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Nota

CIRCANNUAL SEX DISTRIBUTION OF THE BRAZILIAN FREE-TAILED BAT, *Tadarida brasiliensis* (CHIROPTERA: MOLOSSSIDAE), SUGGESTS MIGRATION IN COLONIES FROM URUGUAY

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ABSTRACT. In the Northern Hemisphere, *Tadarida brasiliensis* is a migratory species, but there are no published data about its migration behavior in Uruguay. Using field data, we found significant and consistent seasonal variation in colony sex ratios across Uruguay. Males composed the majority of colonies sampled in Uruguay during the cold season, whereas females proportionally outnumbered males in these colonies during the warm season. Individuals present during the cold season remain active, and our evidence suggests females migrate out of Uruguay in winter. This is the first contribution to knowledge about circannual use of roosts by *T. brasiliensis* in Uruguay.

RESUMEN. Distribución circanual de sexos del murciélago de cola libre brasilero, *Tadarida brasiliensis* (Chiroptera: Molossidae), sugiere migración en colonias de Uruguay. *Tadarida brasiliensis* es una especie migratoria en el hemisferio norte, pero no existen datos publicados al respecto para Uruguay. A partir de datos de campo mostramos que existe una variación significativa en la composición por sexos a lo largo del año en las colonias del país. Durante la estación fría se observa una composición mayoritariamente masculina de las colonias, mientras que en la estación cálida el patrón se invierte. Se verifica que los individuos presentes durante el invierno están activos todo el año y nuestra evidencia sugiere que las hembras migran fuera de Uruguay durante el invierno. Esta es la primera contribución al conocimiento del uso anual de refugios por esta especie en Uruguay.

Key words: Conservation. Pampas biome. Seasonal variation. South America.

Palabras clave: Bioma Pampa. Conservación. Sudamérica. Variación estacional.

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The Brazilian free-tailed bat, *Tadarida brasiliensis* (I. Geoffroy, 1824), is a species that often aggregates in enormous groups, sometimes forming colonies with many millions of individuals (McCracken 2003). This species is migratory in the Northern Hemisphere, showing long-range movements, especially in southern United States and northern Mexico (Wilkins 1989; Cleveland et al. 2006). Individuals that migrate to the northernmost areas of the species' distribution are mainly females (Wilkins 1989). In some shelters in Texas (USA), probably used as stopovers during migration, despite significant changes in group sizes, no changes in sex-ratios were observed (Scales & Wilkins 2007). Understanding bat migration patterns is important for conservation, particularly in recent years as evidence shows migratory species of bats, including *T. brasiliensis*, are vulnerable to mortality from wind turbines (Arnett & Baerwald 2013; O'Shea et al. 2016). Wind-energy development was identified among the emerging threats to bat conservation in Latin America, including Uruguay (RELCOM 2010; Botto et al. in rev.). Improved understanding of temporal and spatial characteristics of bat migration may help prevent mortality at wind turbines. Although *T. brasiliensis* is widely distributed across South America, with the exception of limited observations from Brazil, there have been no reports of migration by this species in the Southern Hemisphere (Bárquez et al. 1999; Pacheco et al. 2010).

To investigate seasonal occurrence and the possibility that *T. brasiliensis* migrates in Uruguay, we first obtained information on the sex composition of colonies at four locations across Uruguay (**Fig. 1**) through field surveys.

The first field-sampling location was Usina Cuñapirú (CUNAPIRU) in the Department of Rivera (31.523042° S, 55.593042° W), which is the site of an ancient mining industrial complex, the country's first hydroelectric dam (**Fig. S1A**), and first in Uruguay's list of Important Sites for Bat Conservation (SICOM) of the Latin American Network for the Conservation of Bats (RELCOM) (Genta et al. 2017). Clusters of *T. brasiliensis* and *Myotis levis* reside in the tunnels below the dam structure and in some of the adjacent warehouses. We visited these sites in December 2007, April 2014, and July and November 2015. In 2007 clusters of *T. brasiliensis* were occupying the old office building (**Fig. S1B**), as well as some of the tunnels. In 2014, the cluster in the office building was very small, in part because the ceiling there had been removed in an attempt to decrease colony size after a local rabies outbreak in 2007-2008 (**Fig. S1D**). However, a cluster in the tunnels was estimated at several hundred individuals, although an exact count was not made (**Fig. S1C**). In July 2015, the cluster seemed very small, with all the tunnels unoccupied and just some individuals in the warehouses and the old office building. In November 2015, the cluster in the tunnels was large (several thousand individuals), as were clusters in the office building and warehouses. These clusters were sampled using mist nets and direct capture inside the roost structures during July and November 2015.

The second field-sampling location was the Museo y Parque Fernando García (MPFG) in Montevideo (34.866113° S, 56.062376° W). This site has a complex of several buildings, including an abandoned house and two warehouses dedicated to preserving a collection of carriages (**Fig. S1E**). The warehouses are used by a mixed colony of *T. brasiliensis*, *M. albescens* and *M. levis* (**Fig. S1F**). From October 2005 to January 2006 this colony was sampled weekly using mist nets in the entrance of one of the warehouses. The size of the colony was not determined, but was estimated to contain at least several hundred *T. brasiliensis*.

The third field-sampling location was the Complejo Zapicán (ZAPICAN), which is a residential complex in the city of Montevideo (34.881139° S, 56.191114° W). The buildings in the complex have double walls with a ventilated air chamber. The chamber is accessible to bats trough ventilation openings in each of the three stories of the buildings. These roosts are inhabited by *T. brasiliensis*. The number of bats using the buildings has not been quantified, but to our knowledge this is the largest colony in the city, probably numbering several thousand individuals. ZAPICAN was visited in May and July 2008, and bats were captured using mist

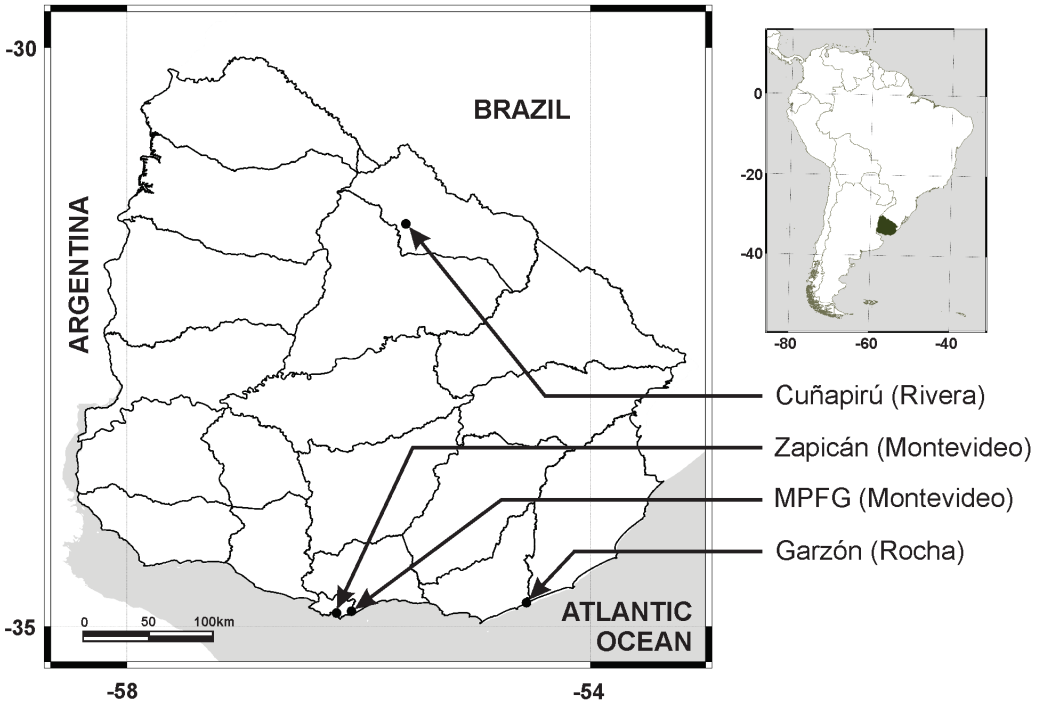

Fig. 1. Locations in Uruguay where colonies of *T. brasiliensis* were seasonally sampled for sex composition: Cuñapirú industrial complex, Department of Rivera; Zapicán residential complex, Department of Montevideo; Museo y Parque Fernando García (MPFG), Department of Montevideo; and Garzón lagoon, El Caracol resort, Department of Rocha.

nets as they flew from the vents on one of the buildings. The sampling was part of a rabies surveillance program on insectivorous bats in Montevideo, carried out by the Veterinary College of the National University.

The fourth field-sampling location, Laguna Garzón (GARZON), is a coastal lagoon located between the departments of Maldonado and Rocha on Uruguay's Atlantic coast. At this site a colony of *T. brasiliensis* inhabits an abandoned house situated between the sea and the lagoon (34.792109° S, 54.553138° W). This colony was visited two times: once in May and once in August 2007. The clusters of *T. brasiliensis* were found in the ceiling of a second-floor room. Both visits were in the cold season, but we were able to catch bats using mist nets placed inside and around the house.

In addition to the field sampling described above, we also summarized sex data on specimens of *T. brasiliensis* in scientific museum collections. The collections of the National Museum of Natural History (MNHN) and the School of Sciences at the Universidad de la República (ZVERT-M) are the two most important mammal collections in Uruguay, both in terms of historical coverage and number of specimens, representing most of the bats ever collected in the country. We visited MNHN and ZVERT-M and collated all records of *T. brasiliensis* from Uruguay that included information on both sex and date of capture. In addition to records from collections in Uruguay, we also included records from three major repositories in the United States that have specimens from Uruguay: Field Museum of Natural History (FMNH), American Museum of Natural History (AMNH), and Smithsonian National Museum of Natural History (USNM). The records from these institutions were obtained by a web search of their catalogues (AMNH 2017; FMNH 2017;

USNM 2017), performed on 11/06/2017 with the terms "*Tadarida*" for genus, and filtered by country. Records missing the information on sex or date of capture were discarded. For the records from FMNH, we assigned the date based on an expedition diary (Sanborn 1929). Records for which we knew the month, but were missing the exact day of capture were included, and we arbitrarily assigned day 01 of the corresponding month for all of them. There is some discrepancy in capture localities for the FMNH specimens between Sanborn's publication and the museum records. Although most of the specimens were labeled "Treinta y Tres, Uruguay", we concluded that those must have come from Maldonado and Rocha cities. We assigned November 1st as the arbitrary day of capture to the presumed Maldonado and Rocha specimens and December 16th to the specimens from a stream at Polanco.

To avoid overrepresentation at our field-sampling locations, we excluded data from museum specimens that were previously collected at those same sites (32 from GARZON, 20 from MPFG, 8 from ZAPICAN). However, those specimens are tallied and included in the observational data table (**Table S1**). We assigned day 01 to 12 records from scientific collections; among them two were from March. The three US institutions housed 45 specimens of *T. brasiliensis* from Uruguay (FMNH = 38, AMNH = 1, USNM = 6). Only 25 of the FMNH specimens had information on sex, and only two of the USNM specimens had information on sex and none on capture date.

To assess the annual distribution of sexes, we calculated the seasonal proportion of males composing clusters sampled, grouped by month and by two seasons: we defined the cold season as 20 March-22 September and the warm seasons as 23 September-19 March. All statistical tests were performed in R version 3.3.1 (R Core Team 2016) and RStudio (RStudio Team 2012). To test for the independence of sex distribution and the month of the year we used a Chi-square test, using the 'chisq.test' function from the 'stats' package. For the monthly distribution, the significance was estimated using a Monte Carlo simulation and a correction for continuity included in the function (using 2000 replicates). For the seasonal distribution, the significance was estimated using the Chi-square distribution with one degree of freedom.

Table S1 shows data from the 477 individuals examined at the four field-sampling locations (MPFG: n = 281, CUNAPIRU: n = 103, GARZON: n = 72, ZAPICAN, n = 21), and the 232 specimens included in the analysis from scientific collections (MNHN, n = 191, FMNH, n = 25, ZVERT-M, n = 15, AMNH, n = 1).

We saw the same pattern at all four field-sampling locations and in different years: males composed greater proportions of the bats sampled from colonies than females in the cold season. During the warm season, the colonies were proportionally dominated by females. Sex ratios of the field data significantly varied by month ($\chi^2 = 210.02$, $p<0.001$, n = 477, **Fig. 2A**) and season ($\chi^2 = 171.16$, $p<0.001$, df = 1, n = 477, **Fig. 2B**). Sex ratios of museum specimens did not significantly vary by season ($\chi^2 = 2.22$, $p = 0.137$, df = 1, n = 232, **Fig. 2D**), and while museum sex ratios did significantly vary by month ($\chi^2 = 31.13$, $p<0.001$, n = 232), a clear trend was not apparent (**Fig. 2C**).

This is the first study to report circannual variation of sex ratios of *T. brasiliensis* in Uruguay. These results show that: (a) there is seasonal variation in the sex ratios and number of individuals composing *T. brasiliensis* colonies in Uruguay, (b) colonies of *T. brasiliensis* sampled in Uruguay are composed of proportionally more males during the cold season and a proportionally more females in the warm season, (c) *T. brasiliensis* present in Uruguay during the winter remain active, and (d) presumed migrations of females away from and then back to the colonies we sampled in Uruguay seem to occur during the start of the autumn (late March and early April) and beginning of spring (late September and early October), respectively.

The observed seasonal change in sex ratios of *T. brasiliensis* in Uruguay is similar to patterns observed in populations of this species inhabiting the Northern Hemisphere (Wilkins 1989; Scales & Wilkins 2007). Our analysis indicates that Uruguayan colonies might be either the origins or destinations for migratory females, rather than migration stopover points. We infer

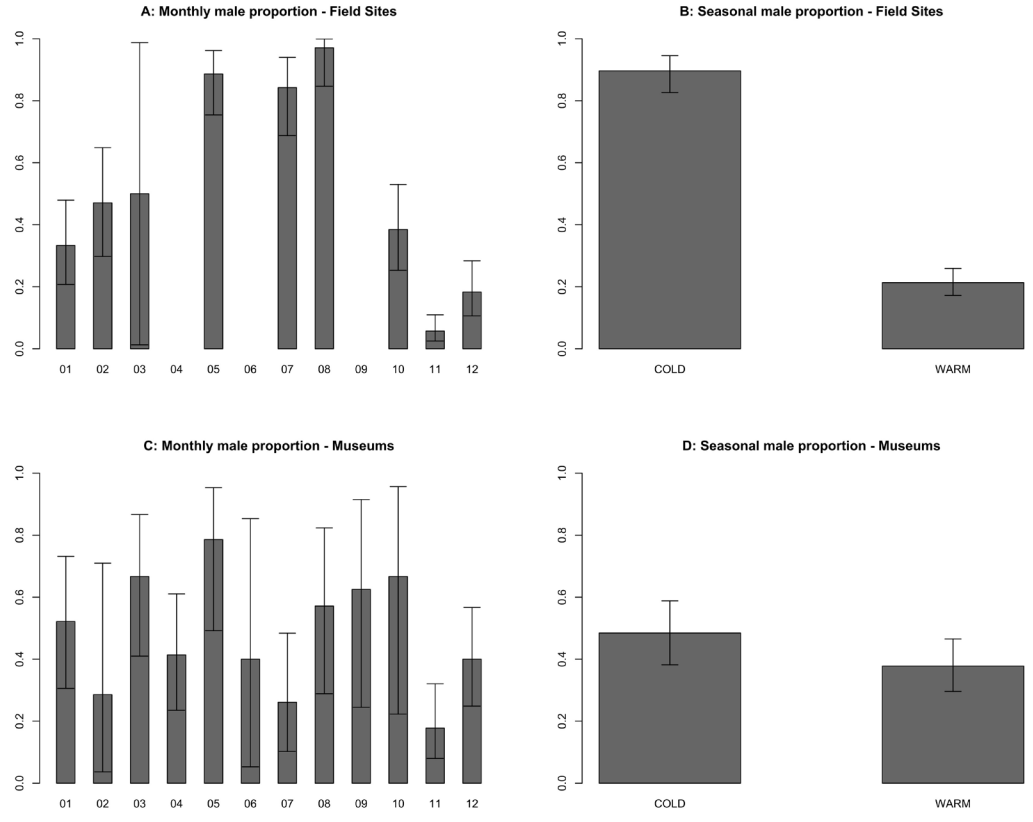

Fig. 2. Male proportions of *Tadarida brasiliensis* sampled at field locations and from specimens housed in museums. A: Monthly proportions from the field samples (only showing the nine months with observational data); B: Seasonal proportions in the observational field sites (COLD = 20 March-22 September, WARM = 23 September-19 March); C: Monthly sex ratio of museum specimens; D: Seasonal sex ratio of the museum specimens. The error bars show exact binomial 95% confidence intervals.

that migration of *T. brasiliensis* in Uruguay mostly involves females, with males remaining in the country and staying active during the winter. This pattern of sex-biased migration was previously observed in *T. brasiliensis* and in other species of migrating bats in subtropical and temperate zones, where females tend to migrate more often or migrate longer distances (Fleming & Eby 2003). The question of where female *T. brasiliensis* go when they disappear from roosts in Uruguay remains unanswered.

Female *T. brasiliensis* give birth and raise young after they seasonally appear in large numbers at roosts in Uruguay. At MPFG we observed peaks in parturition from about November through December, and we consistently saw pups by the end of November at Cuñapirú. This species is known to be monestrous in other parts of its range (Wilkins 1989; Keeley & Keeley 2004), so it is likely that females arrive in Uruguay to raise young after wintering elsewhere, analogous to colonies composed mostly of females that seasonally form during the warm season at higher latitudes of North America. During the winter, colonies we sampled in Uruguay (that were mostly male) contained reproductively inactive individuals.

Our sampling data from repeated visits to several field locations provided a stronger framework for inference than information we were able to obtain from scientific collections. The difference between the field and museum

data may be explained by bias introduced to the latter if prior researchers selectively chose certain individuals to preserve as specimens. For example, the less frequently encountered sex might have been oversampled in order to have good representation of both sexes in collections. Such an effect might be particularly exaggerated in case of smaller museum collections. Regardless potential biases of the museum data, they are generally consistent with the clearer seasonal pattern we observed through field sampling.

Additional studies are needed to establish the origins of female *T. brasiliensis* migrating into Uruguay, to better quantify seasonal changes in colony size, and to precisely establish the timing of the migration events. Detailed knowledge about spatial and temporal aspects of migration by *T. brasiliensis* in the region may be especially important in better understanding the dynamics of wildlife pathogens (e.g., rabies virus) as well as from a conservation perspective, considering the rapid increase in construction of wind energy facilities in Uruguay and southern Brazil.

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SUPPLEMENTARY ONLINE MATERIAL

Supplement 1

Table S1. Records included in the analysis from field sampling of *T. brasiliensis* at four locations in Uruguay: Usina Cuñapirú, Rivera (CUNAPIRU, n = 103), Museo y Parque Fernando García, Montevideo (MPFG, n = 281), Complejo Zapicán, Montevideo (ZAPICAN, n = 21), Garzón Lagoon, Rocha (GARZON, n = 72); and from scientific collections: Sciences College, Universidad de la República (ZVERT-M, n = 15), National Museum of Natural History (MNHN, n = 191), Field Museum of Natural History (FMNH, n = 25) and American Museum of Natural History (AMNH, n = 1).
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Supplement 2

Fig. S1. Images of the four field-sampling locations. (A) Partial exterior view of Cuñapirú industrial complex, department of Rivera. (B) Colony of *T. brasiliensis* in the tunnels under the dam (April 2014). (C) Colony of *T. brasiliensis* in an old office building, before the ceiling was removed (December 2007). (D) View of the office building after the removal of the ceiling (April 2014). (E) Interior view of one of the warehouses of the Museo y Parque Fernando García (MPFG), department of Montevideo. (F) Colony of *T. brasiliensis* in the roof of a warehouse at the MPFG (2004).
https://www.sarem.org.ar/wp-content/uploads/2018/04/SAREM_MastNeotrop_25-1_Botto-sup2.docx