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Original Article
Animal Morphophysiology

Seasonal influence on testicular morphophysiological parameters of bat *Carollia perspicillata* in fragments of the Atlantic Forest, northeastern Brazil [1]

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ABSTRACT.- Arandas M.J.G., Teixeira A.A.C., Texeira V.W., Silva F.R., Marinho K.S.N., Lima Junior N.B., Júnior F.C.A.A & Santos K.R.P. 2019. **Seasonal influence on testicular morphophysiological parameters of bat *Carollia perspicillata* in fragments of the Atlantic Forest, northeastern Brazil.** *Pesquisa Veterinária Brasileira 39(6):429-433.* Departamento de Morfologia e Fisiologia Animal, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros s/n, Dois Irmãos, Recife, PE 52171-900, Brazil. E-mail: julianaarandas@hotmail.com

Bats belong to the order Chiroptera, family Phyllostomidae, and present a wide diversity of reproductive strategies. However, information on the reproductive biology of male bats is scarce, mainly in the Northeast Region of Brazil. Thus, this study evaluated the seasonal testicular histomorphometry of the bat *Carollia perspicillata* in fragments of the Atlantic Forest in Pernambuco state. To this end, adult males were collected, euthanized for removal of the testicles, and later submitted to a routine histological technique. Histomorphometric analysis included assessment of the areas of tubular and intertubular compartment occupation, as well as quantification of spermatocytes, rounded spermatids, elongated spermatids, and Sertoli and Leydig cells. Results indicated that this bat species presents reproductive seasonality, because significantly higher averages of the testicular parameters were observed in the rainy season, which is a period of greater availability of food resources. Such inferences indicate that there is a synchrony between peak spermatogenesis and hormonal inversion in the months of high precipitation; furthermore, a higher carrying capacity of the Sertoli cells is noted. *C. perspicillata* males possibly present greater sperm and androgenic activity in the rainy season, associated with increased tubular area and number of spermatogenic cells, as well as with the intertubular area and number of Leydig cells, respectively.

INDEX TERMS: Morphophysiology, *Carollia perspicillata,* Chiroptera, Phyllostomidae, Atlantic Forest, Brazil, bat, climatic factors, histomorphometry, testicles.

RESUMO.- [**Influência sazonal sobre os parâmetros morfofisiológicos testiculares de *Carollia perspicillata* (Chiroptera: Phyllostomidae) em fragmentos florestais de Mata Atlântica, Nordeste do Brasil.**] Os morcegos pertencem a ordem Chiroptera, família Phyllostomidae, e apresentam ampla diversidade de estratégias reprodutivas. Entretanto, as informações relacionadas à biologia reprodutiva dos machos são escassas, principalmente no Nordeste do Brasil. Dessa forma, o trabalho avaliou a histomorfometria sazonal testicular de *Carollia perspicillata* em fragmentos de Mata Atlântica de Pernambuco. Para tanto, os machos adultos foram coletados, eutanasiados para a remoção dos testículos, e posteriormente submetidos à técnica histológica de rotina. As análises histomorfométricas avaliaram as áreas de ocupação do compartimento tubular e intertubular, assim como a quantificação dos espermatócitos, espermátides arredondadas, espermátides alongadas, células de Sertoli e de Leydig. Os resultados indicaram que a espécie apresenta sazonalidade reprodutiva, visto que maiores médias significativas dos parâmetros testiculares foram encontradas na estação chuvosa, que é um período de maior disponibilidade de recursos alimentares. Tais

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inferências indicam que existe uma sincronia entre o pico de espermatogênese e investimento hormonal nos meses de alta precipitação, atrelado a isso, nota-se ainda, uma maior capacidade de suporte das células de Sertoli. Os machos de *C. perspicillata* possivelmente apresentam uma maior atividade espermática e androgênica na estação chuvosa, associadas ao aumento da área tubular e do número de células espermatogênicas, assim como da área intertubular e do número das células de Leydig, respectivamente.

TERMOS DE INDEXAÇÃO: Morfofisiologia, *Carollia perspicillata,* Chiroptera, Phyllostomidae, Mata Atlântica, Nordeste do Brasil, fatores climáticos, histomorfometria, morcego, testículo.

INTRODUCTION

Bats belong to the order Chiroptera, family Phyllostomidae, present a wide diversity of species, and evolved standards and reproductive strategies that correlate to the occurrence area, climate conditions, and food availability (Neuweiler 2000, Zórtea 2003). In tropical regions, bats reproductive events are associated with periods of increased precipitation (Fleming et al. 1972, Lima Junior et al. 2014).

Despite bat diversity, there is limited information on function, physiological control, and cyclicality of spermatogenesis (Krutzsch 2000). Testicular positioning is an aspect commonly reported in studies addressing male bat reproduction, as these organs present seasonal variation between the inguinal and abdominal region (Lima Junior et al. 2014); however, sexual activity cannot be established only with the external morphological features of the gonads, reason why evaluation of the testis morphological parameters can present inferences about the dynamics of gonadal development and species reproductive capacity (Beguelini et al. 2009, 2010, 2011, 2013a, 2013b, 2013c, 2013d, 2014, 2015, 2016, Morais et al. 2013a, 2013b, 2013c, 2014a, 2014b, Lima Junior et al. 2014, Farias et al. 2015, Notini et al. 2015).

Bats of the Phyllostomidae family are present in temperate and tropical areas, with records of variable reproductive strategies, including male reproductive cyclicality during the whole year (Handley Junior et al. 1991, Zortéa 2003, Oliveira et al. 2009, Duarte & Talamoni 2010, Notini et al. 2015). The species *Carollia perspicillata* (Linnaeus, 1758) belongs to a family widely distributed throughout Brazil, with preferably frugivorous food habit, and thus of great importance for seed dispersion (Charles-Dominique 1991, Mello et al. 2004). Regarding the reproductive biology, studies have indicated bimodal polyestry as the predominant reproductive cycle; their spermatogenesis show ultra-structural features similar to those of other species of the Phyllostomidade family (Mello et al. 1999, Mello & Fernandez 2000, Beguelini et al. 2014).

However, there are few or no studies addressing the seasonal testicular histomorphometry of these bats. Thus, this article evaluated the testicular histomorphometry of the bat *Carollia perspicillata* in the dry and rainy seasons in fragments of the Atlantic Forest in Pernambuco state, Brazil.

MATERIALS AND METHODS

Area of study. The study was conducted in fragments of the Atlantic Forest in the municipality of Sirinhaém (08º 35'27" S; 35º06'58" W), Pernambuco state - submontane and montane, dense ombrophilous (open and seasonal semi-deciduous) forest (Veloso et al. 1991).

Animal collection. Adult male bats (*Carollia perspicillata,* Chiroptera: Phyllostomidae) were captured monthly, during three consecutive nights between 5 PM and 5 AM, using mist nets, from September 2008 to October 2009.

Animals were captured using mist nets (12x3m) authorized by the Chico Mendes Institute of Biodiversity Conservation (ICMBio) and the Biodiversity Information and Authorization System (SISBIO) (no. 2800740). The study was approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of Pernambuco (UFPE) under protocol no. 23076.037360/2014-92.

Meteorological data and weather stations. The dry and rainy seasons were defined by the National Institute of Meteorology (INMet 2008/2009) as per analysis of temperature, humidity and rainfall (Table 1). Two groups were considered: dry season (September to February) and rainy season (March to August).

Reproductive stage. Adult males (n=60) were classified on the basis of testicular position: descending testis (located in the inguinal region) and non-descending testis (located in the abdominal region) (Gannon & Willig 1992).

Euthanasia. The animals selected for histomorphometric analysis were anesthetized with sodium pentobarbital at a concentration of 40mg kg^{-1} intraperitoneally, followed by a potassium chloride saturated solution at 40mg/kg^{-1}.

Histomorphometric and statistical analyses. A total of 26 adult males, comprising at the most two bats per month, were randomly selected according to the two established groups as follows: dry season (n=13) and rainy season (n=13).

After surgical incision from the abdominal region to the inguinal region, the testes were removed and had their tissues fixed in 10% neutral buffered formalin (NBF), routinely processed for histology, and embedded in paraffin (Behmer et al. 1976, Rieder & Schmidt 1987). 5µm-thick sections were obtained, stained with hematoxylin and eosin (HE), and analyzed under optical microscopy.

Table 1. Monthly averages of precipitation, air temperature and humidity determined by the National Institute of Meteorology (INMET) in a fragment of the Atlantic Forest, Pernambuco state, Brazil

Month	Year	Monthly averages		
		Precipitation (mm)	Temperature (ºC)	Humidity (%)
September	2008	47.6	25.3	78
October	2008	53.6	26.2	75
November	2008	16.0	26.9	69
December	2008	18.3	27.2	69
January	2009	85.2	27.3	70
February*	2009	376.1	26.5	79
March	2009	142.8	27.2	76
April	2009	351.8	26.4	83
May	2009	410.1	25.8	88
June	2009	333.0	25.0	86
July	2009	386.8	24.6	86
August	2009	290.2	24.6	82

*Although rainfall was high, it was concentrated in few days, which were followed by dry days, but the temperature patterns were similar to those of the dry season (APAC 2009).

Histological slides were photographed with a total of 100X and 400X magnification using the ScopePhoto software coupled to a camera positioned between the optical microscope and the computer. Thus, 10 photomicrographs were used per animal at each magnification increase.

Testicular histomorphometric analysis was performed using the ImageJ 1.44 software. The following parameters were assessed: number spermatocytes, rounded spermatids, elongated spermatids, and Leydig and Sertoli cells with 400X magnification, as well as percentage of tubular compartment and intertubular compartment occupation areas with 100X magnification.

Variables were submitted to Student's *t*-test and processed using the Statistical Package for the Social Sciences 15.0 software (SPSS Inc., Chicago, IL, USA) for comparison between data for the dry and rainy seasons. Values were considered statistically significant when $p<0.05$.

RESULTS

During the studied period, 60 *Carollia perspicillata* males were captured, 25 in the dry season, of which 80% (n=20) showed descendent testes and 20% (n=5) had non-descending testes, and 35 in the rainy season, of which 65.71% (n=23) presented descendent testes and 34.29% (n=12) showed non-descending testes (Table 2).

Testicular histological results indicated that *Carollia perspicillata* males with descending and non-descending testes observed in the dry and rainy seasons showed Sertoli cells of the spermatogenic lineage at different stages of maturation (spermatogonia, spermatocytes, rounded and elongated spermatids), as well as Leydig cells, regardless of testicular position (Fig.1).

Testicular histomorphometric analysis showed statistically significant differences with respect to tubular compartment occupation area ($p<0.001$), intertubular compartment occupation area ($p<0.001$) and number of spermatocyte ($p<0.001$), rounded spermatids ($p<0.001$), elongated spermatids ($p<0.001$), and Sertoli ($p<0.001$) and Leydig ($p<0.001$) cells, with the highest averages observed in the rainy season (Table 3).

Table 2. Relative frequency (RF) of *Carollia perspicillata* males with descending and non-descending testes in the dry and rainy seasons in a fragment of the Atlantic Forest, Pernambuco state, Brazil

	Dry season		Rainy season	
	RF (%)	N	RF (%)	N
Descending testis	80.00	20	65.71	23
Non-descending testis	20.00	5	34.29	12
Total	100	25	100	35

N= number of individuals.

Table 3. Mean and standard deviation of the areas of tubular compartment (TCOA) intertubular compartment (ITCOA) occupation, quantification of spermatocytes (SPC), rounded spermatids (RS), elongated spermatids (ES), and Sertoli (SC) and Leydig (LC) cells of *Carollia perspicillata* testes in the dry and rainy seasons in a fragment of the Atlantic Forest, Pernambuco state, Brazil

Parameter	Rainy season	Dry season	*p*-value
TCOA %	64.61 ± 4.51^{b}	58.83 ± 7.52^{a}	<0.001
ITCOA %	18.75 ± 4.15^{b}	13.87 ± 2.77^{a}	<0.001
SPC	36.74 ± 6.29^{b}	21.89 ± 3.68^{a}	<0.001
RS	40.38 ± 6.39^{b}	25.86 ± 4.26^{a}	<0.001
ES	46.77 ± 15.48^{b}	28.49 ± 7.04^{a}	<0.001
SC	14.10 ± 3.09^{b}	11.29 ± 2.38^{a}	<0.001
LC	36.89 ± 8.74^{b}	24.31 ± 5.61^{a}	<0.001

[a, b] Different letters in the same line are statistically different by the Student's *t*-test ($p<0.05$).

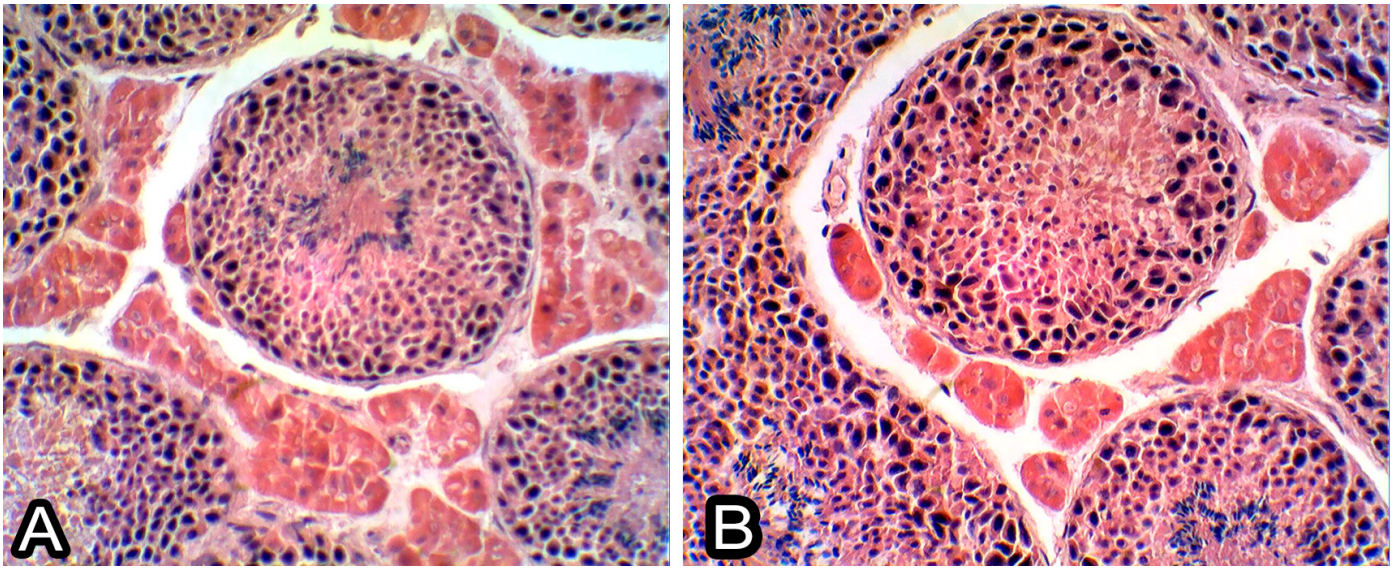

Fig.1. (**A**) Testis in the dry season, (**B**) testis in the wet season. Note the spermatogonia (yellow arrow), spermatocytes (black arrow), rounded spermatids (arrowhead), elongated spermatids (two-headed arrows), and Leydig (asterisks) and Sertoli (blue arrow) cells. HE, obj.40x, bar=20µm.

DISCUSSION

A higher proportion of male bats with descending testes was observed in both seasons; however, the external reproductive characteristics were limited, impairing verification of sexual activity. Recently, a study conducted at the same site of the present research reported that *Phyllostomus discolor* male bats present spermatogenic activity regardless of testis position (Lima Junior et al. 2014). Thus, studies performing testicular histomorphometric analysis become relevant to establish the reproductive dynamics of bats (Morais et al. 2013a, 2013b, 2013c, 2014a, 2014b, Farias et al. 2015, Lima Junior et al. 2014, Notini et al. 2015). Associated with this information, histology of *Carollia perspicillata* testis presents morphological characteristics similar to those described for other bat species (Beguelini et al. 2009, 2011, Bordignon & França 2012).

The higher testicular histomorphometric averages observed in the rainy season indicate an investment in both spermatogenesis and hormone production. These characteristics evidenced the reproductive strategy of this species in generating viable spermatozoa for mating during the months of greater precipitation; in addition, this species presents synchrony of spermatogenesis with the period of greater availability of food resources.

Thus, the larger sperm production may be synchronized with the greater sexual receptivity of females, because *Carollia perspicillata* bats are characterized by polygynous mating system with harem formation (Mello & Fernandez 2000), to which a greater androgenic and spermatogenic investment in the rainy season is essential. These data are associated with the reproductive characteristics of female bats in areas of the Atlantic Forest in the Southeastern Region of Brazil, because there is simultaneity of the reproductive peaks and the rainy season, which is the most favorable period of the year due to the greater availability of food resources (Mello et al. 1999, Mello & Fernandez 2000).

In addition, it is noteworthy the importance of testosterone and Sertoli cells in the maintenance of *C. perspicillata* spermatogenic lineage, especially evidenced by the process of spermatogenesis involving a series of complex biochemical, molecular and cellular events (Mruk & Cheng 2004), and depending on the bat species, Sertoli cells are essential for reproduction, mainly because of the support functions of germ cells, nutrition, and growth factors, as well as formation of the blood-testis barrier, thus providing greater protection to spermatozoa under development (Crichton 2000, Griswold & Skinner 2004, Fijak & Meinhardt 2006).

CONCLUSION

Seasonality influences the testicular morphological and physiological parameters of *Carollia perspicillata* male bats, as evidenced by the increased tubular and intertubular compartment occupation areas, and the increased number of spermatogenic, Leydig and Sertoli cells in the rainy season, suggesting that these bats present greater sexual activity in this period.

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Conflict of interest statement.- The authors declare no competing interests.

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