

Article



# DAILY ACTIVITY PATTERNS IN FREE-LIVING TUCO-TUCOS (RODENTIA: CTENOMYIDAE) FROM ANILLACO, LA RIOJA PROVINCE, ARGENTINA

Juan P. Amaya<sup>1,2</sup>, Pablo M. Lopez<sup>3</sup>, Pablo A. Cuello<sup>4</sup>, Eileen A. Lacey<sup>5</sup> & Verónica S. Valentinuzzi<sup>1</sup>

<sup>1</sup>Centro Regional de Investigaciones Científicas y Transferencia Tecnológica La Rioja (CRILAR), UNLAR, SEGEMAR, UNCa, CONICET, Anillaco, La Rioja, Argentina. [Correspondence: Juan P. Amaya <[jpjuanamaya@gmail.com](mailto:jpjuanamaya@gmail.com)>].

<sup>2</sup>Instituto de Biología de la Conservación y Paleobiología (IBICOPA) DACEFYN-CENIIT-UNLaR, La Rioja, Argentina.

<sup>3</sup>Universidad Nacional de Villa Mercedes (UNVIME), Departamento de Salud, Villa Mercedes, San Luis, Argentina.

<sup>4</sup>Grupo de Investigaciones de la Biodiversidad (GiB), Instituto Argentino de Investigaciones de las Zonas Áridas (IADIZA), CONICET, CCT-Mendoza, Mendoza, Argentina.

<sup>5</sup>Museum of Vertebrate Zoology and Department of Integrative Biology, University of California, Berkeley, CA, USA.

**ABSTRACT.** Daily activity patterns are a fundamental component of a species' biology and have implications for numerous elements of organismal function. Because these patterns may be affected by environmental conditions, the setting in which activity data are collected is critical. Although the daily activity patterns of the Velasco tuco-tuco (*Ctenomys famosus*) have been studied extensively in the laboratory and in semi-natural enclosures, no information on activity is available for free-living members of this species. To characterize activity by these subterranean rodents in their natural environment, we used radiotelemetry to record the locations of 17 adults at hourly intervals for 72 consecutive hours. These data revealed a tendency for members of the study population to be diurnal, with individuals changing locations more often, traveling farther between radio fixes, using larger areas, and spending less time in putative nests during daylight hours. Diurnality index values calculated for these animals also suggested greater daytime activity. Although diurnal behavior has been reported for free-living populations of several other ctenomyid species monitored by radiotelemetry, the strength of this tendency appears to vary, raising interesting questions about the factors that determine daily activity patterns in the genus *Ctenomys*.

**RESUMEN. PATRÓN DE ACTIVIDAD DIARIA EN TUCO-TUCOS DE VIDA LIBRE (RODENTIA: CTENOMYIDAE), DE ANILLACO, PROVINCIA DE LA RIOJA, ARGENTINA.** Los patrones diarios de actividad son un componente fundamental de la biología de una especie, que tienen implicancias en numerosos elementos de la función del organismo. Debido a que esos patrones pueden verse afectados por las condiciones ambientales, el entorno en el cual se toman los datos de la actividad resulta crucial. Aunque los patrones de actividad diaria del tuco-tuco del Velasco (*Ctenomys famosus*) han sido estudiados ampliamente en laboratorio y recintos seminaturales, no hay información disponible sobre la actividad en libertad para los miembros de esta especie. Con el objetivo de caracterizar el patrón de actividad de estos roedores subterráneos en su entorno natural, utilizamos radiotelegrafía para registrar las ubicaciones de 17 adultos a intervalos de una hora durante 72 horas consecutivas. Estos datos revelaron una tendencia de los miembros de la población de estudio a ser diurnos, con individuos que cambian de ubicación con más frecuencia, se mueven una mayor distancia entre puntos fijos, utilizan áreas más grandes y pasan menos tiempo en sus nidos durante la fase clara del día. A su vez, los valores del índice de diurnidad también han sugerido una mayor actividad diurna. Aunque se ha descrito el comportamiento diurno de las poblaciones de vida libre de otras especies de ctenomyidos monitoreadas mediante radiotelegrafía, esta tendencia parece variar, lo que genera interesantes preguntas sobre los factores que afectan a los ritmos diarios de actividad en el género *Ctenomys*.

**Key words:** Daily rhythms, telemetry, *Ctenomys*, subterranean rodents.

**Palabras clave:** Ritmos diarios, telemetría, *Ctenomys*, roedores subterráneos.

**Cite as:** Amaya, J. P., P. M. Lopez, P. A. Cuello, E. A. Lacey & V. S. Valentinuzzi. 2022. Daily activity patterns in free-living tuco-tucos (Rodentia: Ctenomyidae) from Anillaco, La Rioja Province, Argentina. *Mastozoología Neotropical*, 29(2):e0716. <https://doi.org/10.31687/saremMN.22.29.2.01.e0716>

## INTRODUCTION

In many mammals, activity tends to be temporally organized, with species often classified as diurnal, nocturnal, crepuscular, or ultradian based on the portion of the light-dark cycle when most activity occurs (Doyle & Menaker 2007). Determining when during the 24-hour cycle an animal is active can generate important insights into multiple aspects of its biology. For example, the timing of activity can affect the thermal conditions to which an individual is exposed (Hut et al. 2012), the food resources that it consumes (Tyler et al. 2016), and the predation risks that it experiences (Lima & Bednekoff 1999). Accordingly, characterizing temporal activity patterns can improve understanding of the physiological and ecological challenges faced by members of a population (Jannetti et al. 2019). Extending these analyses to include comparative data from multiple populations or species can in turn reveal more general relationships between environmental conditions and activity patterns, such as factors contributing to differences between predominantly diurnal and predominantly nocturnal taxa (Halle & Stenseth 2000).

Tuco-tucos are subterranean rodents in the genus *Ctenomys*. This speciose clade (> 60 named forms) occurs throughout the southern half of South America (Bidau 2015). All species are adapted to hypogeous life, although individuals routinely emerge above ground to eject soil from their tunnels and to forage on surface-growing vegetation (Tomotani et al. 2012). To date, few studies of daily activity patterns have been completed for members of this genus. However, available data suggest that these patterns vary among species, possibly related to differences in social organization. For example, radiotelemetry studies of a free-living population of *C. sociabilis* have revealed that members of this group-living species leave their nests only during daylight hours (Lacey et al. 1997). Similarly, telemetry studies of a group-living population of *C. opimus* indicate that these animals are markedly diurnal in terms of activity outside the nest (O'Brien et al. 2020). In con-

trast, telemetry data from a free-living population of solitary *C. rionegrensis* revealed no significant difference in the frequency with which individuals were encountered outside their nests during the daytime versus the nighttime (Estevan et al. 2016). Although telemetry studies on a free-living population of solitary *C. talarum* also indicate a tendency toward diurnality – in this case based on distance traveled between hourly radio fixes – activity was evident during all phases of the 24-hour cycle (Cutrera et al. 2006). These interspecific differences in activity patterns suggest that comparative studies of *Ctenomys* will prove valuable in elucidating social and other factors that shape variation in daily activity patterns.

The Velasco tuco-tuco (*Ctenomys famosus*) occurs in the Argentine province of La Rioja, where it occupies xeric habitats at the base of the Sierra del Velasco Mountains. The daily activity patterns of these animals have been studied extensively both in the laboratory and in semi-natural enclosures. In the laboratory, individuals exhibit a distinct pattern of nocturnal wheel-running behavior when housed under a standard 12:12 light-dark cycle (Valentinuzzi et al. 2009). Gross motor activity measured under the same conditions using intra-abdominal transponders also reveals a tendency toward nocturnality, although this pattern is less pronounced than for wheel running (Tachinardi et al. 2014). In contrast, studies of animals housed in semi-natural enclosures and monitored by both direct visual observations (Tomotani et al. 2012) and light-logger collars (Flôres et al. 2016) show substantial aboveground activity during daylight hours. The use of accelerometers to monitor individuals in these enclosures has revealed bouts of activity both during the day and the night; the majority (77%) of such bouts occur during the day (Jannetti et al. 2019). This is the only *Ctenomys* species where activity patterns have been examined in detail using multiple methods. However, to date, activity has not been characterized for free-living Velasco tuco-tucos. As a result, despite the comparatively extensive understanding of daily activity in these animals, it is not yet possible to evaluate the activity of free-living members of this species.

As part of efforts to characterize the social organization of the Velasco tuco-tuco, we documented the locations of free-living individuals at regular intervals throughout the 24-hour cycle using radiotelemetry. Specifically, we used the same metrics applied to other natural populations of *Ctenomys* to (1) characterize the daily activity of this species, focusing on below-ground aspects of behavior and (2) examine potential differences in activity related to basic life history parameters such as sex and reproductive status. Our analyses not only provide the first quantitative description of daily activity in free-living Velasco tuco-tucos, but also allow for direct comparisons of activity patterns in this and other *Ctenomys* species. Although Velasco tuco-tucos are not social, neither are they strictly solitary (Amaya et al. 2021), and thus studies of these animals should generate important insights into relationships between social organization and temporal patterning of activity within the *Ctenomyidae*.

## MATERIALS AND METHODS

### Study site and taxon

The population of tuco-tucos studied was located on the eastern outskirts of Anillaco, La Rioja Province, Argentina (26° 48' S; 66° 56' W; 1.445 m.a.s.l.). Anillaco is located within the Monte desert biome in west-central Argentina (Abraham et al. 2009). The climate in this region is arid; average annual rainfall is 100–200 mm, with precipitation occurring almost exclusively during the austral summer (December to February) (Abraham et al. 2009). The study site consisted of an approximately 2-ha portion of an abandoned agricultural field. The soil at the study site was sandy and the vegetation was comprised of a mixture of typical Monte plants (e.g., *Zygophyllaceae*, *Fabaceae* and *Cactaceae* families, Fracchia et al. 2011) and invasive grasses (e.g., *Eragrostis cilianensis*, *Bouteloua aristoides* and *Portulaca* sp.). The site was surrounded by extensive fields of grapes, walnuts, and olives, all of which were occupied by tuco-tucos.

Although the tuco-tucos in Anillaco have frequently been reported as *Ctenomys* cf. *knighti* (Valentinuzzi et al. 2009; Fracchia et al. 2011) or *C.* aff. *knighti* (Tomotani et al. 2012; Tachinardi et al. 2014; Flôres et al. 2016; Jannetti et al. 2019; Yassumoto et al. 2019), a recent analysis based on cranial morphology and mitochondrial *cyt-b* locus sequence data indicates that these animals are part of Velasco sub-clade of *Ctenomys famosus* (Tammone et al. 2022). Accordingly, we refer to the study population as *C. famosus*, but note that previous studies of activity patterns and social organization in these animals indicate the affiliation with *C. knighti*.

### Animal capture and marking

All procedures involving live animals followed guidelines of the American Society of Mammalogists guidelines for the use of wild mammals in research (Sikes et al. 2016).

Individuals were captured using PVC tube traps as described by Amaya et al. (2021). Traps were set by opening the burrow entrance beneath a fresh mound of soil and positioning the trap horizontally along the floor of the tunnel leading to that entrance. Traps were checked every two hours and cleaned and repositioned as needed. Traps that had been plugged with soil were considered indicative of the presence of uncaught animals. Captured tuco-tucos were removed as soon as they were detected. These individuals were transported to the Chronobiology Laboratory at the *Centro Regional de Investigaciones Científicas y Transferencia Tecnológica La Rioja* (CRILAR), located approximately 1 km from the study area. In the laboratory, the sex of each animal was determined. It was then weighed (OHAUS CS series scale, precision  $\pm 1$  g) and permanently marked with a PIT tag (Radiofrequency Identification Microchip; Allflex P/N 86005-001; 12 mm x 2.1 mm; 0.06 g) inserted under the skin in the neck area. Subsequently, the PIT tags were read using a handheld scanner (Global Scan Allflex RFID Reader). The relative age (juvenile or adult) of each animal was determined based on body weight, with males weighing  $> 160$  g and females weighing  $> 108$  g identified as adults (see also Jannetti et al. 2019); in contrast, the mean body weight of individuals identified as juveniles was  $19.7 \pm 2.6$  (range = 17 to 22, N = 4 animals). The reproductive status of adult females was determined by visual inspection of the external genitalia (breeding versus non-breeding) and mammae (lactating) as well as palpation of the abdomen (pregnant). Because the testes of adult males are never externally detectable, the reproductive status of this sex could not be determined. Following these procedures, all adults were fitted with radio-collars as described below.

### Measuring daily activity in the field

Radiotelemetry was used to characterize daily activity patterns among members of the study population. Telemetry data were collected from 5 to 15 December 2015 as part of analyses of the social organization of the study population (Amaya et al. 2021). These dates fall within the annual breeding season for these animals (Amaya et al. 2021). This portion of the year was chosen for analysis to increase the comparability of our results with those of other studies of *Ctenomys* where activity during the annual breeding period was monitored by telemetry (e.g., Lacey et al. 1997; O'Brien et al. 2020). All captured adults were fitted with radio-collars (G3-1V transmitters; AVM Instruments, Colfax, California) that weighed approximately 5 g, which represented less than 4.5% of the minimum body weight for adults in the study population. The collared individuals were kept in the laboratory for up to 12 hours to determine if other individuals were resident in the same burrow system (Lacey et al. 1997). Thereafter, each animal was released at the burrow entrance where it had been captured.

Typically, telemetric monitoring of radio-collared individuals did not begin until  $> 24$  hours after an animal had been released. Animals were located using a handheld 3-element Yagi antenna and a LA12-Q receiver (AVM Instruments, Livermore, California). The location of each animal was recorded to the nearest 0.5 m using a grid (8 x 8 m grid cell size) established on the study site and labeled with a Cartesian coordinate system. Radio-collars placed at known locations revealed the accuracy of this procedure was  $< 0.5$  m (Lacey et al. 1997; Urrejola et al. 2005). In

general, radio fixes were recorded hourly during daylight hours. To characterize daily patterns of activity (the focus of this study), we recorded hourly fixes for 72 consecutive hours from 8 December (16:00 hrs) to 11 December (15:00 hrs). Once data collection ended, the study animals were recaptured and their radio-collars were removed.

Using the radio fixes obtained during the 72-hour data collection period, we examined the activity patterns of all adults monitored. The following four metrics were used to characterize daily activity patterns of members of the study population:

1- *Presence in nest.* Previous analyses of space use by members of the study population have revealed that individuals spend approximately 50% of radio fixes at the same 1-2 locations within their burrow systems, which have been interpreted as putative nest sites (Amaya et al. 2021). The use of more than one nest per 24-hour cycle has been reported for other subterranean rodents (Šklíba et al. 2009); accordingly, for animals characterized by two most frequently used locations, we treated both locations as putative nest sites. At each hourly fix during the focal 72-hour monitoring period, we compared the location of each animal to the location of its nest(s). This information was used to calculate the percentage of fixes during which an animal was located in its putative nest(s). To account for the possible movement of individuals within a nest (O'Brien et al. 2020), we used a conservative error estimate for these analyses; all fixes occurring within 1.5 m of a nest site were considered to be within the nest.

2- *Change in location.* Beginning with the second radio fix recorded during the 72-hour monitoring period, each hourly data point was used to determine whether an animal had changed location since the previous radio fix. Again, a conservative error estimate was used such that fixes occurring within 1.5 m of each other were treated as the same location; no change in location was recorded under these circumstances. These data were used to determine the percentage of inter-fix intervals per individual that included a change in location.

3- *Distance between fixes.* For each radio-collared animal, the distance (m) between each pair of successive radio fixes was calculated. Distances were determined by applying the Pythagorean theorem to the x and y coordinates for successive fixes. To standardize these values, we divided each distance by the maximum distance between successive radio fixes recorded for that individual.

4- *Size of the area used.* To estimate the size of the area used by each animal during the daytime versus the nighttime, individual home ranges were constructed using 95% minimum convex polygons (MCPs), as implemented in the adehabitat HR package in R (Calenge 2006). Home ranges were based on radio fixes taken during the 72-hour observation period; 95% MCPs were used to reduce the effects of outlier locations on estimates of the size of the areas used by radio collared individuals (Girard et al. 2002; Linders et al. 2004).

## Diurnality index

For each of the above metrics of activity, a diurnality index value ( $I_D$ ) (Hoogenboom et al. 1984; Daan et al. 2011) was calculated for each individual monitored using the following equation:

$$I_D = \left[ \frac{\frac{\sum C_L}{h_L}}{\frac{\sum C_L}{h_L} + \frac{\sum C_D}{h_D}} \right] * 2 - 1$$

where  $C_L$  and  $C_D$  are the values of the metric in question during the light (daytime) and dark (nighttime) phases of data collection, respectively, and  $h_L$  and  $h_D$  are the number of hourly data points recorded during the light and dark phases (see below). The part of the equation outside of the brackets is used to convert percentages into an index with values that range from 1 (exclusively diurnal activity) to -1 (exclusively nocturnal activity); these values are not affected by variation in the total number of data points per individual or by changes in photoperiod, making this index suitable for comparisons of activity patterns in free-living animals (Halle & Stenseth 1994). For the categorical variables considered (presence in nest, change in location), a value of one (1) indicated that an individual was outside of its nest or had changed locations since the previous fix. In contrast, a value of zero (0) indicated that an individual was inside its nest or had not moved since the previous fix. For the continuous variables studied (distance between fixes, size of area used),  $I_D$  was estimated using the exact values obtained.

## Data analysis

During data collection, sunrise occurred at approximately 06:00, and sunset occurred at approximately 20:30 (<http://salidaypuestadelso.com/>). Accordingly, radio fixes collected from 06:00 to 20:00 were considered daytime data points, while data collected from 21:00 to 05:00 were considered nighttime data points. For each metric of activity studied, we calculated either the sum (categorical variables: presence in nest, change of location) or the mean (continuous variables: distance between fixes, size of area used) for each animal, with different values calculated for each daytime and nighttime sampling period ( $N = 4$  days and 3 nights). Because the number of radio fixes per individual varied between daytime and nighttime samples, values for each metric were standardized by dividing by the total number of fixes collected during the corresponding (i.e., daytime or nighttime) temporal condition.

To determine whether members of the study population were consistently more active during the daytime than nighttime, values for each metric for these portions of the 24-hour cycle were compared using a Wilcoxon Signed Rank Sum Tests. For these analyses, all daytime data for an individual were pooled; likewise, all nighttime data per individual were pooled. To evaluate activity within each 24-hour period, we compared values for each metric during successive nighttime and daytime intervals (e.g., night 1 vs. day 1) using Wilcoxon Signed Rank Sum Test. The first daytime segment of the 72-hour data collection period (day 0) was not included in this comparison because of the limited number of fixes recorded on that day (data collection began at 16:00; see above). To explore potential differences in activity associated with basic life history parameters such as sex and reproductive status, we compared diurnality index values for males versus females for each metric of activity; for females, these metrics were also examined as a function of reproductive status (non-breeding, pregnant, lactating). Index values were compared using Kruskal-Wallis tests. Finally, to assess individual variability



in activity, we calculated the coefficient of variation for each metric for each animal. Throughout the text, means are reported  $\pm$  1 SD. Statistical analyses were performed using InfoStat (Di Rienzo et al. 2016).

## RESULTS

Activity patterns of 17 adults (6 males, 6 non-breeding females, 3 lactating females, 2 pregnant females; Table S1) were monitored by telemetry. No additional activity was detected at the capture locations for these animals while they were held in captivity, suggesting the presence of no more than one adult per burrow system. During data collection, telemetry was interrupted for one hour (23:00 on the first night of sampling) due to heavy rain, resulting in 46 daytime and 26 nighttime fixes per individual for the majority of monitored animals (6 males, 8 females; Table S1). Because the remaining three adult females in our sample were released with radio collars just before data collection began, these animals were not located during the first two fixes of the 72-hour data collection period, resulting in 44 daytime and 26 nighttime fixes for each of these animals (Table S1).

*Presence in nest.* Approximately half (9 of 17 [53%]) of the individuals monitored used two putative nest sites, with the remaining 48% of individuals using just one putative nest. The mean percentage of radio fixes per individual recorded at putative nest(s) was  $48.7 \pm 16.7\%$  ( $N = 17$  individuals). When the data for each individual were pooled by temporal condition (nighttime versus daytime), there was a significant tendency for animals to spend a greater percentage of fixes at nest(s) during the nighttime versus during the daytime (Wilcoxon Signed-Rank Test,  $N = 17$ ,  $Z = 3.43$ , two-tailed  $P < 0.0001$ ) (Fig. 1A) and Table S1). When this metric was examined per 24-hour sampling period, there was a significant tendency for the percentage of fixes in the nest(s) to be greater during each night versus during the associated day (Wilcoxon Signed-Rank Test,  $N = 17$  animals; night 1 vs. day 1,  $Z = 2.82$ , two-tailed  $P = 0.002$ ; night 2 vs. day 2,  $Z = 2.42$ , two-tailed  $P = 0.010$ , and night 3 vs. day 3,  $Z = 2.82$ , two-tailed  $P = 0.001$ ; Fig. 1B).

*Change in location.* The average percentage of interfix intervals in which an individual changed location was  $58.2 \pm 13.8\%$  ( $N = 41.3 \pm 9.8$  intervals per animal,  $N = 17$  animals). When all data from the same temporal condition (nighttime vs. daytime) were pooled for each individual, the percentage of intervals in which an animal changed location was significantly greater during the daytime compared to the nighttime (Wilcoxon Signed-Rank Test,  $N =$

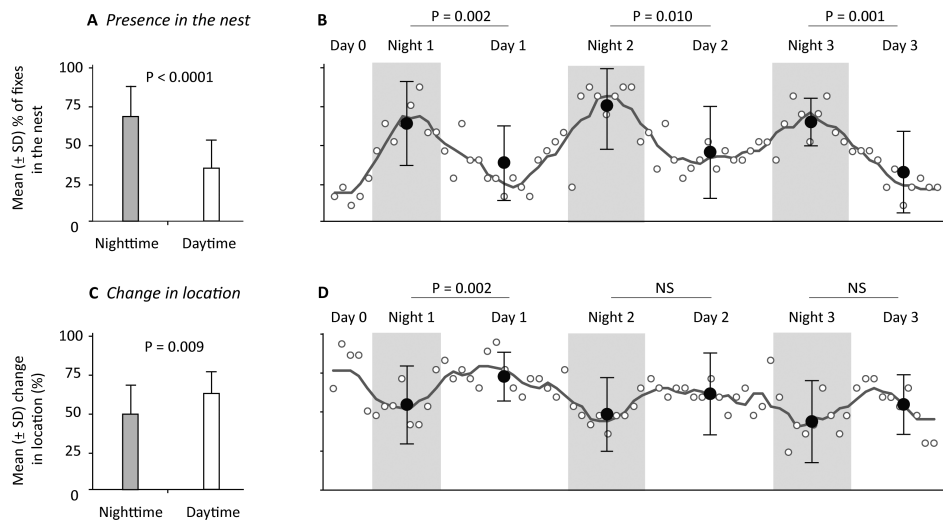
17,  $Z = -2.49$ , two-tailed  $P = 0.009$ ; Fig. 1C and Table S1). When this metric was examined per 24-hour sampling period, there was a significant tendency for the percentage of intervals in which an individual moved to be greater during the daytime in the first 24 hours of data collection (Wilcoxon Signed-Rank Test,  $N = 17$  animals; night 1 vs. day 1,  $Z = -2.51$ , two-tailed  $P = 0.008$ ; Fig. 1D); no significant differences were found for the other two 24-hour sampling periods examined (Wilcoxon Signed-Rank Test,  $N = 17$  animals; night 2 vs. day 2,  $Z = -1.90$ , two-tailed  $P = 0.066$ ; night 3 vs. day 3,  $Z = -1.59$ , two-tailed  $P = 0.119$ ; Fig. 1D).

*Distance between fixes.* When all data from the same temporal condition (nighttime and daytime) were pooled for each animal, there was a significant tendency for the mean standardized distance moved between fixes to be greater during the daytime than the nighttime (Wilcoxon Signed-Rank Test,  $N = 17$  animals,  $Z = -2.67$ , two-tailed  $P = 0.005$ ; Fig. 2A and Table S1). When this metric was examined per 24-hour sampling period, there was a significant tendency for the mean standardized distance traveled by an animal to be greater during each day versus during the associated night (Wilcoxon Signed-Rank Test,  $N = 17$  animals; night 1 vs. day 1,  $Z = -2.22$ , two-tailed  $P = 0.021$ ; night 2 vs. day 2,  $Z = -2.95$ , two-tailed  $P = 0.0002$ , and night 3 vs. day 3,  $Z = -2.27$ , two-tailed  $P = 0.020$ ; Fig. 2B).

*Size of area used.* When the mean sizes of the areas used by an animal during the daytime ( $N = 3$  areas per individual) versus the nighttime ( $N = 3$  areas per individual) were compared, there was a significant tendency for individuals to occupy larger areas during the day (Wilcoxon Signed Rank Sum Test,  $N = 17$  animals,  $Z = -3.62$ , two-tailed  $P < 0.0001$ ; Fig. 2C, Table S1). When this metric was examined per 24-hour sampling period, there was a significant tendency for the area used by an individual to be greater during the day than during the associated night (Wilcoxon Signed-Rank Test,  $N = 17$ ; night 1 vs. day 1,  $Z = -3.20$ , two-tailed  $P = 0.0002$ ; night 2 vs. day 2,  $Z = -3.38$ , two-tailed  $P < 0.0001$ , and night 3 vs. day 3,  $Z = -2.53$ , two-tailed  $P = 0.008$ ; Fig. 2D).

## Diurnality index values

Mean diurnality index values for three of the four metrics of activity examined (change in location, distance moved, size of area used) were positive (Table S2), suggesting an overall tendency among members of the population studied to be more active during the daytime. In contrast, the mean index value for presence in nest was negative (Table S2),



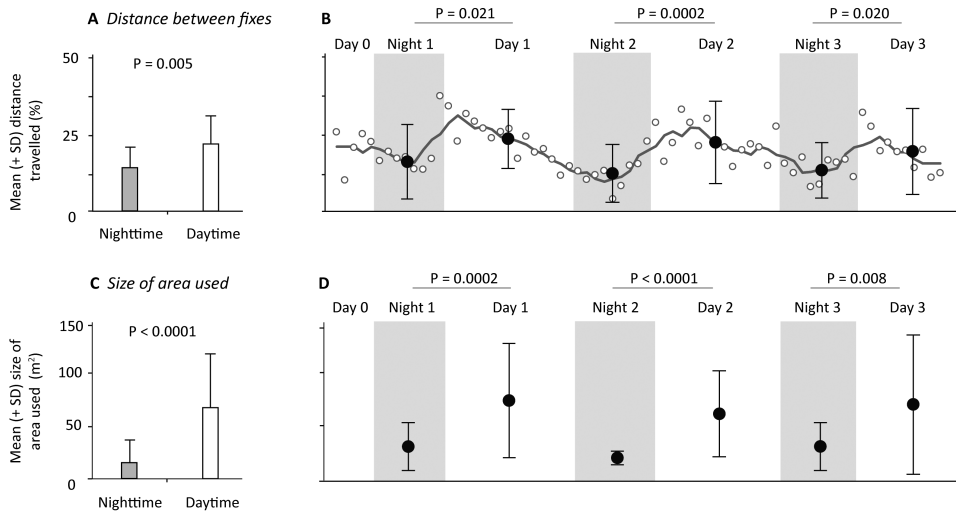
**Fig. 1.** Activity patterns for free-living Velasco tuco-tucos. Data are from 17 adults (6 males, 11 females) monitored hourly by telemetry for 72 consecutive hours. Activity measures presented are (A, B) the mean percentage of fixes during which individuals were detected in their nest(s), and (C, D) the mean percentage of inter-fix intervals during which individuals changed locations. Left panels depict pairwise comparisons (Wilcoxon Signed-Rank Test) of daytime versus nighttime values, with data for each animal pooled by light condition for each animal. The right panels depict activity throughout the 72-hour monitoring period; results of pairwise comparisons (Wilcoxon Signed-Rank Test) of successive nighttime and daytime intervals are indicated. Open circles denote the hourly mean values for each metric. The dark gray line represents the smoothed moving average for the hourly values. Filled circles represent means values  $\pm$  SD for each daytime or nighttime period.

consistent with the tendency of individuals to spend a greater percentage of fixes in the nest during the nighttime (see Fig. 1A). For all activity metrics, index values showed considerable variability at the individual level (Fig. 3). For the two categorical variables examined, index values for presence in nest ranged from  $-0.8$  to  $0.3$  (coefficient of variation =  $41.8$ ), while values for change in location between fixes ranged from  $-0.1$  to  $0.5$  (coefficient of variation =  $13.9$ ; Table S2). No differences between the sexes were observed for these index values (Mann-Whitney U Test, presence in nest,  $U = 47$ ,  $P = 0.499$ ; change in location,  $U = 54$ ,  $P = 0.995$ ) or between females with different reproductive status (Kruskal-Wallis Test, presence in nest,  $H = 1.70$ ,  $P = 0.497$ ; change in location,  $H = 3.35$ ,  $P = 0.226$ ). For the two continuous metrics of activity examined, index values for distance moved between fixes ranged from  $-0.2$  to  $0.4$  (coefficient of variation =  $16.5$ ), while values for size of area used ranged from  $-0.2$  to  $0.9$  (coefficient of variation =  $26.5$ ; Table S2). No differences in index values were found between the sexes (Mann-Whitney U Test, distance moved,  $U = 43$ ,  $P = 0.295$ ; size of area used,  $U = 42$ ,  $P = 0.249$ ). For females, no differences in

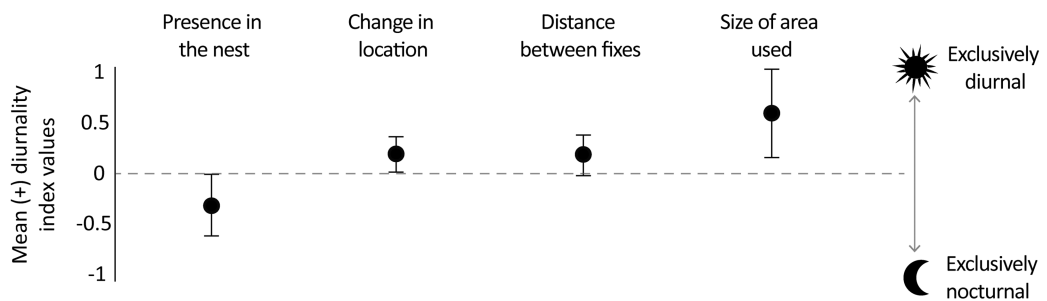
index values were detected for distance moved as a function of reproductive status (Kruskal-Wallis Test,  $H = 0.38$ ,  $P = 0.857$ ). In contrast, size of area used did differ with reproductive status (Kruskal-Wallis Test,  $H = 6.27$ ,  $P = 0.015$ ); pairwise post-hoc tests revealed that diurnality index values for this metric were significantly higher in pregnant than in non-breeding females (Bonferroni,  $P = 0.021$ ).

## DISCUSSION

Our data provide the first characterization of the daily activity patterns of free-living Velasco tuco-tucos. Although members of our study population were active during both daytime and nighttime, each of the four parameters examined revealed a tendency toward more activity during the day. Specifically, members of the study population were present in their nests less frequently, changed locations more often, moved greater distances, and were active over larger areas during the day compared to the night. Consistent with this, the mean diurnality index values for three of these metrics were positive, indicating greater daytime activity. While the mean index value for presence in the nest was negative,



**Fig. 2.** Activity patterns for free-living Velasco tuco-tucos. Data are from 17 adults (6 males, 11 females) monitored hourly by telemetry for 72 consecutive hours. Activity measures presented are (A, B) the mean standardized distance travelled between successive radio fixes and (C, D) the mean size of areas used by individuals (95% MCPs). Left panels depict pairwise comparisons (Wilcoxon Signed-Rank Test) of daytime versus nighttime values, with data for each individual pooled by light condition for each animal. Right panels depict activity throughout the continuous 72-hour monitoring period; results of pairwise comparisons (Wilcoxon Signed-Rank Test) of successive nighttime and daytime intervals are indicated. Open circles denote the hourly mean value for the metric. The dark gray line depicts the smoothed average for the hourly values. Filled circles are mean values  $\pm$  SD for each daytime or nighttime period.



**Fig. 3.** Mean (+ SD) of individual diurnality index values calculated for 17 free-living Velasco tuco-tucos (6 males, 11 females) monitored hourly for 72 consecutive hours. Separate index values were calculated for each of the four metrics used to assess the daily activity patterns of these animals. Values greater than zero indicate a tendency toward diurnality, while values less than zero indicate a tendency toward nocturnality.

this outcome indicates greater use of nest sites during the night, consistent with reduced activity during this portion of the 24-hour cycle. Although

variability in diurnality index values was evident at the individual level, this variation was not associated with differences in sex, and index values for only one

metric (size of area used) varied with differences in female reproductive status.

A similar tendency toward greater diurnal activity has been reported for Velasco tuco-tucos housed in outdoor semi-natural enclosures and monitored using direct visual observations (Tomotani et al. 2012) as well as with light-logger collars (Flóres et al. 2016). Jannetti et al. (2019) used accelerometers on animals housed in the same enclosures. This more sensitive method of measuring movement revealed that although individuals were active both during day and night, most activity occurred during the daytime. More recently, the use of accelerometers to monitor free-living individuals has revealed that the intensity of activity also varies throughout the 24-hour cycle, with periods of most intense activity showing a clear diurnal pattern (Silvério 2022). Overall, these analyses confirm our finding that free-living Velasco tuco-tucos tend to be diurnal, while providing critical information about the temporal patterning of aspects of behavior not observed in this study. These results also raise interesting questions regarding the highly nocturnal pattern of wheel-running that has been reported for captive Velasco tuco-tucos (Valentinuzzi et al. 2009; Tachinardi et al. 2014). Several factors may contribute to differences in the activity patterns of animals living in natural versus laboratory environments, including differences in energetic requirements, lighting and thermal regimes, and perceived risks of being active (Urrejola et al. 2005; Tachinardi et al. 2014, 2017; Jannetti et al. 2019). As a first step toward identifying these factors, we suggest that future studies use the same methods to monitor the activity of free-living and captive conspecifics, thereby minimizing variation arising due to differences in data collection techniques.

In free-living subterranean rodents, activity patterns may be influenced by environmental conditions, particularly differences between conditions at the surface versus below ground. For example, ambient temperatures at the surface are expected to differ from those within burrows due to thermal buffering by the soil (Burda et al. 2007). Previous studies of Velasco tuco-tucos indicate that surface activity is correlated with temperature, with individuals spending more time at the surface when temperatures below ground are colder (Jannetti et al. 2019). Across seasons, a predominantly crepuscular pattern of surface activity during the summer shifts to a more pronounced midday peak during the winter (Jannetti et al. 2019). In general, seasonal differences in surface activity may also reflect temporal changes in food

resource availability or risk of predation (Vassallo et al. 1994). Because our telemetry data only captured information regarding below-ground activity, it is possible that our analyses failed to detect important differences between temporal patterns of surface and subterranean activity in our study population. Studies that employ a range of techniques to document activity both above and below ground as well as studies that track possible seasonal changes in these patterns should be used to clarify the role of environmental conditions in shaping the activity of Velasco tuco-tucos and other subterranean rodents.

*Individual differences in activity patterns.* Although there was an overall tendency for members of our study population to be more active during daylight hours, individual differences in activity patterns were evident. This variation is not unique to Velasco tuco-tucos; pronounced individual-level differences in activity has also been reported for Río Negro tuco-tucos (*C. rionegrensis*; Estevan et al. 2016) and blind mole-rats (*Nannosplax ehrenbergi*; Rado et al. 1993). In our study population, differences in activity patterns were not related to sex and, to the best of our knowledge, no other studies of subterranean rodents have found sex-based differences in activity patterns using metrics similar to those examined here (e.g., Cameron et al. 1988; Cutrera et al. 2006; Šklíba et al. 2007). For females in our study population, reproductive status did not appear to be a consistent correlate of activity, as only diurnality index values for size of area used varied with reproductive status. Our finding that the tendency to use larger areas during the daytime was more pronounced in breeding (pregnant, lactating) females is perhaps not surprising given that reproduction is energetically expensive for female mammals (Perrigo 1987); changing the temporal patterning of space use may serve to reduce these costs or mitigate risks (e.g., predation) associated with activity during some portions of the 24-hour cycle (Perrigo 1987; Gattermann et al. 2008; Tachinardi et al. 2017). Because reproductive status and other phenotypic traits can change seasonally (Zenuto et al. 2001; Tassinio et al. 2011), associated differences in individual activity patterns can vary temporally. Studies that examine the effects of additional phenotypic traits (e.g., age, dispersal history) as well as how those traits change over time should prove valuable in uncovering the causes for individual-level differences in daily activity patterns.

*Comparisons with other subterranean rodents.* Our results suggesting that Velasco tuco-tucos tend to be more active during the daytime are consistent with data from several other subterranean species. For ex-



ample, a general tendency toward diurnality has also been reported for *Spalax ehrenbergi* (Rado et al. 1993), *Spalacopus cyanus* (Urrejola et al. 2005), and *Fukomys anelli* (Šklíba et al. 2014). In contrast, subterranean species such as *Geomys attwateri* (Cameron et al. 1988), *Heliophobius argenteocinereus* (Šklíba et al. 2007), and *F. damarensis* (Lovegrove 1988) have been described as polyphasic, referring to the tendency of these animals to display multiple bouts of activity during the 24-hour cycle. Finally, at least one species – *F. mechowii* – appears to be nocturnal, with activity concentrated during the nighttime (Lövy et al. 2013). Overall, subterranean rodents exhibit considerable variability in their activity patterns, suggesting that comparative studies of these animals should provide important insights into the causes and consequences of this variability (see also Oosthuizen & Bennett 2022).

Comparisons of tuco-tucos and *S. cyanus* could be particularly informative given that the latter is the only truly subterranean species in the family *Octodontidae*, which is the sister taxon to *Ctenomyidae* (Upham & Patterson 2012; Suárez-Villota et al. 2016). Free living cururos are active primarily during daylight hours (Rezende et al. 2003; Urrejola et al. 2005). However, in captivity, activity is concentrated during the dark phase of the 24-hour cycle (Begall et al. 2002). Although activity patterns for most other octodontids have not been characterized in detail, data from common degus (*Octodon degus*) suggest that this burrow-dwelling (but not truly subterranean) species is also diurnal in the field but can be induced to be nocturnal in the laboratory (Kas et al. 1999; Kenagy et al. 2002; Hagenauer & Lee 2008). Because *Spalacopus*, *Octodon*, and *Ctenomys* share a relatively recent evolutionary history but differ in their degree of specialization for subterranean life, comparative studies of these animals may provide important opportunities to examine relationships among phylogeny, burrow dwelling, and a tendency toward diurnality. At the same time, observations suggesting that these species modify their behavior in captivity allow phylogenetically informed exploration of the proximate cues controlling patterns of daily activity.

*Comparisons with other ctenomyids.* Our finding that free-living Velasco tuco-tucos tend to be more active during the daytime is consistent with data from other ctenomyid species whose activity patterns have been documented in the field using radiotelemetry. Although the specific metrics used to quantify activity vary among studies, telemetry-based analyses of *C. talarum* (Cutrera et al. 2006), *C.*

*rionegrensis* (Estevan et al. 2016), *C. opimus* (O'Brien et al. 2020), and *C. sociabilis* (Lacey et al. 1997) all suggest a tendency toward diurnality for at least some elements of behavior. However, the extent to which activity is biased toward daylight hours appears to differ among these species. Although no direct comparisons have been made, the available information suggests that individuals may be more strictly diurnal in *C. sociabilis* and *C. opimus* than in *C. talarum* or *C. rionegrensis*. While the former two species are group living (Lacey et al. 1997; O'Brien et al. 2020), the latter two are not (Cutrera et al. 2006; Tassinio et al. 2011), raising interesting questions about potential relationships between social organization and temporal activity patterns. *C. sociabilis* and *C. opimus* are also unusual among ctenomyids in that individuals tend to spend a large proportion of time at burrow entrances or above ground (Pearson & Christie 1985; O'Brien et al. 2020). As a result, the tendency for these species to be more diurnal may be mediated by ecological factors (e.g., resource availability, predator pressure: Ebensperger & Blumstein 2006) that affect the adaptive costs and benefits of temporal patterning of daily activity. As the number of ctenomyid species whose activity has been characterized using radiotelemetry continues to increase, it should become increasingly possible to conduct phylogenetically informed tests of the effects of these and other behavioral and ecological variables on interspecific variation in activity patterns of this clade of rodents.

## ACKNOWLEDGMENTS

The authors thank the members of the *Binational Argentina-Brazil Chronobiology Laboratory* (G. Oda, P. Tachinardi, M. Jannetti, G. Improta, J. Silvério, D. Flôres, J. Barros) for their enduring generous support with all aspects of our laboratory activities. This work was supported by the *Agencia Nacional de Promoción Científica y Tecnológica* (grant PICT 2013/2753) and *CONICET-Consejo Nacional de Investigaciones Científicas y Técnicas* (grant PIP-11220120100415CO) to VSV. EAL thanks the Museum of Vertebrate Zoology for funding and logistical support.

## LITERATURE CITED

- ABRAHAM, E. ET AL. 2009. Overview of the geography of the Monte Desert biome (Argentina). *Journal of Arid Environments* 73:144-153. <https://doi.org/10.1016/j.jaridenv.2008.09.028>
- AMAYA, J. P., P. A. CUELLO, V. S. VALENTINUZZI, & E. A. LACEY. 2021. Dynamic spatial overlap in a solitary subterranean rodent: the Anillaco tuco-tuco (*Ctenomys* sp.). *Journal of Mammalogy* 20:1-11. <https://doi.org/10.1093/jmammal/gyab011>
- BEGALL, S., S. DAAN, H. BURD, G. I. F. OVERKAMP. 2002. Activity patterns in a subterranean social rodent, *Spalacopus cyanus* (Octodontidae). *Journal of Mammalogy* 83:153-158. [https://doi.org/10.1644/1545-1542\(2002\)083<0153:apiass>2.0.co;2](https://doi.org/10.1644/1545-1542(2002)083<0153:apiass>2.0.co;2)

- BIDAU, C. J. 2015. Family *Ctenomyidae* Lesson, 1842. Mammals of South America (J. L. Patton, U. F. J. Pardiñas & G. D'Elia, eds.). University of Chicago Press, Chicago, Illinois.
- BURDA, H., R. ŠUMBERA, & S. BEGALL. 2007. Microclimate in burrows of subterranean rodents—revisited. In *Subterranean Rodents*. Springer, Berlin, Heidelberg. [https://doi.org/10.1007/978-3-540-69276-8\\_3](https://doi.org/10.1007/978-3-540-69276-8_3)
- CAMERON, G. N., S. R. SPENCER, B. D. ESHELMAN, L. R. WILLIAMS, & M. J. GREGORY. 1988. Activity and burrow structure of Attwater's pocket gopher (*Geomys attwateri*). *Journal of Mammalogy* 69:667-677. <https://doi.org/10.2307/1381621>
- CALENGE, C. 2006. The package adehabitat for the R software: a tool for the analysis of space and habitat use by animals. *Ecological Modelling* 197:516-519. <https://doi.org/10.1016/j.ecolmodel.2006.03.017>
- CUTRERA, A. P., C. D. ANTINUCHI, M. S. MORA, & A. I. VASSALLO. 2006. Home-range and activity patterns of the South American subterranean rodent *Ctenomys talarum*. *Journal of Mammalogy* 87:1183-1191. <https://doi.org/10.1644/05-MAMM-A-386R1.1>
- DAAN, S. ET AL. 2011. Lab mice in the field: unorthodox daily activity and effects of a dysfunctional circadian clock allele. *Journal of Biological Rhythms* 26:118-129. <https://doi.org/10.1177/0748730410397645>
- DI RIENZO, J. A., F. CASANOVES, M. G. BALZARINI, L. GONZALEZ, M. TABLADA, & C. W. ROBLEDO. 2016. InfoStat versión 2016. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. <http://www.infostat.com.ar>
- DOYLE, S., & M. MENAKER. 2007. Circadian photoreception in vertebrates. *Cold Spring Harbor Laboratory Press* 72:499-508. <https://doi:10.1101/sqb.2007.72.003>
- EBENSPERGER, L. A., & D. T. BLUMSTEIN. 2006. Sociality in New World hystricognath rodents is linked to predators and burrow digging. *Behavioral Ecology* 17:410-418. <https://doi.org/10.1093/beheco/arj048>
- ESTEVEAN, I., E. A. LACEY, & B. TASSINO. 2016. Daily patterns of activity in free-living Rio Negro tuco-tucos (*Ctenomys rionegrensis*). *Mastozoología Neotropical* 23:71-80. <https://doi.org/10.1016/j.mambio.2010.06.002>
- FLÓRES, D. E., M. G. JANNETTI, V. S. VALENTINUZZI, & G. A. ODA. 2016. Entrainment of circadian rhythms to irregular light/dark cycles: a subterranean perspective. *Scientific Reports* 6:1-10. <https://doi.org/10.1038/srep34264>
- FRACCHIA, S., L. KRAPOVICKAS, A. ARANDA-RICKERT, & V. S. VALENTINUZZI. 2011. Dispersal of arbuscular mycorrhizal fungi and dark septate endophytes by *Ctenomys cf. knighti* (Rodentia) in the northern Monte Desert of Argentina. *Journal of Arid Environments* 75:1016-1023. <https://doi.org/10.1016/j.jaridenv.2011.04.034>
- GATTERMANN, R. ET AL. 2008. Golden hamsters are nocturnal in captivity but diurnal in nature. *Biology Letters* 4:253-255. <https://doi.org/10.1098/rsbl.2008.0066>
- GIRARD, I., J. P. OUELLET, R. COURTOIS, C. DUSSAULT, & L. BRETON. 2002. Effects of sampling effort based on GPS telemetry on home-range size estimations. *Journal of Wildlife Management* 66:1290-1300. <https://doi.org/10.2307/3802962>
- HAGENAUER, M. H., & T. M. LEE. 2008. Circadian organization of the diurnal Caviomorph rodent, *Octodon degus*. *Biological Rhythm Research* 39(3):269-289. <https://doi.org/10.1080/09291010701683425>
- HALLE, S., & N. C. STENSETH. 1994. Microtine ultradian rhythm of activity: an evaluation of different hypotheses on the triggering mechanism. *Mammal Review* 24:17-39. <https://doi.org/10.1111/j.1365-2907.1994.tb00132.x>
- HALLE, S., & N. C. STENSETH (EDS.). 2000. Activity patterns in small mammals: an ecological approach. Springer, Berlin. <https://doi.org/10.4098/at.arch.01-37>
- HOOGENBOOM, I., S. DAAN, J. H. DALLINGA, & M. SCHOENMAKERS. 1984. Seasonal change in the daily timing of behavior of the common vole, *Microtus arvalis*. *Oecologia* 6:18-31. <https://doi.org/10.1007/bf00379084>
- HUT, R. A., N. KRONFELD-SCHOR, V. VAN DER VINNE, H. DE LA IGLESIA. 2012. In search of a temporal niche: environmental factors. *Prog Brain Res* 199:281-304. <https://doi.org/10.1016/B978-0-444-59427-3.00017-4>
- JANNETTI, M. G., C. L. BUCK, V. S. VALENTINUZZI, & G. A. ODA. 2019. Day and night in the subterranean: measuring daily activity patterns of subterranean rodents (*Ctenomys* aff. *knighti*) using bio-logging. *Conservation Physiology* 7:oz044. <https://doi.org/10.1093/conphys/coz044>
- KAS, M. J. H., & D. M. EDGAR. 1999. A nonphotic stimulus inverts the diurnal–nocturnal phase preference in *Octodon degus*. *Journal of Neuroscience* 19:328-333. <https://doi.org/10.1523/jneurosci.19-01-00328.1999>
- KENAGY, G. J., R. F. NESPOLO, R. A. VÁSQUEZ, & F. BOZINOVIC. 2002. Daily and seasonal limits of time and temperature to activity of degus. *Revista Chilena de Historia Natural* 75:567-581. <https://doi.org/10.4067/s0716-078x2002000300008>
- LACEY, E. A., S. H. BRAUDE, & J. R. WIECZOREK. 1997. Burrow sharing by colonial tuco-tucos (*Ctenomys sociabilis*). *Journal of Mammalogy* 78:556-562. <https://doi.org/10.2307/1382907>
- LIMA, S. L., & P. A. BEDNEKOFF. 1999. Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. *The American Naturalist* 153:649-659. <https://doi.org/10.1086/303202>
- LINDERS, M. J., J. D. WEST, & W. M. VANDER HAEGEN. 2004. Seasonal variability in the use of space by western gray squirrels in south-central Washington. *Journal of Mammalogy* 85:511-516. <https://doi.org/10.1644/1383950>
- LOVEGROVE, B. G. 1988. Colony size and structure, activity patterns and foraging behavior of a colony of the social mole-rat *Cryptomys damarensis* (Bathyergidae). *Journal of Zoology* 216:391-402. <https://doi.org/10.1111/j.1469-7998.1988.tb02437.x>
- LÖVY, M., J. ŠKLÍBA, & R. ŠUMBERA. 2013. Spatial and temporal activity patterns of the free-living giant mole-rat (*Fukomys mechowii*), the largest social bathyergid. *PLoS One* 8(1):e55357. <https://doi.org/10.1371/journal.pone.0055357>
- O'BRIEN, S. L., M. N. TAMMONE, P. A. CUELLO, & E. A. LACEY. 2020. Facultative sociality in a subterranean rodent, the highland tuco-tuco (*Ctenomys opimus*). *Biological Journal of the Linnean Society* 129:918-930. <https://doi.org/10.1093/biolinnean/blaa011>
- OOSTHUIZEN, M. K., & N. C. BENNETT. 2022. Clocks ticking in the dark: a review of biological rhythms in subterranean African mole-rats. *Frontiers in Ecology and Evolution*. <https://doi.org/10.3389/fevo.2022.878533>
- PEARSON, O. P., & M. I. CHRISTIE. 1985. Los tuco-tucos (genero *Ctenomys*) de los Parques Nacionales Lanín y Nahuel Huapi, Argentina. *Historia Natural* 5:337-343.
- PERRIGO, G. 1987. Breeding and feeding strategies in deer mice and house mice when females are challenged to work for their food. *Animal Behaviour* 35:1298-1316. [https://doi.org/10.1016/S0003-3472\(87\)80002-7](https://doi.org/10.1016/S0003-3472(87)80002-7)
- RADO, R., U. SHANAS, I. ZURI, & J. TERKEL. 1993. Seasonal activity in the blind mole rat (*Spalax ehrenbergi*). *Canadian Journal of Zoology* 71:1733-1737. <https://doi.org/10.1139/z93-245>
- REZENDE E. L., A. CORTÉS, L. D. BACIGALUPE, R. F. NESPOLO, F. BOZINOVIC. 2003. Ambient temperature limits above-ground activity of the subterranean rodent *Spalacopus cyanus*. *Journal of Arid Environments* 55:63-74. [https://doi.org/10.1016/s0140-1963\(02\)00259-8](https://doi.org/10.1016/s0140-1963(02)00259-8)
- SILVÉRIO J. T. 2022. Changes in daily activity patterns throughout the year in free-living tuco-tucos. Master thesis defended in March 2022.
- SIKES, R. S., W. L. GANNON, & THE ANIMAL CARE AND USE COMMITTEE OF THE AMERICAN SOCIETY OF MAMMALOGISTS. 2016. Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *Journal of*

- Mammalogy 97:663-688. <https://doi.org/10.1644/10-MAMM-F-355.1>
- ŠKLÍBA, J., R. ŠUMBERA, W. N. CHITAUKALI, & H. BURDA. 2007. Determinants of daily activity patterns in a free-living Afrotropical solitary subterranean rodent. *Journal of Mammalogy* 88:1009-1016. <https://doi.org/10.1644/06-MAMM-A-235R1.1>
- ŠKLÍBA, J., R. ŠUMBERA, W. N. CHITAUKALI, & H. BURDA. 2009. Home-range dynamics in a solitary subterranean rodent. *Ethology* 115: 217-226. <https://doi.org/10.1111/j.1439-0310.2008.01604.x>
- ŠKLÍBA, J., M. LÖVY, E. HROUZKOVÁ, O. KOTT, J. OKROUHLÍK & R. ŠUMBERA. 2014. Social and environmental influences on daily activity pattern in free-living subterranean rodents: the case of a eusocial bathyergid. *Journal of Biological Rhythms* 29(3):203-214. <https://doi.org/10.1177/0748730414526358>
- SUÁREZ-VILLOTA, E. Y., C. A. GONZÁLEZ-WEVAR, M. H. GALLARDO, R. A. VÁSQUEZ, & E. POULIN. 2016. Filling phylogenetic gaps and the biogeographic relationships of the Octodontidae (Mammalia: Hystricognathi). *Molecular Phylogenetics and Evolution* 105:96-101. <https://doi.org/10.1016/j.ympev.2016.08.015>
- TACHINARDI, P., J. E. W. BICUDO, G. A. ODA, & V. S. VALENTINUZZI. 2014. Rhythmic 24 h variation of core body temperature and locomotor activity in a subterranean rodent (*Ctenomys* aff. *Knighti*), the tuco tuco. *PLoS ONE* 9(1):e85674. <https://doi.org/10.1371/journal.pone.0085674>
- TACHINARDI, P., V. S. VALENTINUZZI, G. A. ODA, & C. L. BUCK. 2017. The interplay of energy balance and daily timing of activity in a subterranean rodent: A laboratory and field approach. *Physiological and Biochemical Zoology* 90:546-552. <https://doi.org/10.1371/journal.pone.0085674>
- TAMMONE, M. N., T. FARIÑAS TORRES, A. A. OJEDA, & M. A. CHEMISQUY. 2022. Disentangling the taxonomic status of *Ctenomys* (*Rodentia: Ctenomyidae*) populations inhabiting Northern areas of La Rioja Province, Argentina. *Mammalia*. <https://doi.org/10.1515/mammalia-2021-0169>
- TASSINO, B., I. ESTEVAN, R. P. GARBERO, P. ALTESOR, & E. A. LACEY. 2011. Space use by Río Negro tuco-tucos (*Ctenomys rionegrensis*): Excursions and spatial overlap. *Mammalian Biology* 76:143-147. <https://doi.org/10.1016/j.mambio.2010.06.002>
- TYLER, N. J., P. GREGORINI, M. C. FORCHHAMMER, K. A. STOKKAN, B. E. VAN OORT, & D. G. HAZLERIGG. 2016. Behavioral timing without clockwork: photoperiod-dependent trade-off between predation hazard and energy balance in an arctic ungulate. *Journal of Biological Rhythms* 31:522-533. <https://doi.org/10.1177/0748730416662778>
- TOMOTANI, B. M., D. E. F. L. FLÔRES, P. TACHINARDI, J. D. PALIZA, G. A. ODA, & V. S. VALENTINUZZI. 2012. Field and laboratory studies provide insights into the meaning of day-time activity in a subterranean rodent (*Ctenomys* aff. *knighti*), the tuco-tuco. *PLoS one* 7(5):e37918. <https://doi.org/10.1371/journal.pone.0037918>
- VASSALLO, A. I., KITTLEIN, M. J., & BUSCH, C. 1994. Owl predation on two sympatric species of tuco-tucos (*Rodentia: Octodontidae*). *Journal of Mammalogy* 75:725-732. <https://doi.org/10.2307/1382522>
- UPHAM, N. S., & B. D. PATTERSON. 2012. Diversification and biogeography of the Neotropical caviomorph lineage Octodontoidea (*Rodentia: Hystricognathi*). *Molecular Phylogenetics and Evolution* 63:417-429. <https://doi.org/10.1016/j.ympev.2012.01.020>
- URREJOLA, D., E. A. LACEY, J. R. WIECZOREK, & L. A. EBENSBERGER. 2005. Daily activity patterns of free-living coruros (*Spalacopus cyanus*). *Journal of Mammalogy* 86:302-308. <https://doi.org/10.1644/BWG-222.1>
- VALENTINUZZI, V. S., G. A. ODA, J. F. ARAÚJO, & M. R. RALPH. 2009. Circadian pattern of wheel-running activity of a South American subterranean rodent (*Ctenomys* aff. *knighti*). *Chronobiology International* 26:14-27. <https://doi.org/10.1080/07420520802686331>
- YASSUMOTO, T. I., P. TACHINARDI, G. A. ODA, & V. S. VALENTINUZZI. 2019. Acute effects of light and darkness on the activity and temperature rhythms of a subterranean rodent, the Anillaco tuco-tuco. *Physiology & Behavior* 210:112645. <https://doi.org/10.1016/j.physbeh.2019.112645>
- ZENUTO, R. R., A. I. VASSALLO, & C. BUSCH. 2001. A method for studying social and reproductive behavior of subterranean rodents in captivity. *Acta Theriologica* 46:161-170. <https://doi.org/10.1007/BF03192425>

**Supplemental Material**

**Table S1:** Summary of activity measures used to characterize the daily activity patterns of 17 adult free-living Velasco tuco-tucos (*Ctenomys famosus*) monitored hourly by radiotelemetry for 72 consecutive hours. Sex and body weight are given for each observed animal; reproductive condition is also reported for females. Categorical measures used to quantify activity were the percentage of fixes in nest and the percentage of inter-fix intervals during which an animal changed location. Continuous measures used were the distance from the nest at each fix and the size (95% MCP) of the area used by an individual. Data collected during the same portion of the 24-hour cycle (daytime or nighttime) are pooled for each animal.

	Reproductive status	Weight (g)	# of radio fixes		% of fixes in nest		Change in location (% inter-fix intervals)		Distance between fixes (m)		Size of area used (m <sup>2</sup> )	
			Day	Night	Day	Night	Day	Night	Day	Night	Day	Night
#1 Male	-	193.0	43	29	5.0	64.4	80.8	57.4	32.9	16.1	133.3	24.4
#2 Male	-	187.0	43	29	21.7	73.6	55.0	38.0	14.4	2.6	33.3	2.1
#3 Male	-	164.0	41	29	54.8	81.5	65.0	56.5	17.2	13.6	21.8	14.0
#4 Male	-	228.0	43	29	31.7	59.3	44.2	65.7	19.9	27.0	112.5	0.6
#5 Male	-	160.0	43	29	46.4	25.0	41.7	11.6	7.2	1.3	39.1	35.2
#6 Male	-	166.5	43	29	25.8	75.9	75.0	42.6	31.7	15.7	90.4	2.1
#7 Female	Non-breeding	153.0	41	29	23.9	49.5	47.5	49.5	14.6	16.7	127.7	19.6
#8 Female	Non-breeding	135.0	41	29	23.5	33.8	52.5	77.8	20.6	22.3	2.3	0.2
#9 Female	Non-breeding	120.0	43	29	40.8	79.6	60.0	42.1	21.1	7.6	157.3	73.6
#10 Female	Non-breeding	162.0	43	29	35.9	58.3	65.8	45.8	11.2	5.9	9.5	1.7
#11 Female	Non-breeding	108.0	43	29	25.6	61.1	50.0	42.6	11.5	12.0	16.2	0.0
#12 Female	Non-breeding	146.0	43	29	10.6	64.8	92.5	92.1	38.4	13.4	5.3	0.0
#13 Female	Lactating	156.0	41	29	12.9	72.2	64.2	47.2	38.9	21.2	44.9	7.1
#14 Female	Lactating	148.0	43	29	49.7	92.6	73.3	42.6	19.8	11.3	50.0	23.7
#15 Female	Lactating	135.0	43	29	54.7	92.6	73.3	43.5	22.0	10.3	86.4	40.7
#16 Female	Pregnant	227.0	43	29	71.1	88.0	60.0	27.8	24.1	20.9	104.0	5.8
#17 Female	Pregnant	159.0	43	29	40.8	60.6	66.7	47.7	24.2	16.1	116.6	0.0

**Table S2:** Individual diurnality index values calculated for 17 adult free-living Velasco tuco-tucos (*Ctenomys famosus*) monitored hourly by telemetry for 72 consecutive hours. Sex and body weight are given for each individual monitored; reproductive condition is also reported for females. Separate index values were calculated for each of four activity measures examined for these animals: presence in nest, change in location, distance between fixes, and size of area used.

Sex	Reproductive status	Diurnality indexes				
		Body weight (g)	Presence in nest (% of fixes)	Change in location (% inter-fix intervals)	Distance between fixes	Size of area used (m <sup>2</sup> )
#1 Male	-	193	-0.8	0.2	0.3	0.1
#2 Male	-	160.0	-0.7	0.2	0.0	0.8
#3 Male	-	165.0	-0.1	0.0	0.1	-0.1
#4 Male	-	228.0	-0.5	-0.1	-0.2	-0.2
#5 Male	-	160.0	0.3	0.5	0.2	0.8
#6 Male	-	166.5	-0.4	0.3	0.2	0.9
#7 Female	Non-breeding	153.0	-0.2	0.1	0.1	0.8
#8 Female	Non-breeding	135.0	-0.1	0.0	0.0	0.1
#9 Female	Non-breeding	120.0	-0.3	0.2	0.3	0.1
#10 Female	Non-breeding	162.0	-0.2	0.2	0.4	0.6
#11 Female	Non-breeding	108.0	-0.3	0.0	-0.2	0.5
#12 Female	Non-breeding	146.0	-0.7	0.0	0.4	0.5
#13 Female	Breeding	156.0	-0.7	0.3	0.3	1.0
#14 Female	Breeding	148.0	-0.2	0.3	0.1	1.0
#15 Female	Breeding	135.0	-0.2	0.3	0.4	0.6
#16 Female	Breeding	227.0	-0.1	0.4	0.2	1.0
#17 Female	Breeding	159.0	-0.2	0.1	0.2	1.0
		Mean	-0.3	0.2	0.2	0.6
		SD	0.3	0.2	0.2	0.4
		CV	41.8	13.9	16.5	26.4