly limited to order Chiroptera, in some species sperm stores for some days and other species stores for some months [4].

Scotophilus heathii is a monoestrous bat, which forage forcefully from August to October, which results to storage of white adipose tissue from November to January. Before winter hibernation, body mass of bat also increases 1.5 times before winter hibernation. [5]. Bats living in moderate area are monoestrous due to need of hibernation in winter season. Many bat species living in tropical areas are also monoestrous. The young one's of these species born in summer, when the environmental conditions are favorable. Reproductive cycle of chiropterans bat also disturb due to hibernation in winter when food shortage occurs. The estrus begins in late summer in these bats and mating also occurs. Females store spermatozoa in their reproductive tract and delay fertilization to the arrival of spring. After fertilization, the implantation occurs in favorable environmental conditions.

In males, sperm production is seasonal and occurs in summer, so the climatic changes can modulate the breeding season [6]. So this study was planned to determine the morphological and histological changes in the testes over a year for better understanding of breeding phenology of this specie. This study also emphasizes areas where knowledge is lacking or incomplete, with the hope of fostering additional research on this exciting and fruitful area of this specie.

Material and Methods

Study Area

Faisalabad stands in the rolling flat plains of North Punjab, Pakistan between longitude 73°74 east, latitude 30°31.5 North, with an elevation of 184 meters (604 ft) above sea level. The climate of the district can see extremes, with a summer maximum temperature 50°C (122°F) and a winter temperature of −2°C (28°F). The average yearly rainfall lies only at about 300 mm (12 in) and is highly seasonal with approximately half of the yearly rainfall in the two months July and August.

Experimental design

A total of six viz.,12m (n=1), 9m (n=2) and 6m (n=3) long high quality deep black UV stable at strong mist nets (Ecotone 716/6, 716/9 and 716/12) was used to capture bats .each of these five shelved, 16×16 mm mesh sized and 2.5m high net was erected either in "L" or "V" shape at strategic position on a pair of 3m long bamboo poles in such a way that the last shelf of each net remained one foot above the ground. The total mist net area in season was 120 m^2. The nets were ready to operate half an hour before sunset. All the nets were opened simultaneously at sun set and continued to operate depending on the weather condition for two hour after sunset. Nets were checked continuously to disentangle any captured bat. The sampling effort remained the same throughout the study period. The global position of each roost was also determined using Germin etrax H Global position system (GPS). A total of 25 specimens were captured in which 9 were male *Scotophilus heathii.*

Morphological Assessment

External body measurements

All the captured specimens were brought to the laboratory and autopsied. Next day recording their external body measurements using the Digital Vernier calipers (0-150 mm). these measurements include head and body length, ear length, tragus length, forearm length, thumb along with claw length, length of each metacarpal including its phalanges, wing span, penis length, tibia length, calcar length, hind foot, tail length and free tail length (in case the tail is completely or partially free of uropatagium); these measurements were used to ascertain the species of the bat captured from a particular subarea and compared with those already available in literature. The age of each captured bat specimen was determined following Dietz [] (2005).

Gross features of Testes

The testes from individual bat were obtained and observed carefully for their gross features (testis weight, body weight and position of testis, length of testis, width of testis). The weight of each sample was recorded in grams (g) using an electrical weighing balance.

Histological measurements

Testes were washed with normal saline solution and fixed in Bouin's solution [7] respectively. Different slides were prepared using tissue paraffin technique and photomicrographs of testes captured using Nikon Optiphot 2 microscope at 200X. These photos were used to determine the length (μm) of seminiferous tubules, width of seminiferous tubules and lumen area of seminiferous tubules of testis with the help of automated image analysis system Image J® version 1.43n (Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA).

Statistical Analysis

Data are expressed as Mean ± SE. One way analysis of variance (ANOVA) was used to compare the means of parameters, while significance differences were observed by Fischer (LSD) test using SAS (9.1).

Results

External body features of male *Scotophilus heathii* captured from Faisalabad

The mean values of all different external body parameters used for ascertain the specie is given in table 1. These parameters include the head and body length, ear, tragus height, forearm, thumb along claw, 2nd meta carpel, length of 1st phalange on 2nd metacarpal, length of 3rd metacarpal, length of 1st phalange on 3rd metacarpal, 2nd phalange on 3rd metacarpal, length of 4th metacarpal, 1st phalange on 4th metacarpal, 2nd phalange on 4 meta carpel, length of 5th metacarpal, 1st phalange on 5th metacarpal, 2nd phalange on 5th metacarpal, wing span, length of tibia, length of hind foot and length of tail.

Body parameters	Mean ± SE	Range
HBL	70.98 ± 0.98	66.78-75.45
E	16.35 ± 0.66	12.46-18.76
TRH	8.11 ± 0.36	6.1-9.56
FA	49.73 ± 0.73	46.07-53.15
TH+C	9.97 ± 0.43	8.50-12.15
2MT	47.66 ± 0.52	45.87-50.24
1PH2MT	15.13 ± 1.02	11.56-19.67
3MT	53.85 ± 1.42	48.34-60.23
1PH3MT	18.74 ± 0.88	15.73-23.56
2PH3MT	16.17 ± 0.94	13.12-20.21
4MT	52.44 ± 1.46	46.10-58.98
1PH4MT	14.87 ± 1.19	8.43-20.52
2PH4MT	11.67 ± 1.42	6.45-18.94

5MT	49.32 ± 1.35	43.7-55.71
1PH5MT	13.17 ± 0.84	8.32-16.37
2PH5MT	10.70 ± 1.02	6.82-15.12
WS	35.44 ± 0.60	32.96-38.45
TIB	22.78 ± 0.72	18.32-25.21
HF	12.31 ± 0.42	10.18-14.00
T	53.70 ± 0.64	50.12-56.31

Table 1: *Combined mean ± SE and range of external body measurements in (mm) of nine specimens of S. heathii.*
The abbreviations used in this table along with explanation are as follows.
HBL-head and body length; E- ear; TRH- tragus height; FA-forearm; TH+C- thumb+claw; 2MT- 2nd meta carpel; 1PH2MT- length of 1st phalange on 2nd metacarpal; 3MT- length of 3rd metacarpal; 1PH3MT- length of 1st phalange on 3rd metacarpal; 2PH3MT- 2nd phalange on 3rd metacarpal; 4MT- length of 4th metacarpal;1PH4MT- 1st phalange on 4th metacarpal; 2PH4MT- 2nd phalange on 4 meta carpel; 5MT- length of 5th metacarpal; 1PH5MT- 1st phalange on 5th metacarpal; 2PH5MT- 2nd phalange on 5th metacarpal; WS- wing span; TIB- length of tibia; HF- length of hind foot; T- length of tail.

Morphological evaluation of the testes

Monthly variations in body weight, testicular weight and position of testes

The mean monthly variation in body weight, testes weight and testes position was like In February, mean body and testes weight was 4.45g and 0.021g respectively. Testes were in abdominal position. In March, mean body and testes weight was 4.62g and 0.018g respectively. Testis position was abdominal. In September, mean body and testes weight was 5.13 g and 0.026g respectively and testes was in abdominal position. In October, mean body and testes weight was 5.46g and 0.032g respectively and testes was in scrotal position. In November, mean body and testes weight was 6.25g and 0.039g respectively and testes position was scrotal.

Month	LT (um)	WT (um)	LS (um)	WS (um)	A (um)	Weight (g)
Feb	683.01	325.18	31.02	13.01	9.0	0.021
Mar	676.695	318.7	28.7	12.315	9.75	0.018
Sep	943.16	449.775	48.425	19.115	8.3	0.026
Oct	973.14	470.01	53.12	21.25	7.5	0.032
Nov	1251.01	609.1767	70.78333	34.61	4.67	0.039

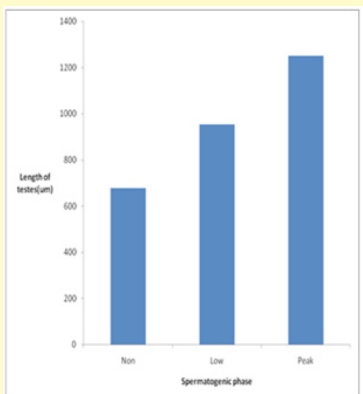
Table 2: *Monthly variations in Major histological parameters of testes of Scotophilus heathii:*
The abbreviations used in this table 2: LT; Length of testis, WT; Width of tesis, LS; Length of seminiferous tubules, WS; Width of seminiferous tubules, A; lumen area of seminiferous tubules.

Histomorphometric Evaluation

Statistical analysis showed the highly significant values P<0.005 of all parameters like as length, width of testes, length (μm) of seminiferous tubules, width of seminiferous tubules and lumen area of seminiferous tubules of testis during different phases of spermatogenesis. Parameters showed the lowest values except the area of lumen, during non spermatogenic phase. Values were gradually increased during low spermatogenic phase. Maximum values were observed in peak spermatogenic phase, except area of lumen which was almost filled in peak phase as indicated in table 3.

Seasons	N	LT (um)	WT (um)	LS (um)	WS (um)	Area (um)	Weight (g)
Non spermatogenic	3	678.8 ± 2.94	320.8 ± 2.61	29.47 ± 1.13	12.54 ± 0.25	9.5 ± 0.28	0.019 ± 5.77
Low Spermatogenic	3	953.15 ± 10.05	456.52 ± 6.7	49.99 ± 1.63	19.82 ± 0.73	8.03 ± 0.29	0.025 ± 6.6
Peak Spermatogenic	3	1251.01 ± 3.46	609.17 ± 8.9	70.78 ± 1.81	34.61 ± 1.63	4.67 ± 0.33	0.034 ± 5.7

Table 3: *Mean ± SEM variations in histological parameters of testes during Non-, low and peak spermatogenic period: The abbreviations used in this table 3: LT; Length of testis, WT; Width of tesis, LS; Length of seminiferous tubules, WS; Width of seminiferous tubules, A; lumen area of seminiferous tubules.*



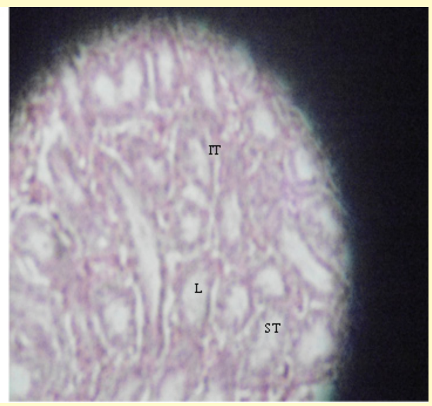


Photo micrograph of testis of the Scotophilus heathii during non spermatogenic phase showing regressed activity of seminiferous tubules (ST), absence of spermatogenesis in lumens (L) and more interstitial tissue (IT). Hematoxylin and Eosin (H&E) 200X

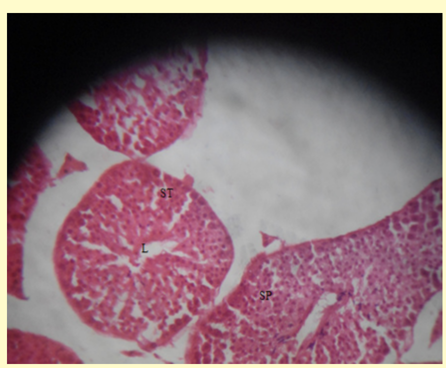


Photo micrograph of testis of the Scotophilus heathii during low spermatogenic phase showing increase size of seminiferous tubules (ST), small lumens (L) and more interstitium. Spermatid (SP) as in low spermatogenesis phase, primary and secondary spermatocytes are visible, found at the periphery of seminiferous tubules, lumen was empty. Hematoxylin and eosin (H&E) 200X

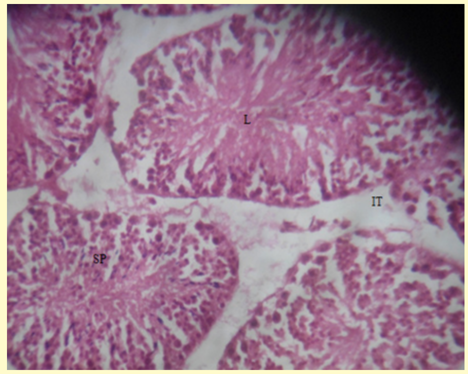


Photo micrograph of testis of the Scotophilus heathii during the peak spermatogenesis showing larger diameter of seminiferous tubules (ST) along their lumens (L) filled with mature spermatids (Sp) and minimum interstitial tissue (IT). In peak spermatogenesis phase, germinal layer was thick, mature spermatid having tail also visible, lumen was filled with spermatozoans. Hematoxylin and eosin (H&E) 200X

Discussion

Many chiroptera species have same reproductive characteristics that are found in temperate region species, such as hibernation and regression in the seminiferous tubules in which only spermatogonia observed. [2]. Spermatogenesis is a complex process in which germ cells (*spermatogonia*) divide mitotically, undergo meiosis, and then differentiate into spermatozoa [8]. The present morphometric study of external body of bat confirmed that the experimental bat is male *Scotophillus heathii.* As another scientist reported the same result about the *Scotophilus heathii* collected from Pakistan.

Histomorphometric data obtained from this study of testes of male *Scotophilus heathii* captured from Faisalabad showed that non spermatogenic period occurred in February and March. During this period, no spermatogenic activity showed, testis weight, length and width was small, seminiferous tubules according to length and width was small in size, spermatocytes were near the epithelial cell wall, and lumen of seminiferous tubule was large [9]. Studied the spermatogenesis in *Plecotus auritus* (Chiroptera: Vespertilionidae) and found that testis size was small and there were no any spermatogenic activity in testis during early spring. Another scientist [10-12] reported that in non spermatogenic period, testes size was small and no spermatogenic activity showed in this period.

Low spermatogenic period start from September to October, in this period spermatocytes became larger in size, seminiferous tubules length, width also increased, testis weight, length and width increased and lumen of seminiferous decreased in size. Raissa., *et al*. studied the spermatogenesis of male *Myotis levis* and observed the increase in length and width of seminiferous tubule as compare to non spermatogenic period. Lumen was also decreased as compare to non spermatogenic period [12-16]. Reported that in low spermatogenic period, bats testes size started to increased and spermatogenesis process has been activated at this stage.

Peak spermatogenic period was in November, in this period testis weight and size increased as two fold from non spermatogenic period, length and width of seminiferous tubule also increased more than two fold from non spermatogenic period [17] also examined more than two fold increment in testis length and width during non spermatogenic to peak spermatogenic period.

Lumen of seminiferous tubule was very small, spermatids transformed into spermatozoa, were mature and occupied in the lumen. Earlier [18] was also reported that maximum testis length and width is also associated with maximal spermatogenic activity. Krishna and Singh [13] examined the spermatogenisis in male scotophilus heathii and reported the same result. He also observed non spermatogenesis in March, low spermatogenesis from September to October, and peak spermatogenesis period in November. Barrosa., et al. [15] examined the testicular activity of Molossus molossus (chiroptera), which showed increased testis weight and seminiferous tubules during fall and winter season. It is associated with higher sperm production and showed that mating occurred in late fall and early winter. The present findings revealed the existence of annular cycle which is influenced by the seasonal changes and breeding activities are linked with body condition and favorable environment. The testicular activities defined by three distinct reproductive phases: resting phase (February and March), low reproductive period (September and October) and peak reproductive period was observed (November and December) as the testes were found in maximum spermatogenic activities during peak reproductive phase in greater Asiatic yellow house bat (Scotophilus heathii) [19-22].

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