

## ACTIVITY PATTERNS IN FREE-LIVING TUCO-TUCOS (*CTENOMYS SP.*)

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*“O sol afundou mais um pouco. As sombras se alongaram, atravessando a caldeira de sal. Linhas de cores vibrantes espalharam-se sobre o horizonte do poente. A cor foi se transformando num dedo de trevas a experimentar a areia. As sombras cor de carvão se alastraram, e o desmoronamento compacto da noite obliterou o deserto.”*

— Frank Herbert, Duna.

# **Agradecimentos**

Gostaria de agradecer a X

## ABSTRACT

## ABSTRACT INGLES

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# Capítulo 1

## Titulo Tese

### 1.1 Introduction

Cyclic environmental changes can impose different restrictions and trade-offs on an animal's physiology and behavior. Light, for example, has a clear 24-hour daily variation, in the form of the day-night cycle. In fact, some animals are morphologically and physiologically adapted to the diurnal or nocturnal way of life (Kronfeld-Schor and Dayan 2003). In terms of physiology, animals have evolved a internal time keeping mechanism, named the circadian clock or circadian oscillator. The circadian clock generates a variety of physiological, metabolic, and behavioral rhythms — such as hormonal rhythms, body temperature rhythms, and activity rhythms (REF). Rhythms driven by the circadian clock, and thus generated endogenously, are referred as circadian rhythms (i.e. circa 24-hour). One main characteristic of the circadian clock is that it is synchronized with environment cycles, this way the circadian clock act as an internal time keeper and maintains a stable temporal relationship between the internal physiology and the external environmental cycle. The presence of circadian clock is thought to enable organisms to anticipate daily environmental challenges (Jabbur, Zhao, and Johnson 2021; Pittendrigh 1993), so changes in physiology and behavior occurs in anticipation to predictable environmental changes. The reactive response to environmental challenges, however, is also important in shaping activity rhythms.

Animals are constantly interacting with multiple environmental cycles or non-cyclic challenges and, not surprisingly, can also respond acutely to these external stimulus, being inhibited or stimulated. Light have both effects, it is the main synchronizer of the circadian clock and can also inhibit nocturnal animals' activity. Consequently, in nature, the observed circadian rhythm, such as the activity rhythm, is an outcome of the influence and interaction of these two mechanisms: the circadian clock, driving endogenous rhythms, and from direct response to environmental stimulus, modifying existing circadian rhythms.

Circadian clock and biological rhythms studies have been majorly conducted in laboratory conditions, which is a powerful approach that enabled the characterization of the clock and its mechanism of synchronization. Studies on circadian rhythms generally relied heavily on manipulating the light-dark cycle and controlling for other variables to investigate synchronization with the light-dark cycle. However, there are limitations to laboratory approaches, specially when we want to extrapolate what we learned in the laboratory to an ecological context. In fact, the ecological significance of circadian rhythms still lacks understanding (Enright 1970; Halle and Stenseth 2000; Helm et al. 2017). In nature, animals are exposed to multiple cyclic and non-cyclic environmental challenges, including biotic factors such as predation, competition, social interaction and food availability; and abiotic factors such as temperature, humidity and light. Consequently, the observed circadian rhythm in nature has significantly more influence from direct responses to external stimulus than in the laboratory, where these effects are often viewed pejoratively (Hut et al. 2012; Kronfeld-Schor and Dayan 2003). For example, interspecific competition between *Dypomys microps* and *Dypomys merriami* appears to drive activity of *D. merriami* to the last part of the night (Kenagy 1973). A more extreme case is exhibited by the golden spiny mouse, *Acomys russatus*, where it completely changes its activity pattern from nocturnal to diurnal in areas where the common spiny mouse, *Acomys cahirinus*, is absent (Kronfeld-Schor and Dayan 2003; Shkolnik 1971). A second aspect that hinders the unders-

tanding significance of circadian rhythms in nature is that the behavior and physiology of laboratory animals might be unrepresentative of wild animals (Calisi and Bentley 2009). A growing number of rodents have completely different activity patterns between laboratory and nature, some species exhibit nocturnal running wheel activity when in the laboratory but field observations reveals diurnal activity in nature, and vice versa (Hut et al. 2012). There is also cases where the same animal transferred from field to the laboratory changes completely changes its activity pattern, as is also the case for *Acomys russatus*. The reasons for this dramatic change in activity patterns is still unknown, it is hypothesised, however, that changes to a more diurnal activity pattern might have energetic advantaged, helping reducing thermoregulatory costs (Hut et al. 2012). There has also been a lot efforts in advancing the studies of chronobiology in the wild and the ecology and adaptive significance of circadian rhythms (Dominoni et al. 2017; Helm et al. 2017; Kronfeld-Schor, Bloch, and Schwartz 2013). Overall, these examples illustrate that it is hard to translate some aspects of chronobiology from the lab to the wild and that more natural studies are still needed to better disentangle the influences and relevance of the endogenous clock versus direct responses to external stimulus in natural conditions.

Circadian activity rhythms studies requires a continuous recording of individual activity for a prolonged time, thus it renders studies in the wild a practical and technological challenge. Recording individual fine-scale activity data on some species of animals is hard, specially because some species are hard to capture, observe or record for long time periods. More recently, however, advancements and miniaturization of animal-borne sensors allowed researchers to investigate fine-scale activity and physiology in free-living animals (Rutz and Hays 2009; Wilmers et al. 2015). These sensors, also known as *biologgers*, have enabled the remote fine-scale measurements of animal data with relatively low disturbance. Two kinds of biologgers widely used in ecological and, more recently, in chronobiological studies are lightloggers and accelerometers. Lightloggers, as

the names imply, records the light exposure of animals, they are often used in migration studies when the timing of light exposure (e.g. going out of nest) is of importance. Accelerometers, in turn, are devices that record animal movement in three dimensions, which can be used to calculate and quantify general activity, energy expenditure and estimate movement paths (Brown et al. 2013). Albeit fine-scale time series data from biologgers is more rich ecologically it also generally required more complex methods and techniques to be analyzed (Brown et al. 2013). One technique used to deal with such type of data is the Hidden Markov Models (HMM). HMMs can be used to uncover state changes in a system, if we think about activity we may want to model the dynamics between rest and active states, for example (Brett T. McClintock et al. 2020). All in all, the possibility of recording individual fine-scale data and the recent statical methods available open a lot of opportunities to study activity rhythms in free-living animals.

The Anillaco tuco-tuco (*Ctenomys* aff *knightii*) is an endemic rodent species from Anillaco, a semi arid region in the north west of Argentina. The genus *Ctenomys* is endemic to South America, distributed in diverse habitats, from deserts to high altitudes (de Freitas, Gonçalves, and Maestri 2021). Species from this genus are subterranean and spend most of their time inside their tunnels, going out of their tunnels to forage and maintain their tunnel systems (de Freitas, Gonçalves, and Maestri 2021). The activity patterns and circadian system of the Anillaco tuco-tuco has been studied in laboratory and semi-natural enclosures. In the laboratory, standard chronobiological experiments showed that their running wheel activity rhythm is driven by the circadian clock and synchronized by the light-dark cycle (Tachinardi et al. 2014; Valentinuzzi et al. 2009). In these controlled settings tuco-tucos are nocturnal, running in the wheel almost exclusively during the night (Valentinuzzi et al. 2009). In semi-natural enclosures, however, direct observation and automated recordings of light exposure shows that the Anillaco tuco-tuco emerges to the surface during the day (Flôres et al. 2016; Tomotani et al. 2012), and hence it is characterized as diurnal, opposed to what is recorded in

the laboratory. In semi-natural enclosures it has also been shown that the surface temperature influences the tuco-tuco's surface emergence. In winter tuco-tucos generally goes out of their tunnels during the middle of the day, in the hottest hours (Jannetti et al. 2019). In the summer, though, tuco-tucos appears to avoid the hottest hours and emerge to the surface in the beginning and end of the day (Flôres et al. 2021). Altogether, laboratory and semi-natural enclosures are crucial to understanding the chronobiology and activity patterns of tuco-tucos the next natural step is to study these animals in the nature. Using available *biologgers* we can now record activity and surface emergence simultaneously in free-living tuco-tuco, which might shed some light into the range of biotic and abiotic factors that might shape tuco-tucos activity and validate the observed surface emergence in semi-natural enclosures.

In the present work our first aim is to develop a field methodology to study free-living tuco-tucos using *biologgers*. Our second aim is to describe the activity and surface emergence patterns in free-living Anillaco tuco-tucos using *biologger*. To do this we deployed animal-borne accelerometers and light loggers on 21 Anillaco tuco-tucos in different months of the year and described their activity and surface emergence patterns. We also used hidden Markov models to further analyse and describe the different behaviors and their change between months of the year.

## 1.2 Methods

### 1.2.1 Study Species

The studied *Ctenomys* population lacks a formal phylogenetic and taxonomic classification but there are some lines of evidence suggesting that the study area is occupied by a single unidentified species (Amaya et al. 2016). In other studies, the studied *Ctenomys*' species has been referred informally as the Anillaco tuco-tuco (Amaya et al. 2016) and as *Ctenomys* aff. *knightii* (Tomotani et al. 2012) or

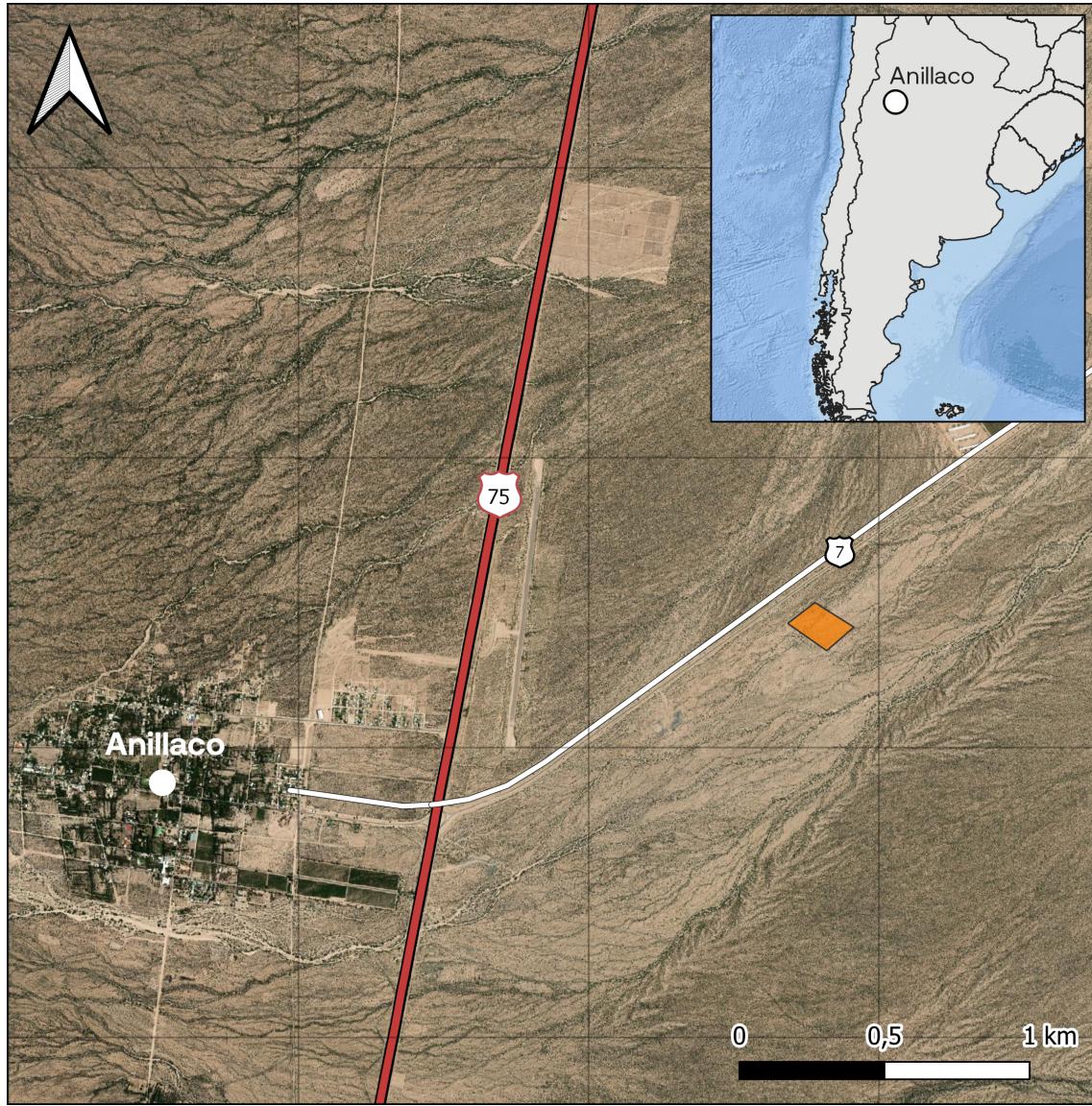
*Ctenomys* cf. *knightii* (Valentinuzzi et al. 2009).

### 1.2.2 Study Site

Field work was conducted at a site located approximately 5km away from the village of Anillaco, in the province of La Rioja, Northwest Argentina. The study site (-66.95°, -028.80, 1325m; Figure 1.1) is a relatively undisturbed natural area, with little human disturbance and no artificial light source, and a area of approximately 75m<sup>2</sup>. The study site is surrounded by the Sierra de Velasco mountain range, located within the Monte Desert biome. The Monte Desert is characterized as an open shrubland dominated by Zygophyllaceae (*Larrea cuneifolia* Cav., *Tricomaria usillo*), Fabaceae (*Prosopis torquata*, *Senna aphylla*) and Cactaceae (*Trichocereus* spp, *Tephrocactus* spp) (Abraham et al. 2009; Fracchia et al. 2011; Aranda-Rickert and Fracchia 2011). At the study site a non-extensive survey of the plant community divided in three transects showed a dominance of the families Zygophyllaceae (*Larrea cuneifolia*, *Tricomaria usillo*), Poaceae (*Microchloa indica*, *Aristida mendocina*) and Fabaceae (*Zuccagnia punctata*) (Figure B.1). The climate is arid with marked daily and seasonal cycles in temperature and rainfall (Figure B.2). The monthly mean temperature ranges from 12°C in the winter months to 23°C in the summer months, with a clear difference in the daily temperature range (Abraham et al. 2009). The mean annual rainfall ranges from 145 to 380mm concentrated almost exclusively in the summer months (Fracchia et al. 2011).

### 1.2.3 Animal Capture and Handling

A total of 47 tuco-tucos were captured between March 2019 and March 2020. Out of these, 30 were part of the present study. Trapping was conducted in four different campaigns to the study site. Three campaigns were done in 2019 during March-April (Autumn), July (Winter) and October (Spring). A fourth campaign was done in February 2020 (Summer). A fifth campaign was planned to occur in May 2020 but had to be canceled due to the COVID-19 outbreak. Tuco-tucos were



*Figura 1.1:* Study site location (orange polygon) at the Monte Desert, approximately 5km away from the village of Anillaco, northwest of Argentina. Study site has an area of approximately 75 m<sup>2</sup>. Geographical information retrieved from ESRI satellites (Datum SIRGAS 2002 UTM 19s).

captured using a custom-made PVC pipe trap (35cm length, 10cm diameter) with a spring-loaded aluminum door at one end and a PVC-lid at the other. Before setting the traps, the study site was scouted for active tuco-tuco's burrows. Active burrows could be identified by the presence of freshly excavated soil mounds at the burrow's entrance. Once found, burrows were excavated to open the access to the underground tunnels and a trap was placed horizontally at the burrow's entrance following the tunnel's orientation. Traps were placed at all active burrows found at the study site, limited to a max of 20 traps available. Traps were set in the field during the morning and checked every 2 hours, when they were reset if they had been plugged with soil or if they had been activated without any tuco-tuco capture. Traps were checked for a last time at dusk and then taken out if no animal had been caught.

After capture, adult tuco-tucos (>120g) were lightly anesthetized in order to be carefully examined and receive a biologging collar. We used a clear plastic anesthesia chamber (318.5cm<sup>3</sup>) with a clip-on lid and a cotton ball affixed inside of the chamber, out of the animal's reach. The cotton ball received approximately 0.5 mL of isoflurane before transferring the animal from the trap to the chamber. While in the chamber animals were observed for breathing, blinking and loss of righting reflex. Once the tuco-tucos could not right themselves they were removed from the chamber. Anesthetized animals were weighted (CSseries, OHAUS, ± 1 g precision), sexed, assessed for reproductive status, marked with a subcutaneous identification PITTag (Passive Integrative Transponder. Allflex, Brasil) and fitted with a collar bearing biologgers (See Biologists).

Animals were released in the same burrow they were originally captured. They were left in the field for 5-18 days before being recaptured for collar recovery. All animal captures, procedures and animal handling were authorized by the local authorities at *Dirección General de Ambiente y Desarrollo Sustentable – Secretaría de Ambiente del Ministerio de Producción y Desarrollo Local – La Rioja, Argentina* (#00501-17). All procedures were also approved by the Ethics Commit-

tee at the *Instituto de Biociências* (#308-2018) and *Faculdade de Medicina Veterinária* (#2045300519) of the *Universidade de São Paulo*.

### **1.2.4 Biologgers**

Accelerometers (Axy-4, TechnoSmart, Italy) and lightloggers (W65, Migrate Technology, UK) were used to record general motor activity and light exposure, respectively. These biologgers were attached to a collar made of a cable tie inserted through silicon tubing [Jannetti et al. (2019); Williams et al. (2014); Figure 1.2]. A telemetry transmitter (SOM-2011. Wildlife Materials, USA) was also attached to the collar to assist in animal location during recapture and minimize sensor's loss. The complete collar setup (accelerometer, lightlogger and telemetry) weighed approximately a total of 6g. Collars without the lightlogger weighted 5.3g. All accelerometers recorded tri-axial acceleration at a 10Hz sampling frequency and 4G sensitivity. Lightloggers were set to sample illuminance every minute but only recorded the maximum sampled value each 5 minutes. The lighlogger possible recording range was 1-19000 lux.

### **1.2.5 Data Processing**

Data were recorded on board of the sensors and later downloaded and converted to raw text files using the software provided by the manufacturers. Acceleration data was used to measure gross motor activity. Tri-axial acceleration data was first reduced to one dimension using the Vectorial Dynamic Body Acceleration (VeDBA, Qasem et al. 2012). VeDBA, measured in gravitational g, is commonly used as a proxy for the animal's activity level and energy expenditure (Wilson et al. 2006; Williams et al. 2016). VeDBA was calculated by: (i) Estimating the effect of the gravitational force over the accelerometer, also known as static acceleration. The static acceleration can be estimated by applying a moving average over the raw acceleration data. There is not a consensus over the number of points used to calculate the moving average, which can be dependent on the study species



*Figura 1.2:* Collar setup and example of field deployment. Upper photo shows the complete collar setup with accelerometer, lightlogger and the telemetry transmitter. Bottom photo shows a tuco-tuco wearing a collar. In the bottom photo it is possible to see the acceleromer and the lightlogger attached to the collar.

and device's recording frequency. In this study we used a 4-second moving average after following the methodology proposed by (Shepard et al. 2008, Figure C.1). (ii) Calculating the acceleration correspondent to the animal's movement, also known as Dynamic Body Acceleration (DBA). The DBA was calculated by subtracting the static acceleration from the raw data. (iii) Lastly, we calculate the VeDBA by the vectorial sum of the DBA over the device's axes (1.1).

$$VeDBA = \sqrt{Xd^2 + Yd^2 + Zd^2} \quad (1.1)$$

Once VeDBA was calculated, the 1Hz acceleration data was downsampled by taking the median over a 1-minute non-overlapping sliding window. All VeDBA datapoints were classified as occurring during the daytime or nighttime based on the daylength of the recording dates. Daylength was calculated using the *maptools* package in R (Bivand and Lewin-Koh 2020), which uses the National Oceanic and Atmospheric Administration (NOAA) equations for estimating twilight times. We used civil twilight times, defined as the times in which the center of the sun is 6° below the horizon, as thresholds to calculate daylength and classify datapoints as occurring during the day or nighttime. Annual daylength variation at the study site can be seen in the Appendix (Figure B.3).

Light exposure was used to analyse patterns of surface emergence, the time tuco-tucos spend on the surface and to further classify VeDBA data points as above or below ground. We classified each 5-minutes data point as being above or below ground using a 2 lux threshold (Jannetti et al. 2019).

Accelerometer and lightlogger data were merged accordingly to the date and times of recordings using purposely written R scripts (R Core Team 2020). Time of recordings between both devices were not synchronized to the minute. Consequently, we had to round lightlogger recording times to the nearest 5 minutes to merge both data streams.

In order to exclude any effects that capture and recapture can have in the animal's activity, we removed the first and last days of all datasets. We also excluded

the data corresponding to the days we were attempting to recapture the animal in cases where the recapture attempts took longer than one day. Animals that had data excluded due to recapturing efforts were FEV05 (5 days), JUL16 (5 days) and JUL23 (2 days).

### **1.2.6 Surface Emergence & Time On Surface**

Visualization of the daily temporal pattern of surface emergence was done using the 5-minute above ground data (see Data Processing). Data points classified as aboveground were treated as presence/absence and used to generate a density histogram with a smoothed kernel density estimate. These visualizations were done individually, for each animal's data, and also for the population level, using the pooled data from all animals of a given month of the year.

Time on surface was calculated presuming that each 5-minute data point classified as aboveground was spent entirely outside the animal's burrow. Accordingly, we deduced the daily time on surface by summing the number of above ground data points multiplied by 5 minutes. Lastly, we also calculated the mean daily percentage of time on surface in relation to the experienced day length for each animal.

### **1.2.7 General Activity**

General activity levels was analyzed using the calculated VeDBA (see Data Processing). We used the mean hourly VeDBA to visualize the temporal patterns in general activity. We also calculated each animal's mean daily VeDBA and the mean daytime VeDBA for month-group comparisons.

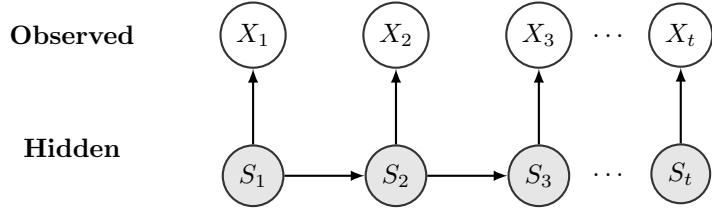
## 1.2.8 Behavioral State Classification

### Hidden Markov Models

We used Hidden Markov Models (HMMs) to further analyze and classify the 1-minute VeDBA data. HMMs are a type of time series model, therefore, they take into account the temporal dependency of the observations (Leos-Barajas et al. 2017). HMMs are also well suited to model accelerometer data given their intrinsic temporal dependency (Leos-Barajas et al. 2017; A. Patterson et al. 2019). HMMs are composed of two time series: the observable *state-dependent process* ( $X_t$ ), VeDBA in our case, and an underlying, or hidden, *state process* ( $S_t$ ). The *state process* is what drives the observations and what we are interested in estimating, which roughly corresponds to behavioral states (Figure 1.3).

The *state process* follows the Markov Property and take temporal dependency into account (Zucchini, Iain MacDonald, and Roland Langrock 2016). The Markov property denotes that a state  $S_t$  depends only on the previous state  $S_{t-1}$  (Zucchini, Iain MacDonald, and Roland Langrock 2016). In the case of accelerometer and animal movement studies the states are representations of the animals' behavior and can take on finite number ( $N$ ) of possible values. The number of states can be chosen *a priori* or based on model selection (Pohle et al. 2017). The changes in probabilities between states are also part of the of HMM formulation, summarized by a Transition Probability Matrix that gives the probability of transitioning from the current state to a possible future state.

In the basic HMM formulation the observable *state-dependent process* comes from a mixture of  $N$  distributions, one for each state. These distributions come from a common distribution family (e.g. Normal, Weibull or Gamma) and each one have their own set of parameter values. The active distribution is determined by the state of the system at a given time  $t$ . Therefore, the observations are a realization from one of these distributions. The distribution parameters, state transition probabilities and other model parameters can be estimated by numerical maximization of the Likelihood (Zucchini, Iain MacDonald, and Roland Lan-



*Figura 1.3: Basic dependence structure for a Hidden Markov Model*

grock 2016). With the model parameters in hand, the most probable state sequence can be found by the Viterbi algorithm (Brett T. McClintock et al. 2020; Zucchini, Iain MacDonald, and Roland Langrock 2016).

### Model Formulation and State Classification

In our models we have chosen VeDBA as our activity metric. We determined *a priori* a possible number of three different states ( $N = 3$ ). This decision was made based on our research question, in the VeDBA distributions (REF Figure ??) and in the biological interpretability of the states. It is important to note, however, that the states do not correspond directly to specific behaviors (e.g. feeding, foraging or digging) but can be assumed to roughly correspond to behavioral states (e.g. activity levels) that can encompass a range of different behaviors (Leos-Barajas et al. 2017; Papastamatiou et al. 2018). We labelled the states as roughly corresponding to “Rest,” “Medium Intensity Activity” and “High Intensity Activity.”

HMMs can be fitted individually (e.g. van de Kerk et al. 2015) or to a pool of animals (Langrock et al. 2012). The models can also include covariate effects that modify either the *state-dependent* distribution parameters or the transition probabilities (T. A. Patterson et al. 2009; Langrock et al. 2012). We fitted a 3-state HMM to the 1-minute VeDBA data using a ‘complete pooling’ approach. This means that the *state-dependent* distribution parameters are common to all animals. Therefore, we assume that individuals are independent and behaviors are the same to all individuals and across the year. However, given that the season/month of the year seems to be an important feature influencing the VeDBA distribution

(Figure ??) we included season as a covariate in the *state process*. Hence, we let the probability of changing from one state to another vary in relation to the season/month of the year. We also fitted an empty model, with no covariate effects, and used Akaike's Information Criteria (AIC) to select the model with best fit to the data (Burnham and Anderson 2002).

Models were fitted using the momentuHMM package in R (Brett T. McClintock and Michelot 2021). We used the gamma distribution, parametrized with mean and standard deviation, to model VeDBA. The gamma distribution is a flexible distribution, that accommodates positive right-skewed data. Appropriate starting values for likelihood maximization of model's parameters were found by following procedures suggested by Michelot and Langrock (2019). Season was included as a categorical variable, its influence over the transition probabilities was summarized using stationary probabilities plots (Leos-Barajas et al. 2017). The most probable state sequence was decoded using the Viterbi algorithm (Zucchini, Iain MacDonald, and Roland Langrock 2016). The decoded sequence was then used to conduct other *post-hoc* analysis of time spent in each state, aboveground activity, diurnality and rhythmicity. We checked model assumptions and goodness of fit by visual inspection of the pseudo-residuals (Zucchini, Iain MacDonald, and Roland Langrock 2016).

### 1.2.9 Aboveground Activity

We also calculated the prevalence of each behavioral state when animal's were aboveground, outside of their tunnels. Above ground activity was defined as any activity in which animals were exposed to light with intensity greater than 2 lux (see Data Processing). Using the state-labelled and light logger data combined we calculated the mean time animals spent per day in each behavioral state when aboveground and the percentage in relation to the total time spent above ground.

### 1.2.10 Diurnality Index

We defined diurnality index (DI) as the percentage of daytime the animals spent in one of the states in relation to the total time spent in the same state during both daytime and night-time, corrected by the daylength of each season Jannetti et al. (2019). The DI ranges from 0 to 1, with 0 meaning that all activity happens during the night and 1 the opposite, that all activity happens during the day.

Diurnality calculation is shown in equation (1.2), where  $ts_{day}$  and  $ts_{night}$  are the time spent in the state during the day and night respectively.  $L_{day}$  and  $L_{night}$  are the day length and night-length, determined by the civil twilight.

$$Diurnality = \frac{ts_{day}/L_{day}}{ts_{day}/L_{day} + ts_{night}/L_{night}} \quad (1.2)$$

### 1.2.11 Circadian Rhythmicity and Period Estimation

We used autocorrelation analysis (Levine et al. 2002; Dowse 2009) and Lomb-Scargle periodograms to assess the robustness and periodicity of activity rhythms. The autocorelation was calculated by comparing the data to itself lagged by a unit of time. The autocorrelation coefficient ranges from 0 to 1 and it is higher as the two time series are more similar to each other. When visually analyzing the autocorrelation plot, recurring peaks indicates that the data is periodic. The height of the peak shows how robust the rhythms is (Dowse 2009). The robustness of the rhythm, also referred as the Rhythmicity Index (RI), is defined as the autocorrelation coefficient at the third peak of the autocorrelation plot (i.e. the height of the third peak).

Before estimating the RI, we applied a 3-hour low-pass Butterworth filter to remove periodicity lower than 3 hours in the data. Autocorrelation plots were first visually analyzed, if they showed recurring peaks in the 24-hour range were labeled as rhythmic. Animals that showed no recurring peaks were classified as

arhythmic in the circadian range. Next, we estimated the period of each behavioral state, for animals that were classified as rhythmic, using the Lomb-Scargle periodogram (Leise 2017). For comparison with the labeled data, we also calculated the RI for the unlabeled VeDBA data

All analysis were done in R (R Core Team 2020). Butterworth filtering was done using the *dlpR* package (Bunn 2008). Autocorrelation function and plots were done in base R. The peaks in the autocorrelation plots were found using the *pracma* package (Hans W. Borchers 2019). Lomb-Scargle periodograms were calculated using the *lomb* package (Ruf 1999).

### 1.2.12 Statistical Analysis

We used ANOVA and post-hoc Tukey's HSD to test for differences between month-groups in time on surface, general activity levels, time spent on behavioral states, time spent on aboveground activity and diurnality. Rhythmicity were compared between states only, we did not perform a month-group comparison given that, as some animals were classified as arrhythmic, the sample number for each season was too low to perform a meaningful statistical analysis. We also visually compared daily patterns of general activity levels, time on surface and behavioral states based on the shape of distributions along the 24-hours. All analysis were done in R using the base packages (R Core Team 2020).

## 1.3 Results

We captured and deployed collars to 20 females and 10 males. We were able to recapture 24 tuco-tucos and recover 21 collars (Table 1.1). One collar was found malfunctioning because one animal got predated. The other two lost collars fell or were taken out of the tuco-tuco's neck between the time of capture and recapture. A complete list of number of recorded days per animal can be seen in the Appendix A.1.

*Tabela 1.1:* Number of captured animals and sensors deployed in the field. There was a higher number of females captured independent of the season. Recapture rates in February 2021 are lower because field work had to be interrupted due to the covid outbreak. Not all recaptured tucos still had their collars. Some collar were taken out by the animals between the time of captured and recaptured. One tuco was predated and the collar was found 1km away from the initial capture burrow malfunctioning.

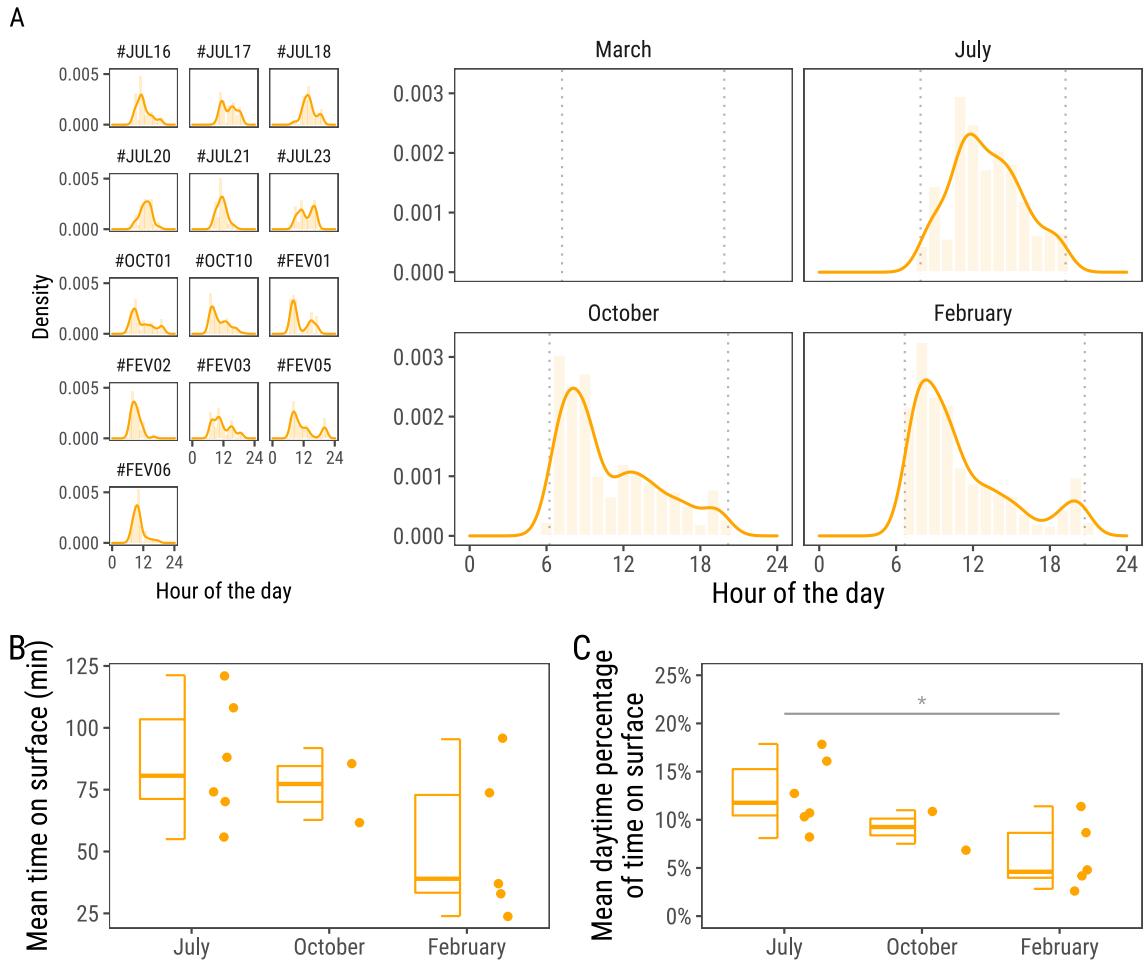
	Captured		Recaptured		Recovered Collars	Accelerometers	Lightloggers
	Males	Females	Males	Females			
February	3	7	2	5	5	5	5
July	4	5	4	5	8	8	6
March	0	2	0	2	2	2	0
October	3	6	1	5	6	6	2

### 1.3.1 Time On Surface

Surface emergence shows a changing temporal pattern along the year. In July, the peak of surface emergence is concentrated in the middle of the day. In other seasons the peak of surface emergence is bimodal, with a higher peak in the first hours of daylight and a smaller peak at the end of daylight (Fig. 1.4A).

Time on surface, measured light exposure, shows no difference along the year. The mean daily time on surface shows no significant differences among month groups (ANOVA,  $F = 2.148$ ,  $p = 0.167$ ), the overall mean and standard deviation of all animals is  $71.98 \pm 29.21$  minutes on the surface during day. In contrast, the daily percentage of the time on surface in relation to day length was significantly different between groups (ANOVA;  $F = 4.38$ ,  $p = 0.0429$ ). Post hoc Tukey's HSD shows a significant difference between July-February ( $p = 0.035$ ; CI = [-0.12; -0.04]). For these groups the mean daily percentage of time on surface is  $13\% \pm$

4% for July, and  $6\% \pm 4\%$  for February.

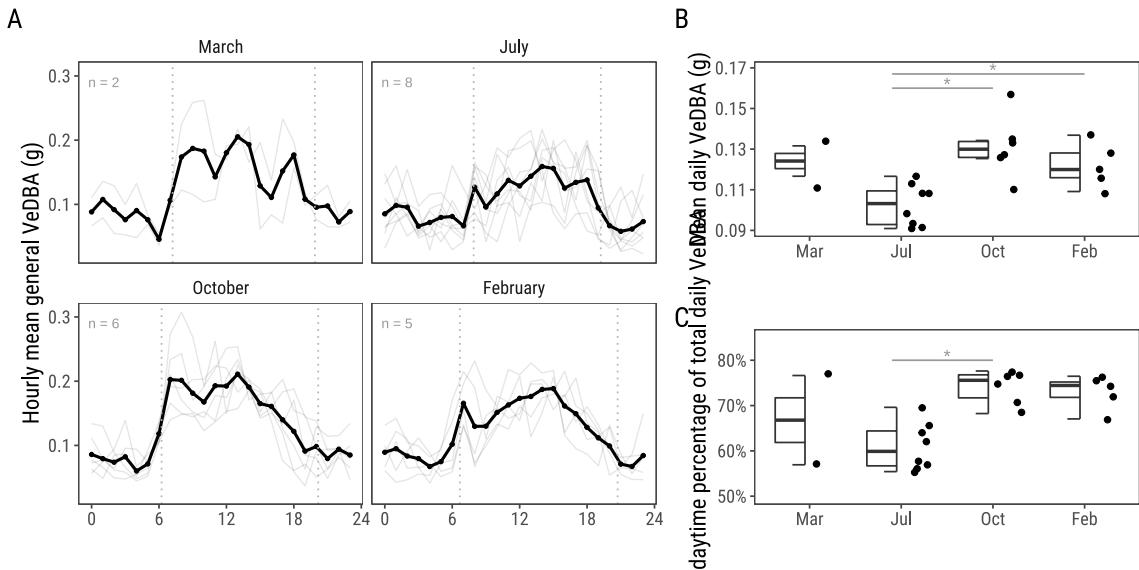


*Figura 1.4:* Tuco-tuco's daily patterns of surface emergence. (A) Density histogram and kernel estimates of surface emergence showing the relative probability of surface emergence. On the left: Individual daily patterns of surface emergence, pooling all daily counts of each animal. On the right: population-level patterns of surface emergence in each month of the year. Population-level is the pooled data for all days of all animals of each month of the year. Solid lines indicate the Gaussian kernel density estimates as a function of time. Light-colored bars shows observed surface emergence binned per hour. X-axis is time of the day in Anillaco, La Rioja (UTC-3). Dotted vertical lines show times of civil twilights. (B) Mean daily time on surface per animal. Each point is an animal's mean daily time on surface (ANOVA;  $p = 0.167$ ). (C) Mean daily percentage of time on surface in relation to the daylength. Each point is an animal's mean daily percentage of time on surface (ANOVA;  $p = 0.043$ ). Asterisks indicates pairwise significant statistical difference (Tukey's HSD;  $p < 0.05$ ).

### 1.3.2 General Activity

Tuco-tuco's general activity follows a typical diurnal pattern. Hourly mean VeDBA is lower during the night time, rises just before dawn, remains generally higher during the daytime and falls just after dusk. The average daily VeDBA is significantly different among month-groups (ANOVA;  $F = 7.182$ ,  $p = 0.002$ ; Figure 1.5B). There is a significant month-group difference between July-October and July-February (Tukey's HSD,  $p < 0.05$ ). In both pairwise comparisons July's average daily VeDBA is lower, with a difference in means of 0.029 g in comparison to October and 0.019 g in comparison to February.

The percentage of daytime VeDBA is also significantly different between months (ANOVA;  $F = 8.288$ ,  $p = 0.001$ ; Figure 1.5C). Differently from the the daily mean VeDBA, however, there is only a significant difference between July-October (Tukey's HSD,  $p < 0.05$ ), with a lower July VeDBA level and mean different of 0.13 g.

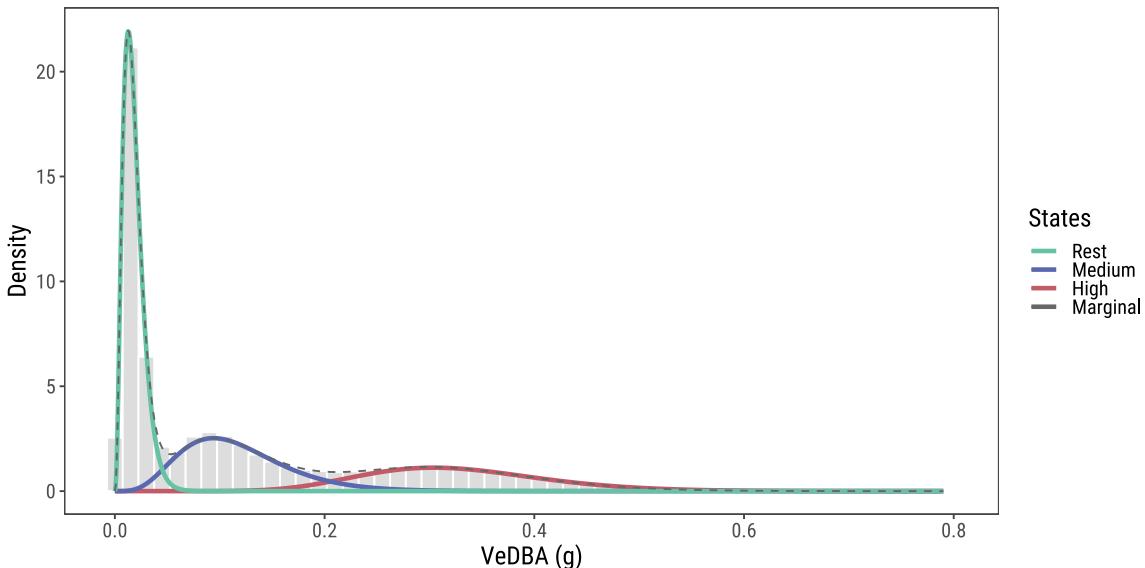


*Figura 1.5:* Tuco-tuco's Daily VeDBA levels. (A) VeDBA was binned by hour (0-23). Background lines show data for individual animals. Thick lines show mean hourly VeDBA. (B) Distribution of mean daily VeDBA. Points show daily VeDBA mean for each animal. In July Tuco-tuco's exhibited lower Daily VeDBA than October and February. (C) Distribution of percentage of VeDBA occurring during the daytime. Points show mean daily percentage of VeDBA during the daytime. During July tuco-tuco's have significant lower VeDBA levels during the day than in comparison to other monthes of the year. Vertical dashed lines shows time of civil dawn and dusk. Asterisks shows significant statistical difference between groups in an ANOVA followed by Tukey-Kramer's Test.

### 1.3.3 Behavioral State Classification

We fitted two different HMMs to VeDBA data, one empty model, with no covariates, and a second one with ‘*season*’ as a covariate in the transition probability matrix. The second model was selected based on informational criterion ( $\Delta\text{AIC} > 2$ ; REF Tabela AIC nos supps).

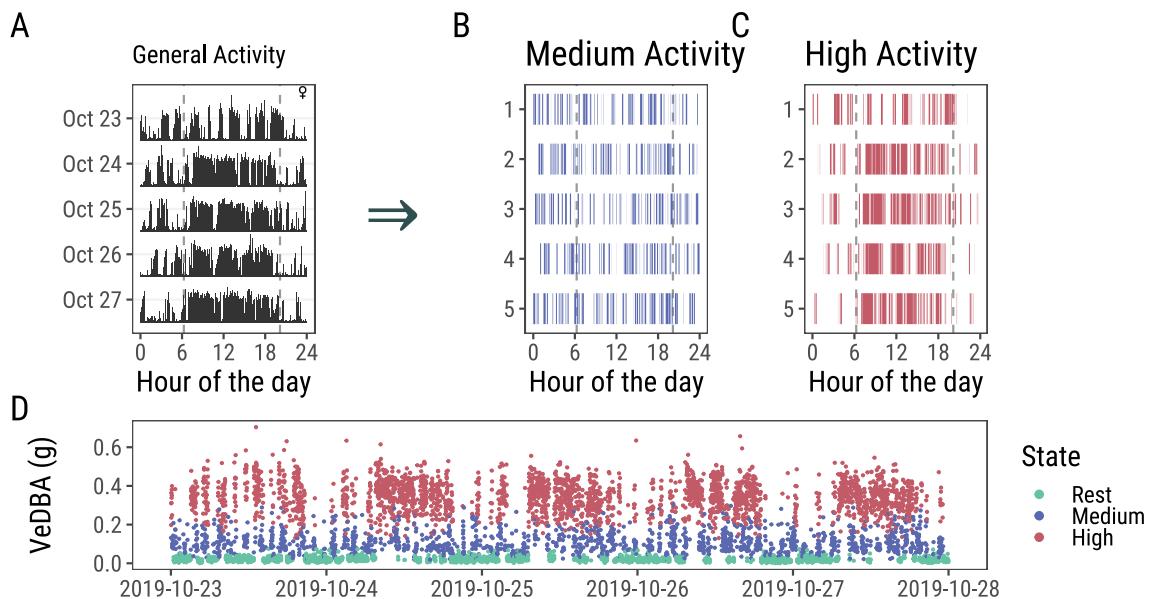
The estimated state-dependent distributions are shown in Figure 1.6. We interpreted and labelled these states as ‘Rest,’ ‘Medium intensity activity,’ and ‘High intensity activity’ corresponding to low, intermediate, and high VeDBA values respectively. The marginal distribution (Figure 1.6; dashed line) has a good correspondence to the empirical VeDBA distribution. A visual analysis of the Pseudo-residuals (Figure D.1) shows that the residuals deviate from the expected normal distribution, especially in the lower end values, and that there is still significant residual autocorrelation. Nevertheless, the overall fitting seems to be reasonable. The estimated state-dependent parameters are shown in the Appendix (Table D.1).



*Figura 1.6:* State-dependent distributions of the selected Hidden Markov model fitted to the VeDBA acceleration metric. Histogram, in grey, shows the empirical VeDBA from the pooled data of 21 Anillaco’s tuco-tuco. State-dependent gamma distributions are shown above the histograms. These distributions are weighted accordingly to the proportion of observations assigned to each state.

With the state-labeled data we were able to dissociate and visualize the daily patterns of each different state. An example of how VeDBA levels and the behavioral states are related is shown in Figure 1.7.

A visual inspection of all individual actograms (Figure E.1) indicates that the high activity state rhythm is more robust than the Medium Activity rhythm. Nonetheless, neither of these behavioral states occurs exclusively during the daytime. The high activity state, despite being more concentrated during the daylight hours, also occurs sporadically during the night. Medium Activity, in turn, is spread throughout the day and night with no clear 24-hour rhythm.

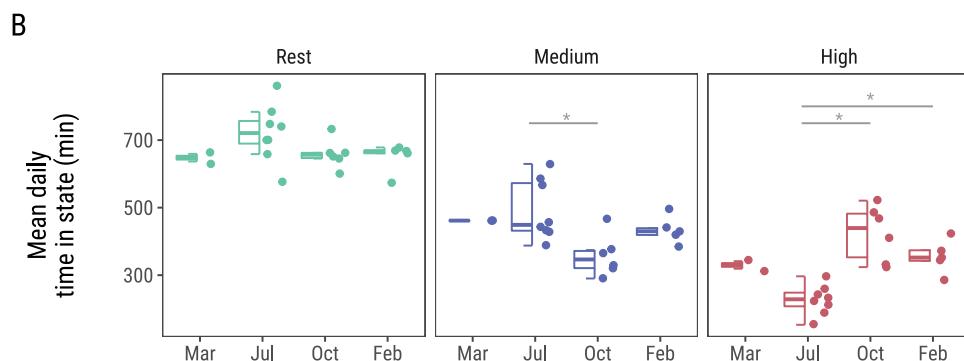
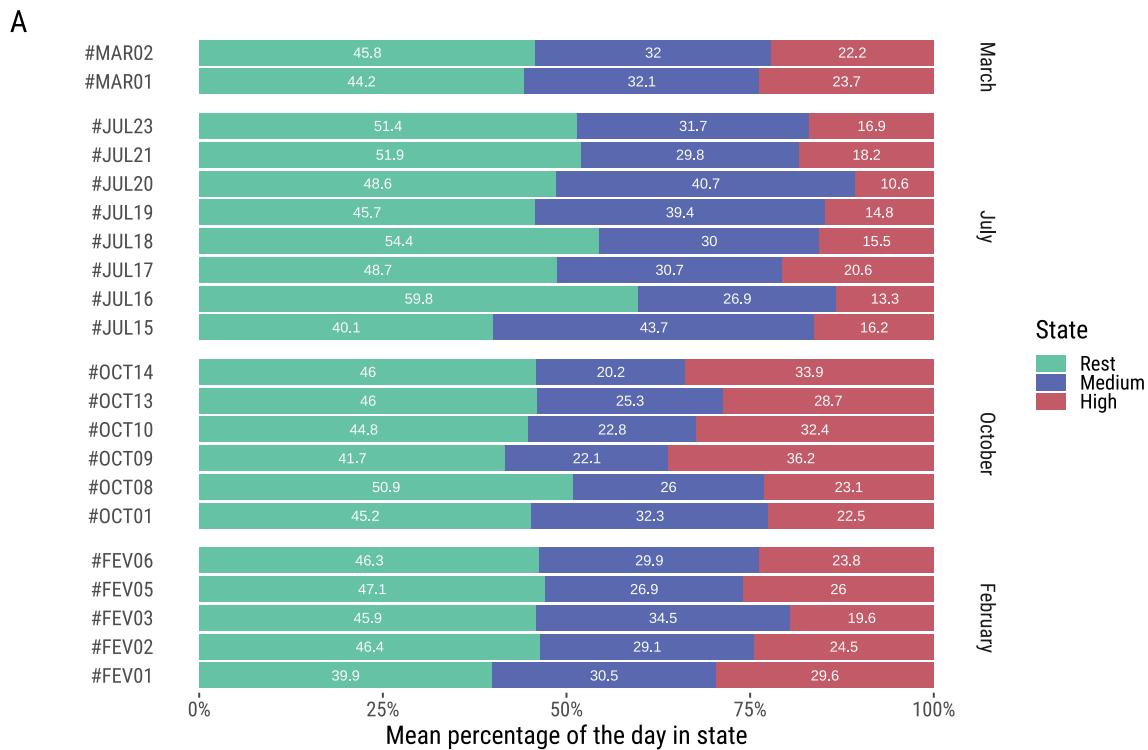


*Figura 1.7: Actograms and Time Series Plot of general activity, measured by VeDBA, and state-labelled data of a representative animal (#OCT09). The actograms shows daily patterns of VeDBA (A) and of Medium and High State occurrences (B and C) classified using HMM. Medium Activity State shows no clear pattern of a daily rhythm, with episodes spread throughout the day. High Activity, on the other hand, has a higher concentration during daylight hours. The time series (D) shows 1-minute VeDBA measures colored by behavioral state. Dashed lines shows time of dawn and dusk.*

### **1.3.4 Daily Time-Activity Budgets**

On average, tuco-tucos spent between 45-50% of the 24 hours in the Rest state, with no statistical difference between the percentage of time spent resting between seasons (ANOVA;  $F = 1.93$ ,  $p = 0.163$ ). The remaining time is spent in an active state, either Medium or High Activity State.

Tuco-tucos spent a variable percentage of their daily active time in one of the two active states, High or Medium, across seasons. Daily time spent in High Activity was lower in July (15.8%) and higher in October (29.4%). In contrast, daily time spent in a Medium Activity State was higher in July (34.1%) and lower in October (24.8%). There is a significant difference in the percentage of time spent in Medium (Figure 1.8; ANOVA:  $F = 4.457$ ,  $p = 0.0175$ ) and High Activity State across seasons (Figure 1.8; ANOVA:  $F = 13.62$ ,  $p = < 0.001$ ). Tukey's post hoc test shows that the mean percentage of time spent in the Medium Activity State is 9% lower in October than in July ( $p = 0.01$ ). For the High Activity State, pairwise Tukey's test shows a significant difference between October-July ( $p < 0.001$ ) and February-July ( $p < 0.01$ ). In comparison to July the mean daily percentage of time spent in a High Activity State is 13% higher in October and 8% higher in February (Figure 1.8).

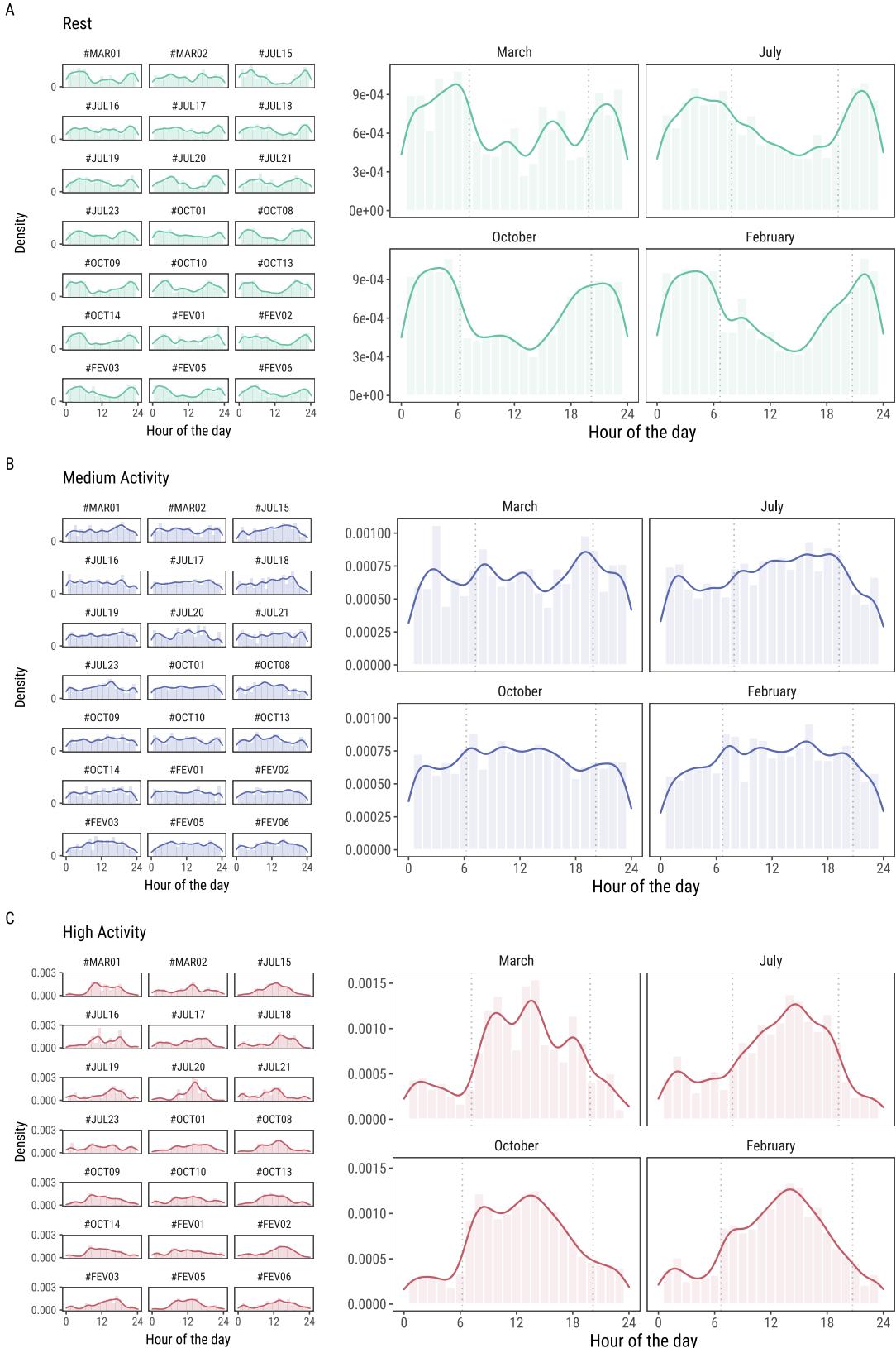


*Figura 1.8:* Daily time-activity budgets for the behavioral states. (A) Percentage of time spent in each behavioral state per animal. (B) Boxplot and individual points of the distribution of the mean percentage of time spent in each behavioral state. Asterisks shows statistical significant pairwise comparison between Months. The mean percentage of time spent in the High Activity State is lower in July in comparison to October and February. The mean percentage of time spent in the Medium Activity State, however, is higher in July in comparison with October.

### 1.3.5 Daily Activity Patterns

Daily activity patterns for each behavioral state are shown in Figure 1.9. These plots show that, qualitatively, the timing of occurrence of High Activity and Light Exposure episodes follow a diurnal pattern. Medium Activity, however, is spread out along the 24h and do not follow a daily (24-hour) rhythm. It is important to note that the timing of peak occurrence of High Activity behavior does not appear to change dramatically along the year. In all four Months the peak of High Activity is around 14:00.

- REF calculate peak
- REF adicionar linha do meio dia solar



*Figura 1.9:* Density estimates of daily activity patterns of tuco-tucos' behavioral states. Solid lines indicate the Gaussian kernel density estimates. Light-colored bars show observed distribution of each behavioral state occurrence. The x-axis is hour of the day in Anillaco, La Rioja (UTC-3). Dotted vertical lines show time of civil twilights. (A) High Activity State shows a diurnal pattern independent of the time of the year. (B) Medium Activity State shows no daily pattern. (C) Daily pattern of light exposure changes according to the season.

### 1.3.6 Aboveground Activity

Outside of their tunnels, when exposed to light, tuco-tucos are mostly in a active state, either high or medium state (Fig. 1.10). The percentage of the high activity state that occurs on the surface is higher than the other activity states. The mean percentage of the high state that occurs aboveground is 0.59, whether for the medium state the mean is 0.32. Rest, in contrast, is the behavioral state that happens the least on the surface with a mean percentage of 0.08.

- aqui ainda falta um teste. Precisa ser um não parametrico ou test-t dois a dois. O ANOVA não cabe nesses dados.
- adicionar estatistica descritiva do tempo

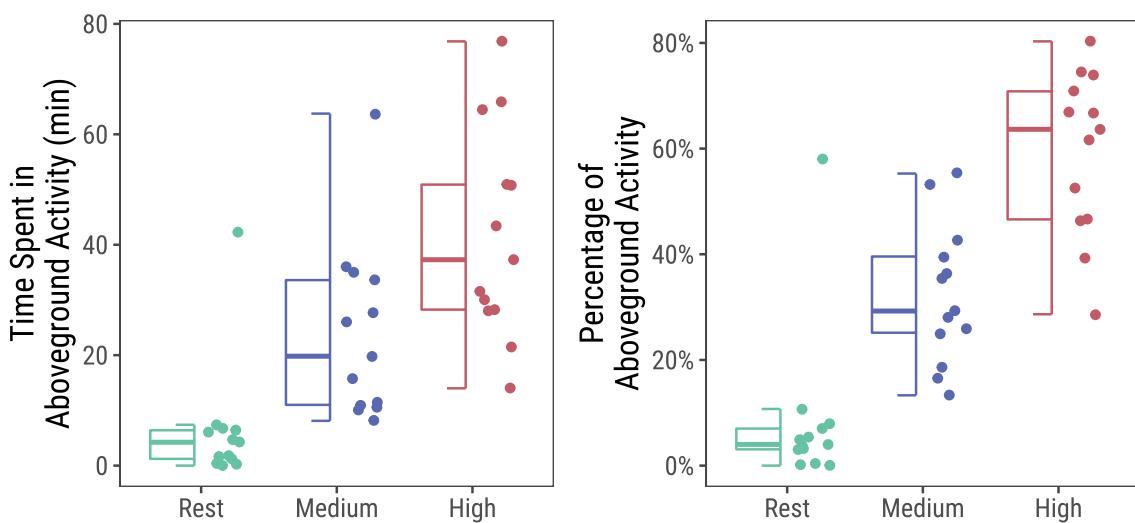


Figura 1.10: Distribution of the percentages of each activity state that happens aboveground during daytime.

### 1.3.7 Diurnality

High Activity State is predominately diurnal ( $DI > 0.5$ ). The average diurnality for the High Activity State is higher than 0.7 for all seasons. The Medium Activity State, however, has a diurnality index that ranges from 0.5 in March to 0.56 in July and February. The Rest State is predominantly nocturnal with Diurnality lower than 0.38 in all seasons. There is no statistical difference between seasons (ANOVA;  $p > 0.2$  for all states; Figure 1.11).

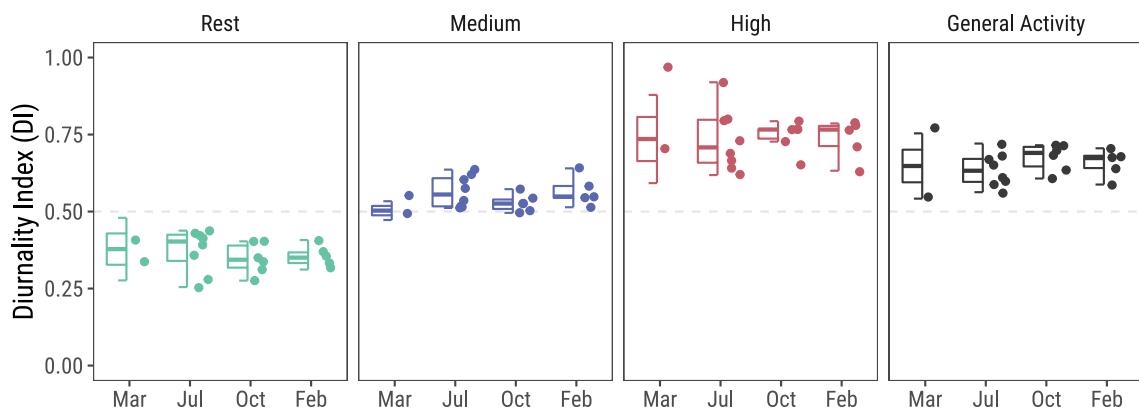


Figura 1.11: Distribution of calculated diurnality index (DI). Between labelled states, only the High Activity State is predominantly diurnal, with diurnality greater than 0.7 across all season. General activity, measured by the unlabelled VeDBA, is also predominantly diurnal,

### 1.3.8 Circadian Rhythmicity

Circadian rhythms are present in all states as seen in the visual analysis of the autocorrelation plots (REF APPENDIX - plotar ACFs individuais). The percentage of animals classified as rhythmic is higher for the high activity state, where a total of 18 animals were classified as rhythmic (85.7%). In contrast, the medium activity state had, in total, 11 animals (52.4%) classified as rhythmic.

We calculated the RI only for animals classified as rhythmic (Figure 1.12A). It is important to note that animals classified as arrhythmic were excluded from this analysis, which is why there is a different number of samples between each category. Nonetheless, the Medium Activity State is significantly different from the other states and VeDBA (Figure 1.12A; ANOVA;  $p < 0.05$ ). We did not test for differences between seasons (See Methods).

Lastly, we used Lomb-Scargle periodograms to estimate the periodicity of each state (Figure 1.12B). All estimated periods, independent of state, were in the 24-hour range. The high activity state has a mean period  $\pm$  standard deviation of  $23.87\text{h} \pm 0.34$ . The medium activity state has a mean period of  $23.91\text{h} \pm 1.22$ .

We also estimated RI and periodicity for VeDBA. In comparison with state-labelled data VeDBA analysis are more similar to the high activity state. The number of animals classified as rhythmic based on the autocorrelation plot is similar between VeDBA and high activity state data. The distribution of RI shows no difference between High state and VeDBA (Figure 1.12 A). For VeDBA the mean estimated period was  $23.91\text{h} \pm 0.31$ .

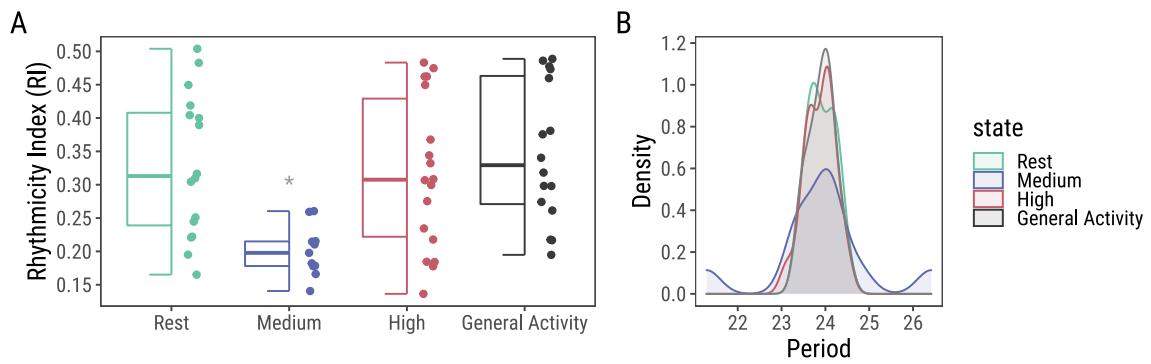


Figura 1.12: Distribution of Rhythmicity Index for state-labelled data and VeDBA. The distribution of the Medium State is statistically different from all other states and VeDBA. Graphs shows half boxplots and individual data

## 1.4 Discussion

- mencionar que tudo que é medido foi a primeira vez em vida livre
- Optamos pelo tipo de modelos mais simples com outras a analises a posteriori. Existem outros métodos interessantes Patterson 2009. Extensions to our model could include (...)
  - selecionamos os fatores mais relevantes baseados nas analises exploratorias
- limitações dos dados de lightlogger: não sabemos se os picos podem se extender durante a noite tbm.
- discutir as semelhancas entre arena e freeliving
  - o padrão é igual, explicar o que foi visto nas arenas
- diferença ritmicidade vedba vs estados na ritmicidade
- falar que nem sempre o HMM é ideal para ritmicidade e diurnalidade
- uma boa mensagem para deixar pro futuro
- reler artigo catemeralidade
  - nesse artigo foi percebido varios bouts de atividades
  - tamiris: while not on the wheel they are doing different things
    - \* não podemos assumir que quando nao está na roda ele está parado
    - \* erkert artigo + voles lehmann (trabalhos de catemeralidade)
    - \* esse ponto é essencial para conectar com o que o HMM fez
    - \* HMM trouxe a tona outros ritmos presentes ao longo de todo dia
      - + o componente circadiano que tbm vemos na roda, p.e.
    - \* tbm falar que não era possível medir -> tecnologias diferentes.
- Deixar os eixos como Atividade Geral (VeDBA)

- colocar apenas na legenda que atividade geral é medida pelo VeDBA
- Erkert, H.G.; Cramer B. 2006. Chronobiological background to cathemerality: circadian rhythms in *Eulemur fulvus albifrons* (Prosimili) and *Aotus azarai boliviensis* (Anthropoidea). *Folia Primatologica* 77: 87–113.
- lehmann
- A atividade “catemera” não era prevista então isso é uma coisa interessante
  - falar que é novo e interessante
  - tudo isso foi medido pq fomos ao campo
  - “qual a relacao entre o circadiano e os ultradianos?”

- 
- os resultados do indice de ritmicidade devem ser interpretados com cuidado já que existe diferença na classificação dos animais em primeiro lugar.
    - Isso principalmente na hora de interpretar o grafico de boxplot dos RI, entre HIGH e VEDBA.

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# **Apêndice**

# Apêndice A

## Animals

*Tabela A.1:* List of all tuco-tucos captured, the corresponding attached biologgers and the number of days recorded

ID	Total Days recorded	Season	Sex	Capture Weight (g)	Accelerometer	Lightlogger	Recaptured	Collar Recovered
MAR01	6	March	f	120	Yes	No	Yes	Yes
MAR02	6	March	f	137	Yes	No	Yes	Yes
JUL15	6	July	f	110	Yes	No	Yes	Yes
JUL16	5	July	m	162	Yes	Yes	Yes	Yes
JUL17	8	July	f	132	Yes	Yes	Yes	Yes
JUL18	6	July	f	133	Yes	Yes	Yes	Yes
JUL19	4	July	m	220	Yes	No	Yes	Yes
JUL20	4	July	m	147	Yes	Yes	Yes	Yes
JUL21	4	July	f	152	Yes	Yes	Yes	Yes
JUL23	5	July	f	168	Yes	Yes	Yes	Yes
OCT01	18	October	f	202	Yes	Yes	Yes	Yes
OCT08	15	October	m	208	Yes	No	Yes	Yes
OCT09	7	October	f	144	Yes	No	Yes	Yes
OCT10	11	October	f	164	Yes	Yes	Yes	Yes
OCT13	11	October	f	189	Yes	No	Yes	Yes
OCT14	5	October	f	144	Yes	No	Yes	Yes
FEV01	5	February	f	175	Yes	Yes	Yes	Yes
FEV02	9	February	f	147	Yes	Yes	Yes	Yes
FEV03	7	February	m	123	Yes	Yes	Yes	Yes
FEV05	13	February	m	165	Yes	Yes	Yes	Yes
FEV06	9	February	f	153	Yes	Yes	Yes	Yes

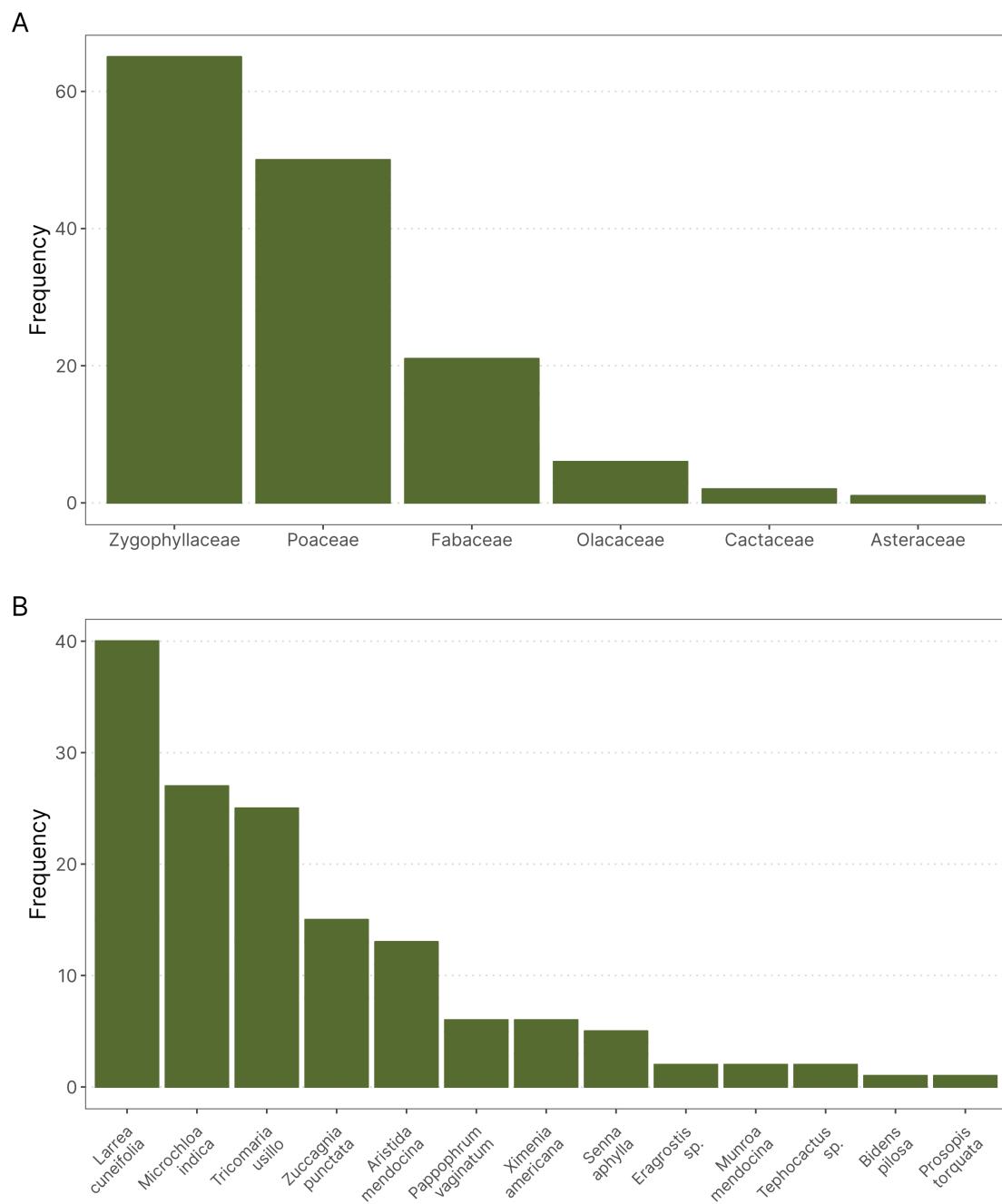
# **Apêndice B**

## **Anillaco's Environment**

### **B.1 Anillaco's Plant Community**

Following methods similar to (Aranda-Rickert, Diez, and Marazzi 2014) a non-extensive survey of the plant community was done in May 2019. Three perpendicular 50m transects were defined near the study site (COORDINATES). A point-intercept method was used to record plant species present in the transects, species right below the sampling points were registered in the data. Sampling points were defined every 1m along the 50m transects. Plant species were identified in the field by a Botanist, except for a few members of the Poaceae family.

The results for the plant survey is in line with what has been described in the literature for the region (Abraham et al. 2009; Aranda-Rickert, Diez, and Marazzi 2014; Fracchia et al. 2011). The results show a dominance of Zygophyllaceae, Poaceae and Fabaceae families. The relative frequency of plant families and species recorded in the area are shown in the graphs below (Fig. B.1).



*Figura B.1:* Relative frequency of plants family (A) and species (B) in three transects near the Study Site. The plant community is dominated by members of the Zygolhyllaceae, Poaceae and Fabaceae families and is in accordance with what has been described in the literature. (n = 145)

## B.2 Anillaco's Weather

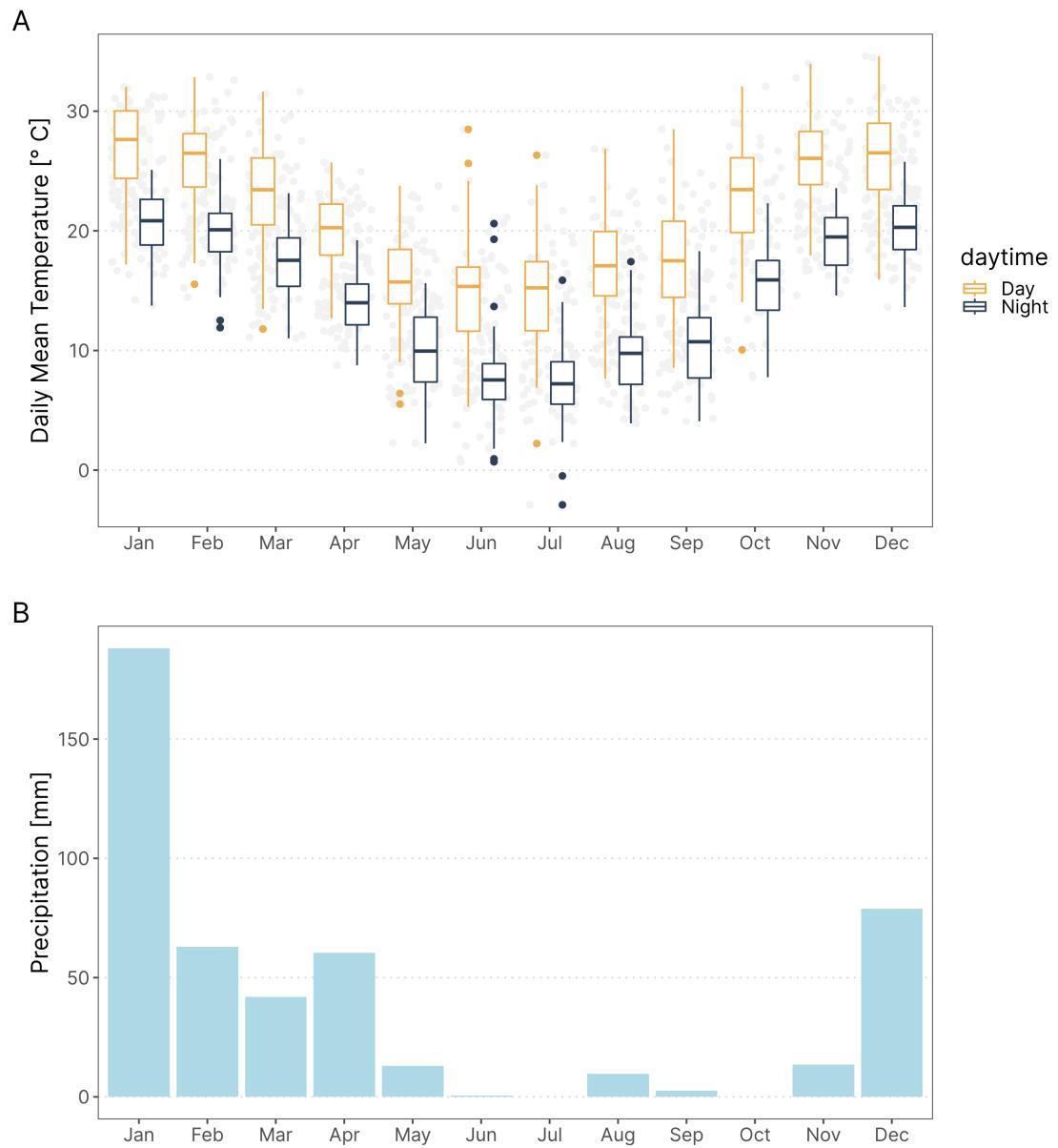


Figura B.2: Temperature and Rainfall yearly trends in Anillaco, Argentina. Data was collected in the years 2017 and 2019 from a weather Station (Vantage Pro 2, Davis Instruments. USA.) maintained in CRILAR, approximately 5km away from the study site.

### B.3 Anillaco's Yearly Daylength Changes

- Adicionar tabela com duração do dia nas datas de coleta

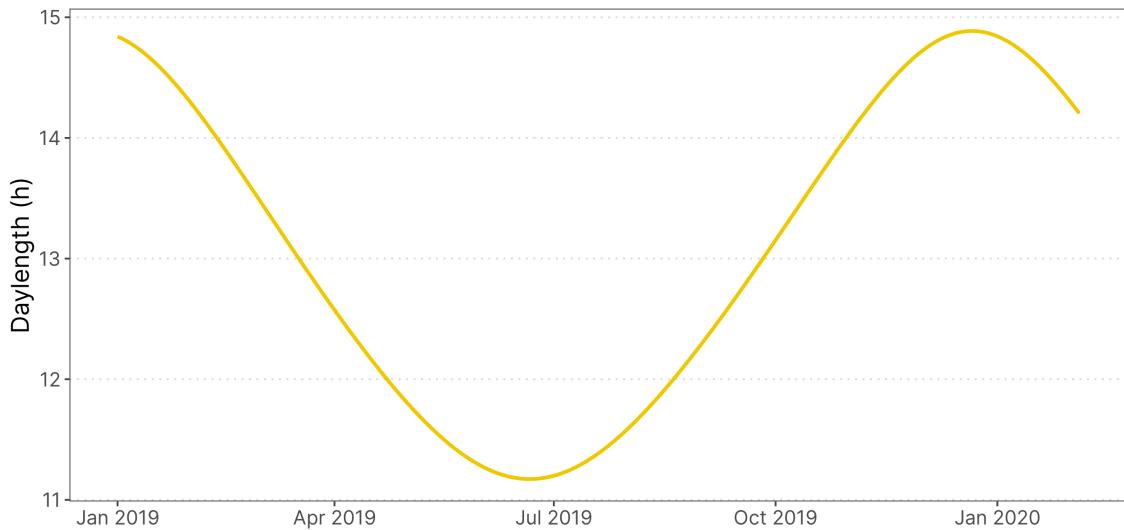


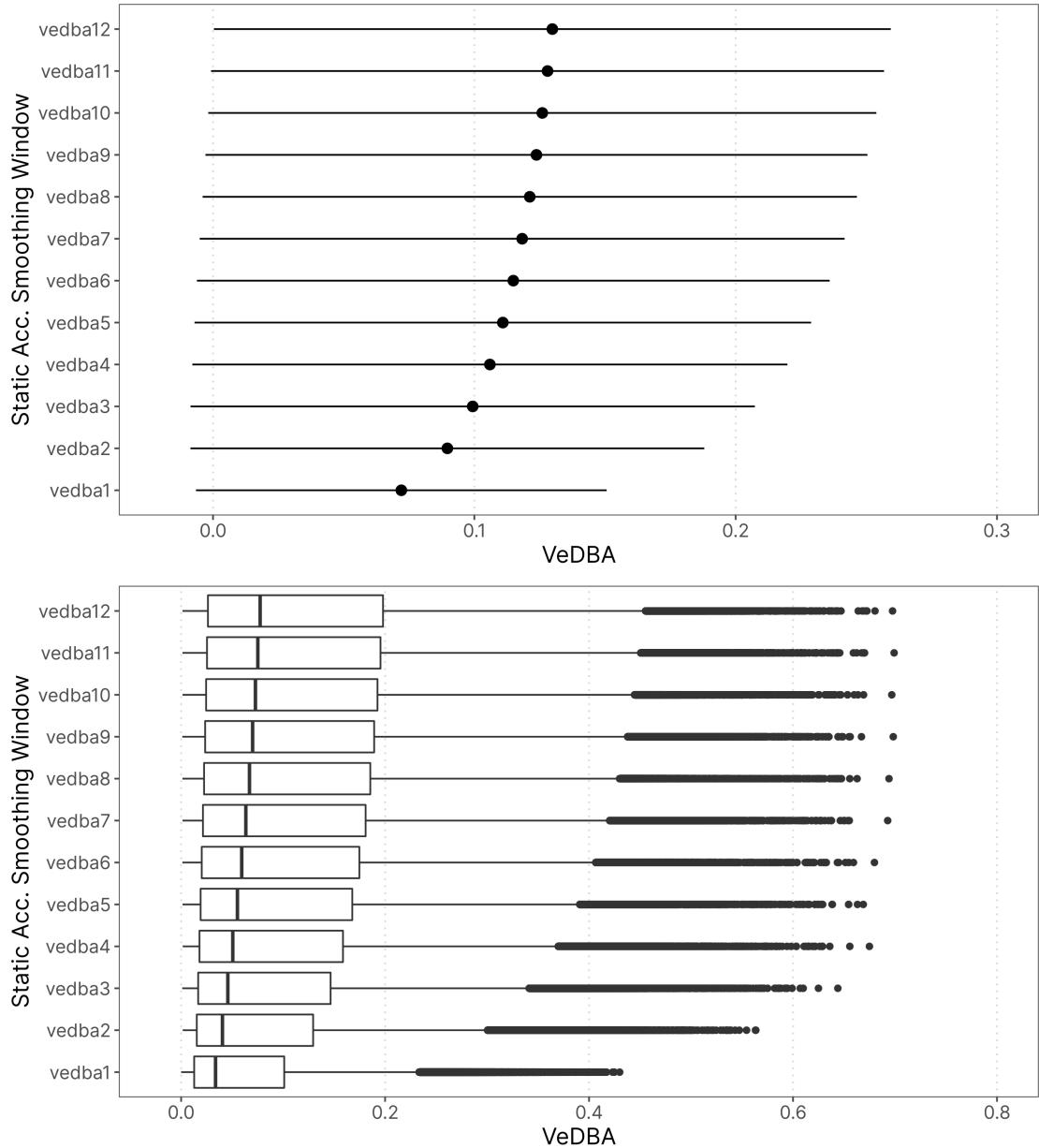
Figura B.3: Changes in daytime changes across the year in Anillaco, La Rioja. Maximum duration of daytime, during summer, is 14 hours and 53 minutes. Minimum duration of daytime, during winter, is 11 hours and 10 minutes.

# **Apêndice C**

## **Accelerometer Data Exploration**

### **C.1 Static Acceleration Smooth Window Assessment**

### **C.2 Exploratory VeDBA data Analysis**



*Figura C.1:* Smooth window assessment. We chose the 4-second moving average to proceed with analysis.

# Apêndice D

## Hidden Markov Model

### D.1 AIC

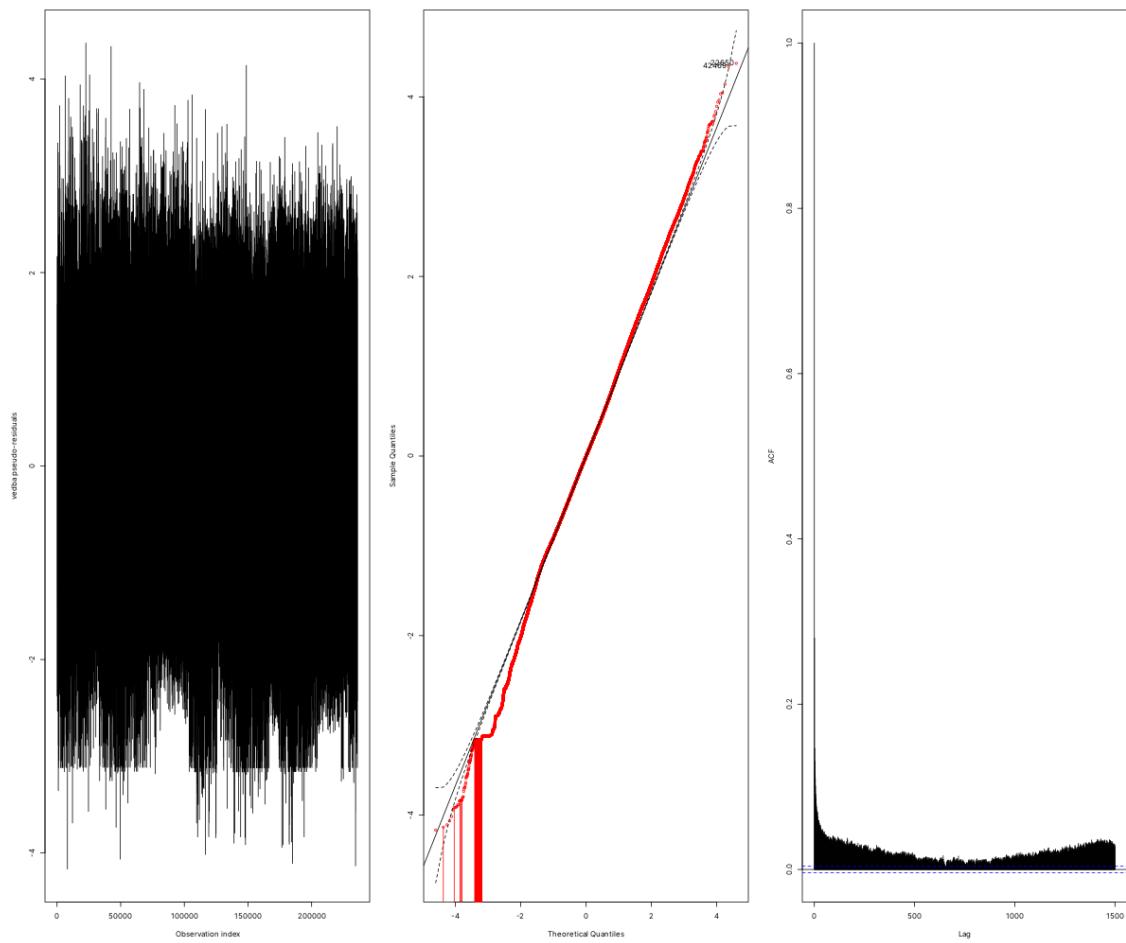
- Adicionar o LL

Model	Formula	AIC
m2	~season	-935612.2
m1	~1	-934800.8

### D.2 Estimated Parameters

Tabela D.1: Gamma State-dependent distribution parameters, mean and standard deviation, estimated by a three-state Hidden Markov Model.

Parameter	State	Estimate	CI
mean	Rest	0.018	[0.018, 0.018]
mean	Medium	0.116	[0.116, 0.117]
mean	High	0.327	[0.326, 0.328]
sd	Rest	0.010	[0.01, 0.01]
sd	Medium	0.051	[0.05, 0.051]
sd	High	0.087	[0.086, 0.087]



*Figura D.1: Model Residuals.*

### D.3 Pseudo-residuals



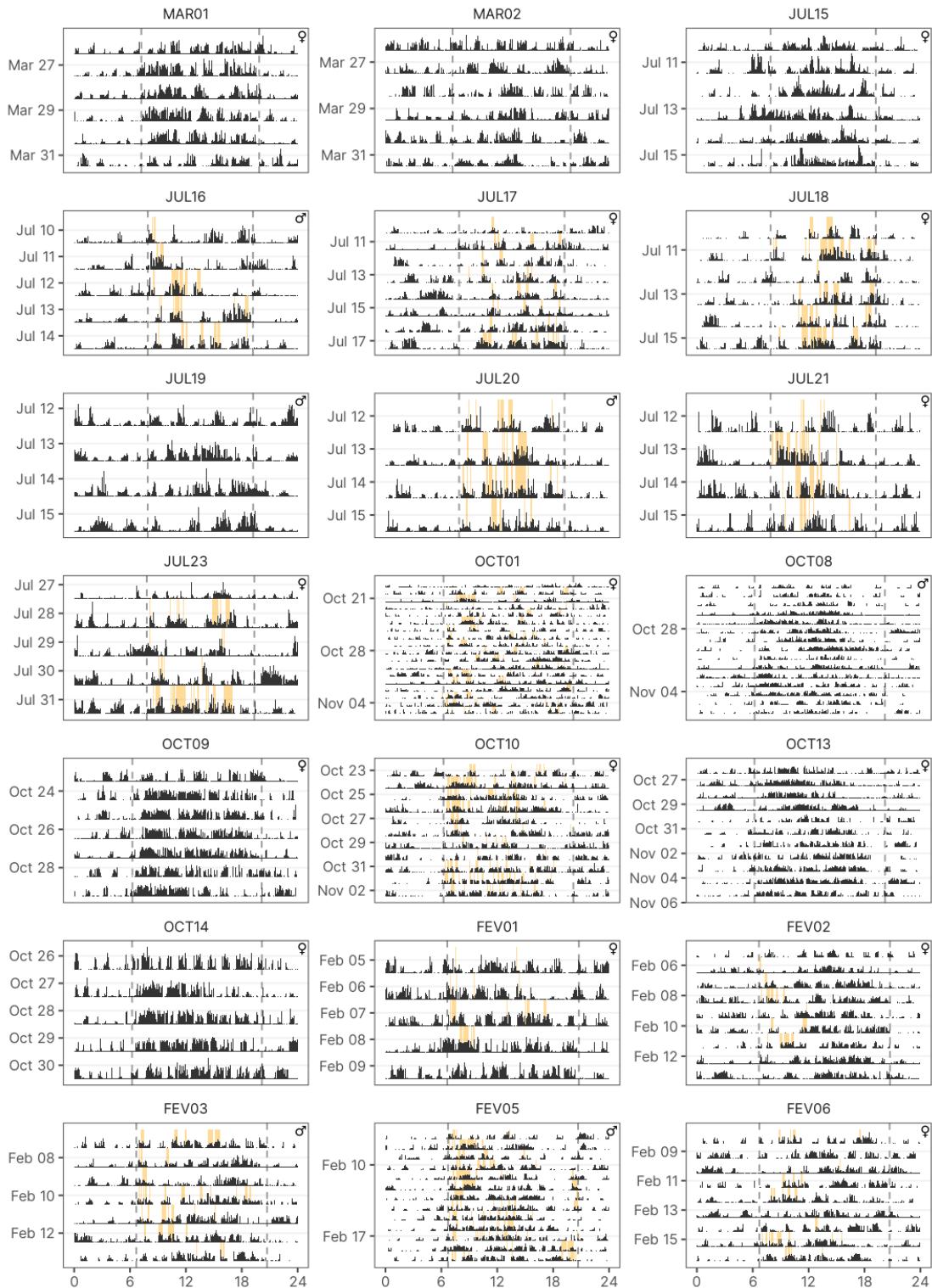


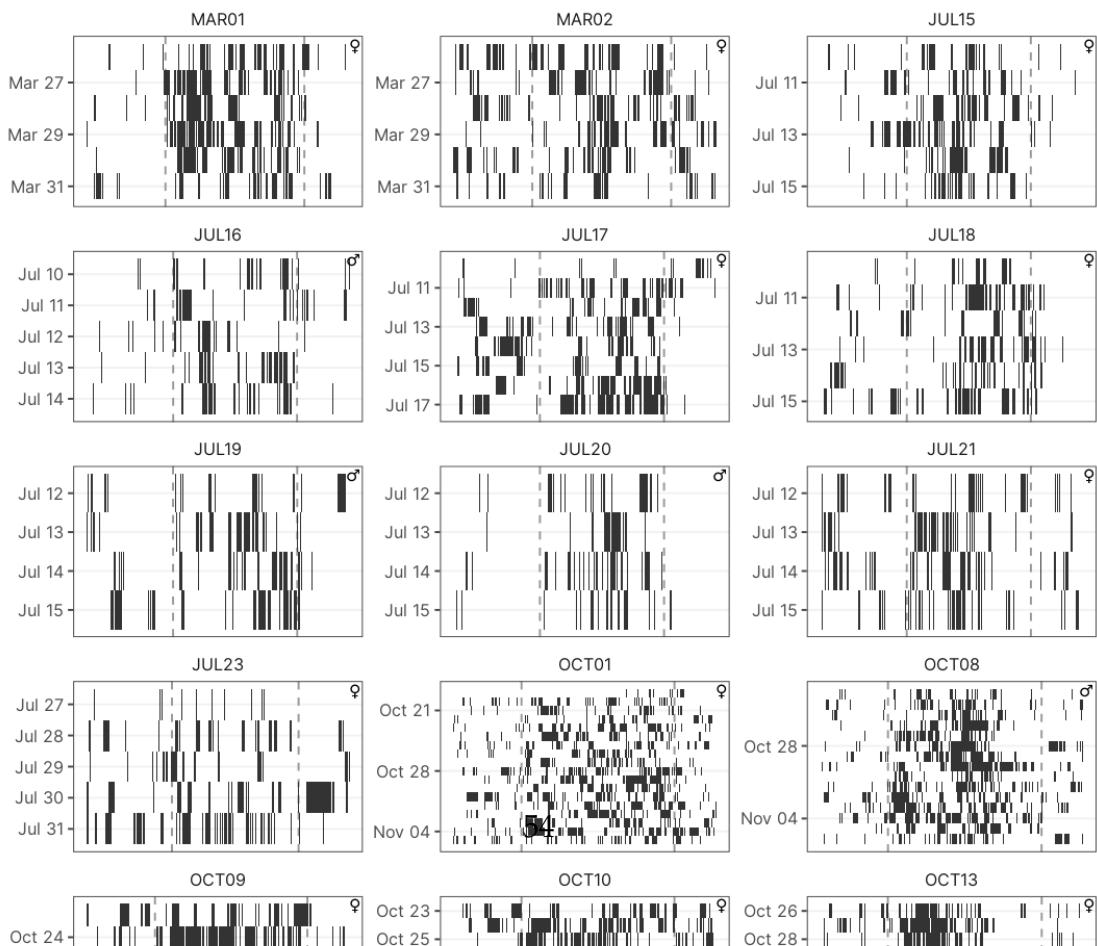
Figura E.1: VeDBA Actograms.

# Apêndice E

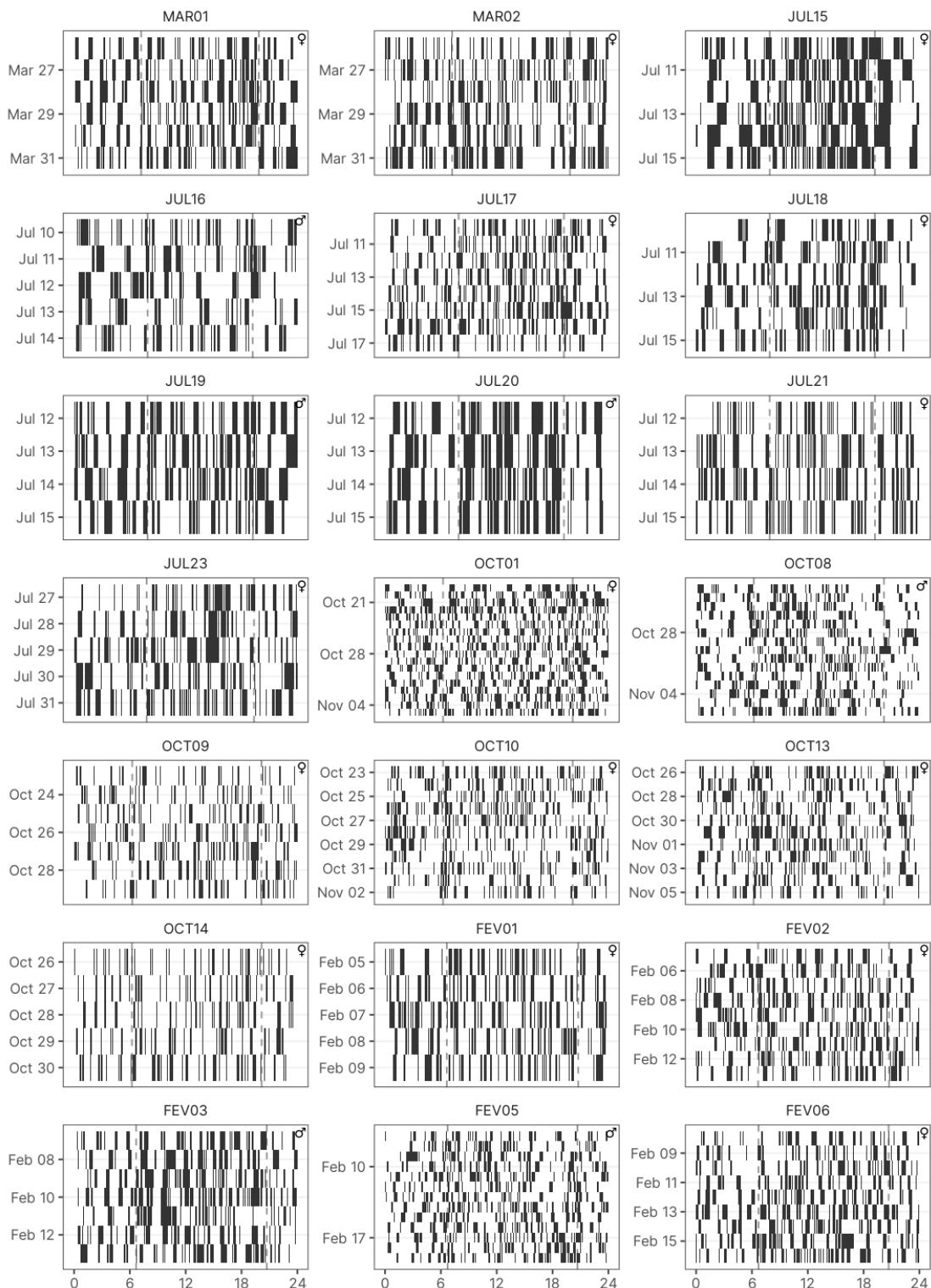
## Individual Actograms and Circadian Analysis Plots

### E.1 VeDBA Actograms

### E.2 High Activity Actograms



### E.3 Medium Activity Actograms



## E.4 Individual Rhythmicity Plots



## E.5 Individual Period Estimation

