Biota Neotropica 16(4): e20160231, 2016
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

short communication

Reproductive biology of *Artibeus fimbriatus* Gray 1838 (Chiroptera) at the southern limit of its geographic range

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LIMA, C., FABIAN, M. E. **Reproductive biology of Artibeus fimbriatus Gray 1838 (Chiroptera) at the southern limit of its geographic range.** Biota Neotropica. 16(4): e20160231. http://dx.doi.org/10.1590/1676-0611-BN-2016-0231

Abstract: Bats of the Phyllostomidae family exhibit different reproductive patterns in Neotropical regions and the strategy adopted depends on the regional climate. Here we studied the reproductive biology of *Artibeus fimbriatus* at the southern limit of their distribution in Brazil. This region has no rainy season, and the climate is characterized by high temperatures and variable photoperiods. We examined 129 *A. fimbriatus* females over several months, and used histological procedures where necessary in order to determine whether bats were pregnant. Females exhibited a long reproductive period and were pregnant from June until February. The reproduction events were found to be dependent on the photoperiod, but independent of annual accumulated precipitation. Our results show that at the southern limit of their distribution, *A. fimbriatus* exhibit seasonal-dependent reproductive patterns, with parturition events occurring during spring and summer, in which the days are longer and temperature is warmer.

Keywords: *Phyllostomidae; Photoperiod; Reproduction; Seasonal.*

Biologia reprodutiva de Artibeus fimbriatus Gray 1838 (Chiroptera) no limite sul de sua distribuição geográfica

Resumo: Os morcegos Phyllostomidae apresentam diferentes padrões reprodutivos no Neotropico e a estratégia adotada depende do clima regional. Neste trabalho nós estudamos a biologia reprodutiva de *A. fimbriatus* no Brasil, na região que determina o limite sul de sua distribuição geográfica, onde não há estação chuvosa e o clima é caracterizado por variações na temperatura e fotoperíodo. Nós examinamos 129 fêmeas de *A. fimbriatus* e utilizamos procedimentos histológicos, quando necessário, para classificar as fêmeas como prenhe ao longo dos meses. As fêmeas exibem um longo período reprodutivo e estão grávidas de junho até fevereiro. Nossos resultados mostram que as fêmeas de *A. fimbriatus* apresentam uma estratégia reprodutiva que é dependente da sazonalidade com os nascimentos ocorrendo quando os dias são mais longos e a temperatura é maior (primavera e verão), no limite sul de sua distribuição geográfica.

Palavras-chave: *Phyllostomidae; Fotoperíodo; Reprodução; Sazonal.*

Introduction

Phyllostomidae are a family of Neotropical bats that are distributed from southern United States to northern Argentina. Within this wide geographic range they inhabit a great diversity of habitats in tropical and subtropical regions, and therefore, are submitted to a variety of abiotic factors, such as variations in latitude, temperature, rainfall and photoperiods (Fleming et al. 1972; Beguelini et al. 2013a). In response to these factors, bats of the Phyllostomidae family have evolved different reproductive patterns in Neotropical regions, such as seasonal polyestry, aseasonal polyestry and monoestry (Fleming et al. 1972; Estrada & Coates-Estrada 2001). Considering the high diversity of bats in this region, the available information about their reproductive biology is lacking (Zortéa 2003), and only few species have had their reproductive aspects analyzed in detail (Godoy et al. 2014).

Reproduction in bats is energetically expensive and is strongly associated with specific environmental conditions (Wilson 1979). Thus, in areas where food production is seasonal, bats meet their energetic requirements for reproduction during periods of maximum food availability (Racey & Entwistle 2000). Bats from temperate latitudes reproduce during periods of higher temperatures, when there is increased abundance of insects. In tropical regions, however, reproductive periods are associated with the rainy season, because variations in rainfall may affect the seasonality of food (Fleming et al. 1972; Racey & Swift 1985). Therefore, variations

in rainfall in the Neotropics affect food availability, directly interfering with the reproductive cycles of the bats over their distribution area and consequently, sub-populations may exhibit different reproductive patterns (Crichton & Krutzsch 2000).

Artibeus fimbriatus Gray 1838 is a Phyllostomidae bat with a wide geographic distribution, found in east Paraguay, Northwest Argentina and in Brazil, from Bahia to Rio Grande do Sul (Rui & Fabián 1999). Although most studies that have investigated tropical species generally focus on aspects of female reproduction (Beguelini et al. 2013b), the reproductive biology of *A. fimbriatus* is poorly studied and is only known in a region in southeastern of Brazil (Ésberard et al. 1998). The southern limit of the geographic range of this species is the south of Brazil, where the climate differs from other regions due to the absence of a rainy season, in addition to its characteristic high variability in temperatures and photoperiods (Kuinchtner & Buriol 2001; Alvares et al. 2013).

There is a lack of information on the reproductive biology of *A. fimbriatus*, and on the effect of the different environment at the southern limit its distribution. Therefore, the objective of this study was to investigate the reproductive biology of *A. fimbriatus* females at their southern limit, and their relationship with environmental factors. Specifically, we aimed to determine: (1) the annual period of female *A. fimbriatus* reproduction at the southern limit of their distribution; and (2) whether the reproductive events are associated with the photoperiod, temperature or annual accumulated precipitation of this region.

Materials and methods

We investigated specimens that had been deposited in scientific collections to investigate the reproductive pattern of *A. fimbriatus* females. These specimens had been deposited at the University of Rio Grande do Sul and Fundação Zoobotânica do Rio Grande do Sul, and were collected between 1989 and 2011. The specimens were from Brazilian localities at the southern limit of the species distribution, located at 28°26'06.94" S 49°11'5.25" W north, 29°22'28.97" S 51°07'31.23" W east and 28°36'55.53" S 49°01'31.73" W west. These regions are characterized by the absence of a rainy season. Instead, the annual accumulated precipitation is constant throughout the year, and the climate (Köppen Cfa) is characterized by high temperatures and photoperiods that vary between the four well-defined seasons.

We determined the age of females by verifying their epiphyseal ossification, and only adults were included in this study (Anthony, 1988). We analyzed their gonads to determine whether the females were pregnant. Histological confirmation was performed on some females to confirm the initial stages of pregnancy. The histological slides were prepared with a Leica® Historesin Embedding Kit or paraffin and were stained with hematoxylin and eosin. Females were classified as either lactating, pregnant, pregnant and lactating or inactive. Fetuses were measured to determine their developmental stage (Reis 1989), and were classified into five groups: group I, initial stages of pregnancy (histologically confirmed); group II, less than 1 cm; group III, between 1 and 2 cm; group IV, between 2 and 3 cm; and group V, between 3 and 4 cm.

We used generalized linear models (GLM) with a binomial distribution to test the effect of the photoperiods and annual accumulated precipitation on the probability of reproductive events over the year. For the residual analysis and model validation, we followed the method described by Zuur et al. (2009). The photoperiods, annual accumulated precipitation (hereafter called rainfall) and temperature data were obtained from the Brazilian National Weather Institute (INMET). All analyses were performed using R software (R Development Core Team 2012) with the lme4 package (Bates et al. 2015).

Results

A total of 129 specimens were included in the analysis. Female *A. fimbriatus* bats were found to be pregnant between June and February (Figure 1) in the south of their geographical distribution range. The reproduction events were dependent on the photoperiod ($P = < 0.001$), and were independent of the annual accumulated precipitation ($P = 0.83$; Figure 2). We only included the photoperiod and rainfall for this analysis as photoperiod and temperature are correlated. As days become longer, the temperature also increases, and therefore, the probability of pregnancy is higher.

Fetuses at different developmental stages were observed throughout the year. Fetuses in advanced stages were found in September and February, whereas fetuses were smaller during the other months of the year (Figure 3). This may suggest the existence of two distinct parturition events during the year. Thus, our results show that *A. fimbriatus* females exhibit a seasonal reproductive strategy, with parturition events occurring around September and October (spring) and February (summer) when the days are longer and the temperature is warmer at the southern limit of their geographic range.

Discussion

Artibeus fimbriatus exhibits seasonally-dependent reproduction at the southern limit of its geographic range, and parturition events occur in September and October (spring) and February (summer), when the days are longer and temperature is warmer. The only previous study that has focused on the reproduction of *A. fimbriatus* was performed in a tropical

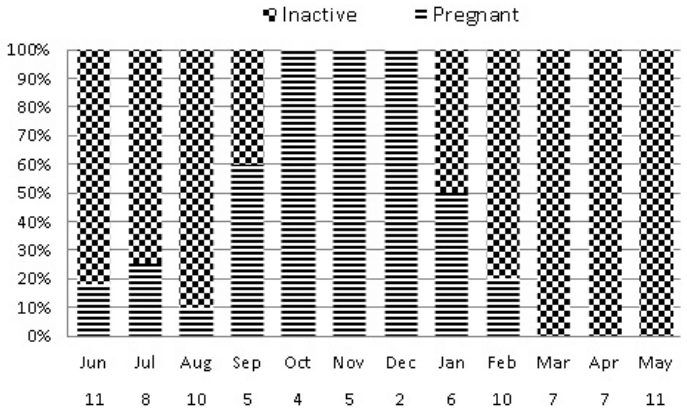

Figure 1. Reproductive status of *Artibeus fimbriatus* females at the southern limit of their distribution over different months of the year.

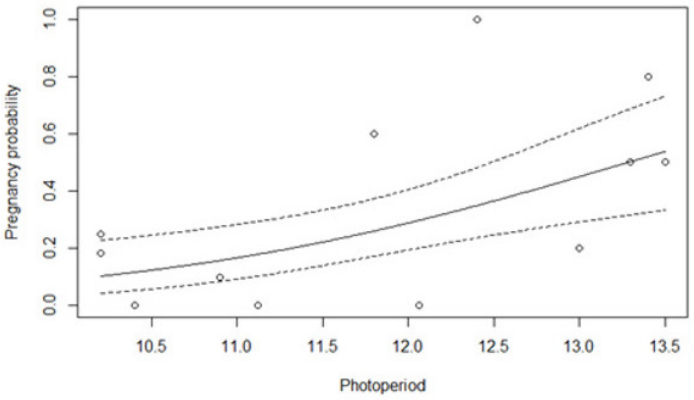

Figure 2. Relationship between reproductive events (pregnancy probability) and photoperiods at the southern limit of *Artibeus fimbriatus* distribution.

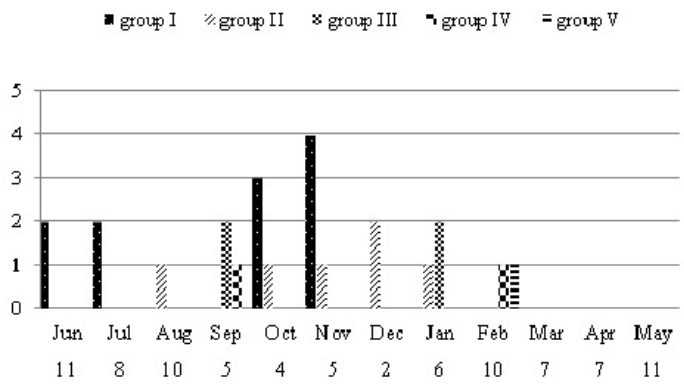

Figure 3. Different developmental stages of *Artibeus fimbriatus* fetuses over different months of the year.

area in Brazil (Rio de Janeiro state), where the species was also reported to exhibit seasonal reproduction, with the parturition events occurring in the rainy season (Esbérard et al. 1998). Other species of the *Artibeus* genus, including *A. lituratus* and *A. jamaicensis* Leach, 1821, also show seasonal reproduction, with the highest rates of pregnancy observed during the warmest seasons in Panama (Fleming et al. 1972; Bonaccorso 1978), Venezuela (Sosa & Ramoni-Perazzi 1995) and in different localities of Brazil (Taddei 1976; Willig 1985; Reis 1989). This pattern has also been observed for other species of the Phyllostomidae family, such as *Uroderma bilobatum* Peters, 1866, *Carollia perspicillata* Linnaes 1758 (Fleming et al. 1972), *Sturnira lilium* (E. Geoffroy, 1810) and *Platyrrhinus lineatus* E. Geoffroy, 1819 (Estrada & Coates-Estrada 2001; Stoner 2001; Costa et al. 2007).

The reproduction of bats is usually synchronized so that parturition and lactation events occur during periods of highest food availability, which coincide with periods of highest rainfall in tropical regions (Fleming et al. 1972; Willig 1985; Zortéa 2003). At the southern limit of *A. fimbriatus* distribution there is little variation in rainfall, however, the photoperiod and temperature can vary significantly (Kuinchtner & Buriol 2001; Alvares et al. 2013). Within these localities we found that the parturition events occur in the spring and summer, when days are longer and the temperature is warmer. Synchronizing parturition and lactation with spring and summer in the south of Brazil, a region in which winter brings low temperatures, also increases the survival rate of growing pups (Racey & Swift 1985). In addition, spring and summer likely correspond to periods of higher food availability in the south of Brazil. While there is no phenological information in the literature about the diet of *A. fimbriatus* in these regions, it is known that the abundance of insects is higher in spring and summer (Fabián et al. 1990), which are consumed by *Artibeus* as part of their diet (Passos et al. 2003; Passos & Graciolli 2004).

Female *A. fimbriatus* bats have a long reproductive period of 9 months, which lasts from June until February at the southern limit of its geographic range. Ésberard et al. (1998) also reported that the reproductive period of *A. fimbriatus* in southeast Brazil lasts about 9 months, however, they reported that the reproductive period lasts from July until March. While the reproduction period of *A. fimbriatus* appears to be the same for these two localities, the reproduction period of other species within the Phyllostomidae family may vary. In Costa Rica, *A. jamaicensis* was reported to have a 9-month reproduction period. In southeast Brazil, *Sturnira lilium* was reported to have an 11-month reproduction period (Godoy et al. 2014) and *Platyrrhinus lineatus* presented a 10-month reproduction period (Costa et al. 2007).

Seasonal polyestry is the most common reproductive strategy in Phyllostomidae bats, with females exhibiting bimodal peaks for pregnancy and also for lactation, as well as monthly increases in embryo size (Fleming et al. 1972). Our data only allowed us to determine the period in which *A. fimbriatus* reproduces at the southern limit of its geographic range, in addition to the embryo size. We were unable to collect information about the lactation period, as the analyzed specimens were from scientific collections, therefore, we cannot verify that *A. fimbriatus* have a bimodal pregnancy pattern. However, Ésberard et al. (1998) also concluded that *A. fimbriatus* exhibit seasonal reproduction, and suggested that the species may exhibit a seasonally polyestry with bimodal peaks. Therefore, although the results presented from our study and the study by Ésberard et al. (1998) are informative about the reproductive biology of *A. fimbriatus*, these questions remains to be answered. A bimodal reproductive pattern has also been observed for other *Artibeus* species, including *A. lituratus* in Panama (Fleming et al. 1972; Bonaccorso 1978), Venezuela (Sosa & Ramoni-Perazzi 1995) and Brazil (Taddei 1976; Willig 1985; Reis 1989). This reproductive pattern has also been recorded for other species of Phyllostomidae, including *Uroderma bilobatum* Peters, 1866 and *Carollia perspicillata* Linnaes 1758 (Fleming et al. 1972).

In conclusion, our study shows that *A. fimbriatus* exhibit seasonal reproduction at the southern limit of their distribution, coinciding with variations in seasonal photoperiods. The parturition events occur during spring and summer when the days are longer.

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Received: 17/07/2016
Revised: 02/10/2016
Accepted: 06/10/2016