UNIVERSIDADE FEDERAL DE MINAS GERAIS
Instituto de Ciências Biológicas
Programa de Pós-graduação em Ciências Biológicas:
Fisiologia e Farmacologia

Cíntia Aparecida de Souza Garcia Piton

A COMPREHENSIVE STUDY OF SLEEP-WAKE BEHAVIOR IN THE BURROWING OWL (*ATHENE CUNICULARIA*)

Belo Horizonte
2022

Cíntia Aparecida de Souza Garcia Piton

A COMPREHENSIVE STUDY OF SLEEP-WAKE BEHAVIORS IN THE BURROWING OWL (*ATHENE CUNICULARIA*)

Thesis submitted to the Graduate Program in Physiology and Pharmacology at the Federal University of Minas Gerais, in partial fulfillment of the requirements for the degree of Doctor Biological Sciences - Physiology.

Advisor: Jerome Baron

Belo Horizonte
2022

043 Piton, Cíntia Aparecida de Souza Garcia.
A comprehensive study of sleep wake behaviors in the burrowing owl (Athene cunicularia) [manuscrito] / Cíntia Aparecida de Souza Garcia Piton. – 2022.
170 f. : il. ; 29,5 cm.

Orientador: Jerome Baron.
Tese (doutorado) – Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas. Programa de Pós-Graduação em Fisiologia e Farmacologia.

1. Fisiologia. 2. Aves. 3. Estrigiformes. 4. Privação do Sono. 5. Vigília. I. Baron, Jerome Paul Armand Laurent. II. Universidade Federal de Minas Gerais. Instituto de Ciências Biológicas. III. Título.

CDU: 612

Ficha catalográfica elaborada pela bibliotecária Fabiane C M Reis – CRB 6 – 2680

UNIVERSIDADE FEDERAL DE MINAS GERAIS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS

PROGRAMA DE PÓS-GRADUAÇÃO EM FISIOLOGIA E FARMACOLOGIA

ATA DA DEFESA DA TESE N° 354 DE CÍNTIA APARECIDA DE SOUZA GARCIA PITON

Às 14:00 horas do dia **18 do mês de fevereiro de 2022**, transmitida através de webconferência pela plataforma Microsoft Teams, realizou-se a sessão pública para a defesa da Tese de **Cíntia Aparecida de Souza Garcia Piton.** A presidência da sessão coube ao **Prof. Dr. Jerome Paul Armand Laurent Baron,** orientador. Inicialmente, o presidente fez a apresentação da Comissão Examinadora assim constituída: **Prof. Dr. Cleiton Lopes Aguiar,** ICB/Universidade Federal de Minas Gerais, **Prof. Dr. Theo Rolla Paula Mota,** ICB/Universidade Federal de Minas Gerais, **Prof. Dr. Edison Rogério Cansi,** Universidade Federal do Sul da Bahia, **Prof. Dr. Sidarta Tollendal Gomes Ribeiro,** Instituto do Cérebro/Universidade Federal do Rio Grande do Norte, e **Prof. Dr. Jerome Paul Armand Laurent Baron,** ICB/Universidade Federal de Minas Gerais, orientador. Em seguida, a candidata fez a apresentação do trabalho que constitui sua **Tese de Doutorado**, intitulada: ***"A Comprehensive Study Of Sleep-wake Behavior In The Burrowing Owl (Athene cunicularia)".*** Seguiu-se a arguição pelos examinadores e logo após, a Comissão reuniu-se, sem a presença da candidata e do público e decidiu considerar **APROVADA** a Tese de Doutorado. O resultado final foi comunicado publicamente a candidata pelo presidente da Comissão. Nada mais havendo a tratar, o presidente encerrou a sessão e lavrou a presente ata que, depois de lida, se aprovada, será assinada pela Comissão Examinadora.

Belo Horizonte, 18 de fevereiro de 2022.

Assinatura dos membros da banca examinadora:

Documento assinado eletronicamente por **Jerome Paul Armand Laurent Baron**, **Professor do Magistério Superior**, em 24/02/2022, às 11:02, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Cleiton Lopes Aguiar**, **Professor do Magistério Superior**, em 14/04/2022, às 13:23, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Theo Rolla Paula Mota**, **Professor do Magistério Superior**, em 15/04/2022, às 14:07, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Edison Rogério Cansi**, **Usuário Externo**, em 18/04/2022, às 23:03, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

Documento assinado eletronicamente por **Sidarta Tollendal Gomes Ribeiro**, **Usuário Externo**, em 05/05/2022, às 14:13, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

A autenticidade deste documento pode ser conferida no site https://sei.ufmg.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **1222596** e o código CRC **F2D8E793**.

This thesis was developed in the Laboratory of Visual Neurodynamics (LANEVI) as part of the Graduate Program in Biological Sciences: Physiology and Pharmacology, at the Institute of Biological Sciences, Federal University of Minas Gerais, under the supervision of Prof. Dr. Jérôme Baron, with the financial support of the National Council for Scientific and Technological Development (CNPq) and Max Planck Institute for Ornithology, Seewiesen, Germany.

ACKNOWLEDGMENTS

First, I would like to thank my supervisor, Professor Jérôme Baron, whose input was invaluable in preparing this thesis and the whole project.

Also, I would like to acknowledge Professor Niels Rattenborg for his support in this research.

I would like to thank Hector, Otávio, Clara, Amanda, and Mateus. I also would like to express my thanks to Mônica and Gabriela for their valuable help. In particular, my very supportive intern deserves special mention, Alice Timponi, without whom I would not have completed the data collection.

From the bottom of my heart, I would like to say big thanks to the owls, mice, rats, and also to my twenty-one cats!

Thanks to my lovely friends and family.

I could not have completed this thesis without the immeasurable support of my brother Alex, my mother Fafá, and my husband, Joffer. Without you, I'm nothing!

AGRADECIMENTOS

Primeiramente, gostaria de agradecer ao meu orientador, Professor Jérôme Baron, dado a sua inestimável contribuição na preparação desta tese e de todo o projeto.

Gostaria também de agradecer ao Professor Niels Rattenborg pelo seu apoio nesta pesquisa.

Meus sinceros agradecimentos ao Hector, Otávio, Clara, Amanda, e Mateus. Gostaria também de expressar meu reconhecimento a valiosa ajuda da Mônica e Gabriela. Em particular, gostaria de destacar a minha exemplar e prestativa IC, Alice Timponi, sem a qual eu não teria completado a coleta e tratamento dos dados.

Do fundo do meu coração, gostaria de dizer um grande obrigado às corujas, ratos, camundongos, e também aos meus vinte e um gatos!

Gratidão aos meus adoráveis amigos e família.

Não poderia ter completado esta tese sem o apoio incomensurável do meu irmão Alex, da minha mãe Fafá, e do meu marido Joffer. Sem vocês, eu não sou nada!

RESUMO

O ciclo sono-vigília é homeostaticamente regulado e universalmente conservado no reino animal, sendo a coordenação bem equilibrada desse processo um imperativo para sobrevivência. Todavia, muitos aspectos desse processo vital ainda permanecem pouco compreendidos. Motivados pelo grande potencial dos estudos comparativos que buscam elucidar várias questões nesta área, o presente trabalho apresenta um estudo comportamental pormenorizado sobre o ciclo sono-vigília da coruja buraqueira (*Athene cunicularia*). A originalidade deste estudo fundamenta-se numa mistura ecléctica e parcimoniosa da literatura primária e de observações pessoais sugerindo que a coruja buraqueira representa um caso raro de catemeralidade em aves - traço comportamental intrinsecamente ligado ao sono polifásico. A fim de fornecer evidências mais diretas e quantitativas sobre essa hipótese, filmamos um total de 7 corujas por dia em um viveiro ao ar livre; e anotamos, com 1 segundo de precisão, o seu repertório comportamental tendo com base um etograma especificamente elaborado para descrever os comportamentos relacionados ao ciclo sono/vigília. Para analisar tal conjunto de dados, utilizamos várias abordagens complementares, incluindo a seleção de modelos circulares, análises quantitativas, probabilidades de transições de estado, e estatísticas multivariadas. Globalmente, os resultados obtidos a partir dessas análises indicam que as corujas buraqueiras fragmentam suas atividades diárias em picos de curta duração, de forma irregular e não aleatória, sendo em sua maioria expressas por movimentos de cabeça. Esse padrão comportamental manteve-se dia e noite havendo ligeiras alterações em cada hora do dia e um expressivo aumento de atividade durante o crepúsculo, corroborando, pois, a noção latente de que a coruja buraqueira é uma ave catemeral. Vale a pena destacar que a marcação das poses vigilante e de repouso mostrou ser uma estratégia valiosa para se obter uma compreensão mais profunda do ciclo sono-vigília das corujas buraqueiras. À luz da complexa natureza da privação de sono em aves, ficamos surpreendidos ao constatarmos que as corujas buraqueiras exibem rebote do sono, ainda que nossos resultados tenham se baseado em indicadores comportamentais. Tudo isso nos permite concluir que o fato das corujas buraqueiras apresentarem um padrão de sono fragmentado, amplamente distribuído ao longo de 24 horas, não as isenta da necessidade de compensação do sono. Este é o primeiro estudo a documentar um efeito rebote após período de privação de sono induzida experimentalmente em corujas.

Palavras-chave: aves, corujas, catemeralidade, privação do sono, homeostasia do sono, comportamento sono-vigília.

ABSTRACT

The well-balanced coordination of the sleep-wake cycle is a mandatory, homeostatically regulated, and universally conserved requirement for animal survival. Yet, many aspects of this vital process remain poorly understood. Motivated by the great potential of comparative studies to clarify various conundrums in this research area, the present study provides a comprehensive behavioral account of the sleep-wake cycle in the burrowing owl (*Athena cunicularia*). The originality of this endeavor rested on an eclectic and parsimonious mix of primary literature and intimate, personal observations suggesting that this owl species may represent a rare of cathemerality in birds, a behavioral trait intrinsically linked to polyphasic sleep. To provide more direct and quantitative evidence for this hypothesis, we videotaped a total of 7 owls over a daily cycle (24h) in an outdoor aviary, and scored their behavioral repertoire, with one-second precision, according to an ethogram tailored to organize specific behaviors into sleep/wake-related categories. To analyze this dataset, we used various complementary approaches, including circular model selection, computation of scalar quantities, probabilities based on state transitions, and multivariate statistics. Overall, the results obtained from this analysis indicate that burrowing owls fragment their daily life into short-lived, irregular, albeit non-random, bursts of activity, mostly expressed by head movements. This behavioral pattern was sustained day and night, with minor alterations, except for twilight hours, during which an expressive increase in wakefulness was detected, demonstrating that the latent notion of burrowing owls being a cathemeral bird is accurate. Moreover, the scoring of vigilant and rest poses proved to be a valuable strategy to gain a deeper understanding of the sleep-wake profile of burrowing owls. In light of the complex nature of sleep loss in birds, we were surprised to discover that burrowing owls exhibit sleep rebound even though our results are based on behavioral indicators. This leads us to the following conclusion: the fact that burrowing owl species present a fragmented sleep pattern largely distributed over 24 hours does not exempt them from the need for sleep compensation. Indeed, this is the first study documenting sleep recovery after a period of experimentally induced sleep deprivation in owls.

Keywords: birds, burrowing owls, cathemerality, , sleep deprivation, sleep homeostasis, sleep-wake behaviors

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AIC: Akaike information criterion (AIC)

BEC: Binocular Eye Closure

BORIS: Behavioral Observation Research Interactive Software

BS: Bihemispheric Sleep

DR: Diurnality Ratio

DS: Desynchronized Sleep

EEG: Electroencephalogram

GLMM: General Linear Mixed Models

GPS: Global Positioning System

HSD: Tukey's honestly significant difference (HSD)

Ls: Least Squared

MEC: Monocular Eye Closure

M1: Uniform model

M2A: Unimodal

M2B: Symmetric Modified Unimodal

M2C: Modified Unimodal

M3A: Homogenous Symmetric Bimodal

M3B: Symmetric Bimodal

M4A: Homogenous axial bimodal

M4B: Axial bimodal

M5A: Homogenous bimodal

M5B: Bimodal

NREM: Non Rapid Eye Movement

REM: Rapid Eye Movement

RH%: Relative Humidity

SD: Sleep deprivation

SWS: Slow Wave Sleep

SWA: Slow Wave Activity

US: Unihemispheric Sleep

TABLE OF CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

The sleep-wake cycle can be broadly defined as an adaptive, brain-controlled bodily process that plunges recurrently an organism in alternate sleep and wakefulness periods. In general terms, sleep can be conceptualized as a rapidly reversible state characterized by reduced motor activity, reduced metabolism, and increased response threshold to external sensorial stimuli (Tobler, 1995). In contrast, wakefulness can be viewed as a non-monolithic brain state that predisposes an organism to interact with its ecosystem and elaborate appropriate actions to satisfy many vital demands otherwise not fulfilled by sleep.

The sleep-wake cycle is known to be mandatory, homeostatically regulated, and universally conserved among the animal kingdom. However, many aspects of this vital process remain a conundrum. Experimentally, the distinction between sleep and wakefulness can be established on the basis of three non-mutually exclusive criteria:

(1) behavioral changes (e.g. body posture, responsiveness to stimuli);

(2) electrophysiological changes (e.g. cortical activity, eye movement patterns, muscle tension);

(3) physiological changes (e.g. heart-rate, body temperature, and breathing).

Many researchers consider electroencephalography (EEG), developed by Hanns Berger at the beginning of the 20th century, as the gold standard to classify sleep-wake states. EEG allows the non-invasive characterization of electrical activity generated by neural tissue covering the dorsal part of the telencephalon. Voltage traces obtained by this technique exhibit characteristic amplitudes and frequencies, which serve as reliable indicators of sleep-wake states. Moreover, in both mammals and birds, two types of sleep states can be reliably identified based on their distinct electroencephalographic signature: (1) Non Rapid eye movement (NREM) sleep, also called slow-wave sleep (SWS), characterized by low-frequency, large-amplitude waves correlated to muscle relaxation, decreased heart rate and blood pressure; (2) Rapid eye movement (REM) sleep, characterized by decoupled high-frequency, small-amplitude waves associated with muscular hypotonia and characteristic rapid eye movement (Aserinsky & Kleitman, 1953; Dement & Kleitman, 1957). REM sleep is also known as paradoxical sleep because its spectral features are, in many ways, similar to those exhibited during wakefulness (Jouvet, 1965).

Although sleep is a behavioral state in which an individual exhibits greater fragility in the face of predators or other risk situations, sleep occurs in most animals, including invertebrates (Keene & Duboue, 2018; de Souza Medeiros *et al.*, 2021). Hence, it is reasonable to conjecture that sleep must play a functional role essential to survival (Campbell & Tobler, 1984, Lima *et al.*, 2005). There exist several theories and experimental observations concerning the function of sleep (Roth *et al.*, 2010). Among them, some conceive sleep as an essential state for body temperature maintenance (McGinty & Szymusiak, 1990), energy conservation (Berger & Phillips, 1995), immune support (Bryant *et al.*, 2004), memory (Diekelmann & Born, 2010; Stickgold, 2013) as well as the strengthening and weakening of synapses in cortical networks (Tononi & Cirelli, 2003; 2006; Cirelli & Tononi, 2021).

The sleep-wake cycle is regulated by complex interactions between homeostatic and circadian mechanisms (Borbély, 1982a). However, these two types of mechanisms also seem to work independently as sleep homeostatic regulation is not abolished when the circadian cycle is disrupted (Tobler *et al.*, 1983). The circadian cycle controls the length of sleep-wakefulness, whereas sleep homeostasis acts opposing a permanent wake state and largely determines the intensity of sleep (Edgar *et al.*, 1993; Achermann & Borbély, 2003). In mammals and birds, two main variables increase after sleep deprivation: sleep intensity, measured by the amplitude of slow waves, and the sleep duration or amount of REM sleep (Borbély & Neuhaus, 1979; Tobler & Borbély, 1988; Rattenborg *et al.,* 2009). Moreover, sleep regulation is flexible, meaning that some animals may not present a clear sleep rebound depending on the survival conditions or ecological demands (Lyamin et al., 2005; Rial et al., 2007; Siegel, 2009; Lesku *et al.*, 2012; Rattenborg *et al.*, 2016).

Comparing sleep patterns among different species has been of tremendous utility to provide overarching principles underlying the functions of sleep and their evolutionary determinants. Analyzing the extraordinary variety of sleep traits encountered in the animal kingdom has opened up new research avenues. Comparative studies on sleep unequivocally contribute to generating higher-order concepts of significant importance for sleep research. For example, it is now recognized that the human EEG signature of REM and non-REM sleep is not a universal feature across the animal kingdom. It is a specific trait only shared among mammals and birds (Campbell & Tobler, 1984; Lesku et *al.*, 2009a). In the past, sleep was associated with a global activity pattern distributed uniformly throughout the neocortex (Mukhametov *et al.*, 1977; Rattenborg *et al.*, 2016). In more recent years, several comparative studies have shown that sleep is also associated with regional differences in the dorsal pallium of birds and mammals. The discovery of this local neural modulation reinforced the notion that sleep may provide a means to ensure that functionally specific cortical networks or even cell types are being homeostatically regulated according to their specific use-dependent needs (Lesku *et al.*, 2011) . This concept is still highly debated but has been supported by empirical evidence in several mammalian and avian species. For example, an increase in slow-wave amplitude is consistently found in cortical areas most predominantly recruited for the fulfillment of specific behavioral tasks during prior wake periods (Tononi & Cirelli 2003, Butler & Hodos, 2005; Rattenborg *et al.*, 2009; Stickgold, 2013; Huber *et al.*, 2004; Vyazovskiy & Tobler, 2008). Therefore, comparative studies incorporating different species and experimental techniques are very important for understanding this complex phenomenon cyclically generated by the nervous system.

1.2 Objectives

Motivated by the great potential of comparative studies in unveiling essential aspects of the sleep-wake cycle, the purpose of this work is to provide a comprehensive behavioral account of this process in the burrowing owl (*Athene cunicularia*). More specifically, we intended to fulfill this goal by:

- Providing a fine-grain, quantitative analysis of the 24-h distribution of sleep-wake behavioral markers;
- Characterizing the temporal structure and transition probabilities of these markers;
- Assessing the extent to which these markers are affected by sleep deprivation.

1.3 Justification and contributions

We chose the burrowing owl as an experimental model because several lines of evidence (see CHAPTER 3) and our laboratory observations suggest that this owl species may represent a rare case of cathemerality in birds, a behavioral trait intrinsically linked to polyphasic sleep.

An animal is referred to as cathemeral when its life-sustaining activities are distributed approximately evenly throughout the 24 hour day. Compared to diurnality and nocturnality, cathemerality is relatively rare in the animal kingdom . Moreover, only mammalian species have been the subject of studies specifically dedicated to investigating this activity pattern. Nevertheless, it is reasonable to conjecture that cathemerality is not limited to the evolutionary history of mammals. Hence, birds exhibiting no clear-cut distinction between diurnal and nocturnal activity patterns are crucially needed to be studied. In this respect, ecological evidence available on the diel activity of burrowing owls is somewhat parsimonious and often contradicting. Some studies indicate that burrowing owls are diurnal; others describe them as nocturnal with pronounced crepuscular trends. However, none of these studies was tailored to score the rich behavior repertoire of burrowing owls uninterrupted during 24 hours. We expect that the present study will fill in this knowledge gap. More generally, we hope to provide sufficiently convincing data to bring, unprecedentedly, the avian taxonomic class at the center of the discussions about cathemerality.

In 1972, Berger and Walker published a polysomnographic study of the burrowing owl. This study revealed unique particularities: frequent, short, and irregular sleep-wake cycles (> 200 cycles/day), non-REM sleep with opened eyes, and few second-long REM episodes. Although the study mentioned above did not present a complete analysis for the distribution of sleep episodes over 24 hours, it suggests that burrowing owls may have a polyphasic sleep pattern. Polyphasic sleep is defined as sleep-wake episodes with an approximately homogeneous distribution throughout 24 hours (stricter definition) or when less than 50% of sleep occurs continuously (Ball, 1992; Stampi, 1992a). Newborns of species that sleep monophasically tend to exhibit a polyphasic sleep pattern, including human beings (Kleitman & Engelmann, 1953; Parmelee *et al.* 1964; Ball, 1992; Liu *et al.*, 2000). However, little is known about the endogenous and exogenous components which govern the alternation and the prevalence of sleep staging in episodic sleepers, as most studies have been conducted in humans who purposely adopted a polyphasic sleep pattern (Weitzman et al. 1974; Webb & Agnew 1977; Campbell 1992).

From an ontogenetic and evolutionary point of view, it is not well-understood what would be the determining functions and factors (ecological and social demands, genetics, maturation level, and complexity of the nervous system) for changing the sleep phase (polyphasic *vs.* monophasic) in animals (Stampi, 1992a; Capellini *et al.,* 2008). But, it is well known that sleep fragmentation may be advantageous in situations where an animal is at constant risk so that it cannot sleep continuously for a long time (Lima *et al.*, 2005).

The present work provides an untapped opportunity to gain comparative insights into the physiological implications of polyphasic sleep, more commonly encountered in mammalian species, including human infants.

1.4 Organization of the thesis

This chapter set out the background to the present work, identified the problem that the work intends to investigate, and pointed out its foreseeable contributions. The rest of the dissertation is organized into four chapters, summarized as follows:

- Chapter 2 describes the methodology used to obtain and analyze the data.
- Chapter 3 presents results on the daily activity pattern of activity of burrowing owls and discuss their consistency with the concept of cathemerality.
- Chapter 4 presents a set of results on the dynamical structure of behavioral sleep-wake bouts.
- Chapter 5 presents results about sleep deprivation effect.

- Chapter 6 presents an overview of the entire work and some perspectives for future works are proposed.

CHAPTER 2

MATERIALS AND METHODS

2.1 Subjects

Data were obtained from a total of 7 burrowing owls (average weight, 185 g). Three of them were males, the rest were females. Four owls were born in captivity, and the other threes were obtained from the Wild Animal Screening Center (CETAS) of the Brazilian Institute for the Environment and Natural Renewable Resources (IBAMA) in Belo Horizonte. All the owls that took part in this study were housed in the aviary for at least one year before the experiments began and at most five years. Those who were born in captivity were, on average, three years old. Preliminary observations let us conclude that the owls were adults highly active within their colony, so none of them showed signs of aging.

All experimental procedures were approved by the UFMG Ethics Committee for Animal Experimentation (CEUA, license nº 178/2017, see appendix A). The permission for maintaining owls in captivity for scientific purposes was granted by the IBAMA (license nº 290344, see appendix B). The project was also authorized by the National System of Genetic Resource Management and Associated Traditional Knowledge (SisGen, Registration nº A0792B6).

2.2 Description of the experimental site

All the experiments took place in a large outdoor aviary where we maintained other burrowing owls colony. Geographically, the study site was located at 19°52'07.6"S 43°58'01.4"W 818 m asl. Locally, the study set was placed in an urban site of the university.

The experimental owls were housed in a grounded aviary of around 10 m^2 area (measuring 450 cm x 230 cm x 200 cm) featured a metal frame spaced by 2.5 cm. The rooftop was half covered which permitted proper ventilation and protection against extreme weather conditions. The aviary is located toward the Institute of Biological Sciences building and surrounded by a small wood on the west side.

There is indirect lighting coming from some lamp posts in the vicinity during the night, but it does not seem to shed light inside the experimental aviaries. Additionally, it is pertinent to note that, inside the aviary non-experimental rooms, there were burrowing owls, among other owl species (e.g., *Tyto furcata, Asio clamator, Megascops choliba*). However, they were totally separated, and the owls participating in the experiment were habituated to their presence. No one of the birds made direct contact with the other aviary rooms, but it was possible to hear the calls of nearby owls or other animals during the experiment. Aside from this, as the aviary is located close to a woodland, sometimes, free-roaming animals such as opossums, cats, marmosets, and various birds could occasionally be seen exploring the nearby area. These characteristics resemble a more natural setting, so we find them particularly important.

2.3 Experimental protocols

Two weeks before the experimental video recordings took place, individuals were transferred to a dedicated aviary unit empty. The aim of this procedure was to familiarize the bird to the specific (slightly restrictive) conditions imposed by our experimental approach.

Each bird was tethered on a curved bow perch of 50-cm height. The maximum leash extension was about a meter, thereby allowing the (at least) natural expression of behavior and the maintenance of the animal in the field of view of at least one of the two cameras. Despite that owls were leashed during the video recordings, we used falconry techniques to ensure a more natural acclimatization. Food and water were offered *ad libitum* just before the experiment. Weight controlling was held to ensure that all animals were in good health.

2.3.1 Sleep deprivation protocol

The sleep deprivation protocol (SD) consisted in manually stimulating the owl inside the outdoor aviary every time it stands motionless during uninterrupted 24 hours. A thin string was attached in its legs so we could gently stretch the string when the bird was inactive. In this way, the owl could be kept awake with minimum contact with the experimenter. We monitored sleep-deprived owls from the inside of the laboratory via two infrared video cameras throughout the protocol. Before the sleep deprivation protocol a baseline-24 hours video was recorded. One day after the baseline-24 hours video recording, we performed a sleep deprivation protocol (see figure 2.1).

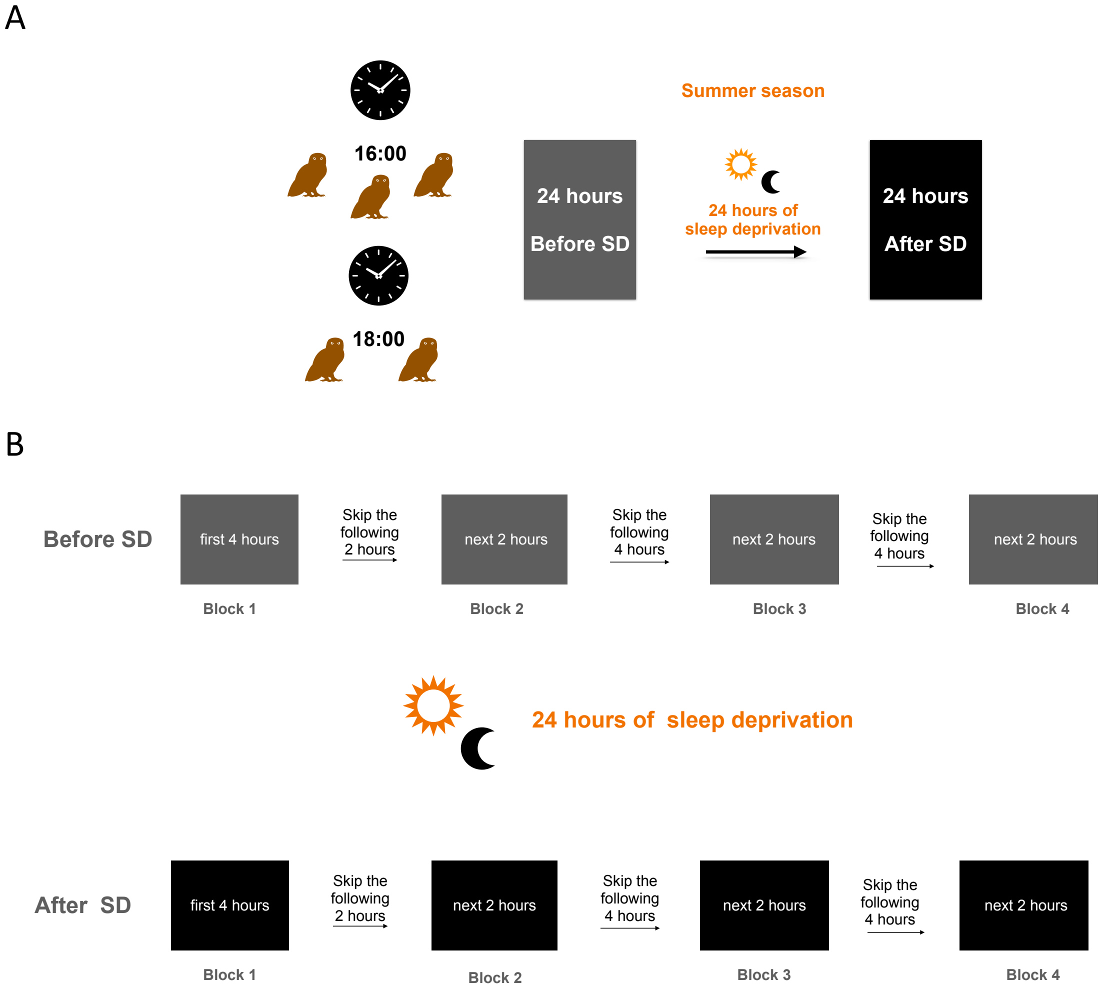


Figure 2.1. Sleep deprivation protocol and the scoring plan.
(A) For 3 owls, sleep deprivation (SD) started at 16:00, and for 2 owls, it began at 18:00. Video recordings were conducted 24 hours prior and 24 hours following SD. **(B)** Scoring plan was divided into four time-windows, referred to as Blocks before and after SD. Block 1 consists of the first four hours of 24h observations before and after SD (n=5 owls). The first and second blocks were separated by a two-hour non-scoring interval. Blocks 2 and 3 had a four-hour non-scoring interval, as well as blocks 3 and 4. Block 1 encompasses a four-hour time-window, while the remaining blocks cover a two-hour time-window.

2.4 Video recordings

Owls were filmed by two infrared video cameras (COLOR 700 SONY - CCD) at a sampling frequency of 25 Hz and for 24-hours. The videos were captured (escape © 1168 USB 2.0 - Video Capture) and stored on a PC for later analysis.

2.5 Environmental data collection

Experiments took place in winter from mid-July to August 2016 and at the same period in 2018. The video recordings started around 16:00 and 18:00. On-site temperature and humidity measurements were recorded for four owls at a three-minute resolution. A data logger (Trotec BL30 - GmbH & Co. KG) installed inside the aviary was used to monitor the ambient temperature (accuracy +/- 1 ^{deg}C) and relative humidity (accuracy +/- 3.5%; Range 0 - 100 % RH). Data storage interval was 3 minutes. Data were resampled at 1 Hz using linear interpolation.

2.6 Behavioral taxonomy and scoring

The major goal of this work was to provide a reliable estimate of the time budget spent in awake and sleep states. Prior inspections of long video clips were used to establish an ethogram (see figure 2.2 and Table 2.1) consisting of two major categories: wake and sleep behaviors essentially defined by specific body postures and movements (e.g., vertical neck protrusion, motionless sentry posture, eyelid closure of more than 1000 ms in duration). We also defined a number of transient behaviors associated with head movements and eye blinks.

The behaviors were grouped in three categories considering the dichotomy of wake/sleep state. The first category, denominated *Overtly Active*, comprised a set of well-defined locomotory and non-locomotory behaviors, ensuring that the animal exhibits a clear level of activity, such as walking, head movements, or feeding (see Table 2.1).

Operationally, an *overtly active* behavior was defined as a sequence of video frames in which the animal moved at least one part of its body without exhibiting a static posture. The second category, *overtly sleeping,* was defined as an unequivocal sleeping state evidenced by the closure of one- or both eyes for more than 1000 ms.

Another issue, often neglected, concerns the fact that an active state is commonly taken as synonymous with body movements. An animal, especially an owl, can be immobile whole alert. For that reason, we considered that specific static poses could also typify an awake state. This notion fits like a glove for describing the behavior of burrowing owls as they spend most of their time motionless as a sentinel or sleeping in an upright posture with their eyes opened (Coulombe, 1971; Berger & Walker, 1972; Haug & Oliphant, 1990; Mrykalo, 2005; Chipman *et al.*, 2008; LaFever *et al.*, 2008). The awake immobile state was not a rare and needed to be treated as a behavioral particular. Accordingly, we defined a behavioral quiescence when no movements were observed across at least 2-s. Based on this criterion, we created a third category named *static posture* composed of two motionless behaviors: a vigilant pose and a rest pose. The vigilant pose was characterized by an observable erect posture whereby the animal is clearly staring at something, with both eyes open. In many circumstances, the animal exhibited an expressive neck extension. The temporal unfolding of successive saccades and vigilant postures can be seen as a visual scanning bout. The rest pose featured a clear neck-muscle relaxation, accompanied by other body phenotypes such as standing on one leg, with fluffy feathers and eyes partially closed. The sleep state periods were therefore represented by the rest pose or the monocular or binocular eye closure.

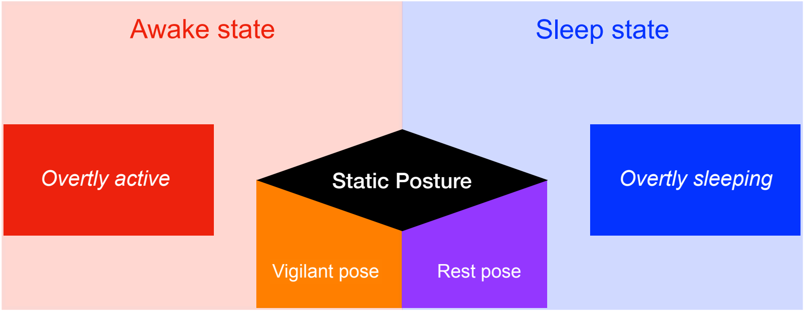


Figure 2.2. Ethogram categories based on the dichotomy of wake and sleep states. Behaviors were divided into three categories: *Overtly Active*, encompassing a set of locomotor and non-locomotor behaviors; *Overtly Sleeping*, defined as an unambiguous sleep state evidenced by the closure of one or both eyes for more than 1000 ms, and *Static Posture*, comprising two motionless behaviors, a vigilant pose and a rest pose.

The scoring of behavioral variables were performed by means of the Behavioral Observation Research Interactive Software (BORIS, version 2.95, http://www.boris.unito.it), an open-source event logging software described in (Friard & Gamba, 2016). Using BORIS, we could score the rich repertoire of behaviors that compose the sleep-wake cycle in burrowing owls' behaviors. We selected the ethogram's most frequent and representative behaviors to determine the distribution of the awake and sleep states in the burrowing owl.

Table 2.1. Ethogram definitions

Category	Behavior	Definition	Event Status	Examples*
Overtly active	Head saccade alone	Shift of the line sight as a result of a head movement without blinking the eyes, which sometimes it is followed by a body movement. Head saccades are scored when the owl is moving only its head (<1s)	Point	https://youtu.be/yFlSbnTPp98
	Head saccade with eye blink	Head saccade associated to eyes blinking (<1s)	Point	https://youtu.be/sdfTWhIrgqw
	Other head movements	The owl makes jerky movements with head and body. The owl moves the head gazing something in a smooth, continuous way. The owl shakes its head.	Duration	https://youtu.be/8p5kku5q5vA
	Locomotion	The owl displays locomotory behaviors such as walking or flying	Duration	https://youtu.be/pxMwZRTrnZ8
	Maintenance behavior	Other maintenance behavior such as beak maintenance, digging, regurgitation, defecation, drinking water or comfort-seeking postural readjustments (e.g. ruffling feather or stretching legs and wings)	Duration	https://youtu.be/RAW5RLV5LsI https://youtu.be/wCEH3tGFFB0
	Feeding	The owl eats.	Duration	https://youtu.be/S3Ls6Mtb-fk
	Cleaning	The owl displays maintenance behaviors as preening or bill wiping.	Duration	https://youtu.be/_7QJwldFLaw
Overtly sleeping	Binocular eyelid closure	Displacement of both eyelids resulting in complete closure for more than 1000 ms. In this condition the owl always exhibits a rest pose.	Duration	https://youtu.be/xlyR_ber3xc
	Monocular eyelid closure	Just one eye is completely closed for more than 1000 ms. In this condition the owl always exhibits a rest pose.	Duration	https://youtu.be/-1f1aBbC4HU
	Bobble head	The head slightly bobbles due to relaxation of the neck or the head turns very slow with eyes closed	Point	https://youtu.be/6HxL9ehxiiI
	Downward jerk	The owl's head nods involuntarily with eyes closed	Point	https://youtu.be/EVf3osmATU0
	Drop off	The owl closes it eyes, relaxes the body and droops one of its wings. In this case, the body relaxation may result in loss of balance, and consequently the opening of one or both wings. Also, it is frequently observed the lowering of its feathers and tail. Alternatively, the relaxation can result of a partial crouch by the owl	Point	https://youtu.be/AR1u06I5dvs
Static posture	Vigilant pose	The owl maintains an immobile upright posture as a sentry. Its neck is raised or shows some degree of extension. Exclusion criterion: eyes can not be closed for more than 1000ms	Duration	https://youtu.be/icG0YNIK-7g
	Rest pose	The owl maintains an immobile upright posture, but unwind. Its neck exhibits relaxed pose, but its head is maintained in an upright position. The eyes are opened or partially closed	Duration	https://youtu.be/gW6a0pl79yU

Out of sight	Out of sight	Animal disappeared from the field of view of the camera	Duration	https://youtu.be/VL6YI7Uc0PE
Eyes out of camera	Eyes out of camera	Period that both of the eyes are not visible, because the animal is standing with its back to the camera shooting plan or the animal turns its head out of the camera shooting plan for some seconds.	Duration	https://youtu.be/Z6J17K_C0Q8

* The quality of videos examples has been lowered in the process of making the clips and uploading them to YouTube.

2.7 Data analysis

Data were analyzed using custom-made or public-domain computational routines implemented in MATLAB (MathWorks, Natik, MA), LabVIEW (National Instruments, Austin, TX) or R, a free, open-source software environment for statistical computing and graphics.

2.7.1 Construction of categorical and binary time series

The analysis consisted of a series behavior events scored by timestamps with a temporal resolution of one second. There were two types of events: the duration events that were specified by a series of consecutive timestamps corresponding to its duration, for example, locomotor behaviors; and the point events which were specified by a single timestamp of its occurrence, for example, head saccades. Here, it is important to highlight that the head saccade alone is often referred to in the text as saccades, since burrowing owls have practically no eye movements (Berger and Walker, 1972; Steinbach & Money, 1973). Also the terms head saccade with blink/associated with blink, saccades with or associated with blinks, are interchangeable, by the same reasoning.

The behavioral data was concatenated in the form of time series of events and mutually exclusive behavioral states. Behavioral budgets were generated for the point events in terms of total numbers of events and numbers of events per unit of time (time rate). Inter-events intervals, i.e., the time elapsed between two subsequent behavior, were also computed. Duration events were quantified as follows: bouts, namely the number of intervals behavior, central tendency measures of the duration, and the proportion of time such behavior happened at a given time range or diel category. For example, the amount percentage spent in the binocular eyes closure behavior every 15-min. In this case a 15 min rate corresponds to the sum of all time spent with eyes closed within each 15 min. Note that the proportion of time spent on 'behavior xx' at a 15 min rate is independent from the number of intervals with eyes closed.

2.7.2 Circular analysis

In this analysis, behavioral occurrences of a particular category (e.g. *overtly active*, *overtly sleeping* behaviors) or a meaningful combination of categories (e.g. *overtly active* and *vigilant pose* behaviors) were initially projected onto a unit circle to assess their distribution around a 24-hour clock. This projection transformed the data into a circular time series (in radians), which we modeled as a finite von Mises mixture distribution. In its general form, this distribution consists of a sum of M von Mises probability distributions which can be mathematically written as

$$f\left(\theta \mid \varphi_m, \kappa_m, \lambda_m\right) = \sum_{m}^{M} \lambda_m \frac{1}{2\pi I_0\left(\kappa_m\right)} e^{\kappa_m \cos(\theta - \varphi_m)} \qquad \text{(Eq. 1)}$$

where θ is the random variable representing the 24 hour clock time in radian units, φ_m, κ_m denote the mean time parameter and the concentration (around κ_m) parameter, respectively, for $m = 1,2,\ldots,M$ components of the von Mises distribution, while λ_m is the mixing proportion parameter ranging between 0 and 1 such that

$$\sum_{m}^{M} \lambda_m = 1 \quad \text{for} \quad (m = 1,2,\ldots,M) \qquad \text{(Eq. 2)}$$

$I_0(\kappa)$ is the modified Bessel function of the first kind and of order zero, which operates as a normalizing constant of $f\left(\theta \mid \varphi_m, \kappa_m, \lambda_m\right)$, and is given by

$$I_0(\kappa) = \frac{1}{2\pi} \int_0^{2\pi} e^{\kappa \cos \theta} d\theta \qquad \text{(Eq. 3)}$$

The main objective of our circular modeling approach was motivated by the tantalizing attempt to find the most parsimonious and reliable mixture of von Mises distributions that could be easily related to canonical categories of diel activity patterns (e.g. nocturnal, cathemeral). With this objective in mind, we used the modeling approach initially described by Schnute and Groot (1992), which defines a set of ten different models (i.e. M1, M2A, M2B, M2C, M3A, M3B, M4A, M4B, M5A, M5B, see table 2.2) from a finite mixture of up to two von Mises distributions, given by

$$f\left(\theta \mid \varphi_1, \kappa_1, \lambda, \varphi_2, \kappa_2\right) = \lambda \frac{1}{2\pi I_0\left(\kappa_1\right)} e^{\kappa_1 \cos\left(\theta - \varphi_1\right)} + (1 - \lambda) \frac{1}{2\pi I_0\left(\kappa_2\right)} e^{\kappa_2 \cos\left(\theta - \varphi_2\right)} \qquad \text{(Eq. 4)}$$

where λ , in this special case, can be thought as the weighting term of the first von Mises component given that, by definition,

$$\lambda + (1 - \lambda) = 1 \qquad \text{(Eq. 5)}$$

To assess the relative fitting performance of each of the 10 models, we used the R package CircMLE v0.2, developed by Fitak and Johnson (2017) and made publicly available on CRAN (https://cran.r-project.org/). The software package implements a maximum likelihood estimation to measure model goodness of fit and ranks each model according to information criteria. Accordingly, fitting was carried out to estimate the free parameter values of the bimodal von Mises which maximize the log-likelihood function, given by

$$L\left(Q\right) = \sum_{i=1}^{n} - \ln\left[f\left(\theta_i \,|\, Q\right)\right] \qquad \text{(Eq. 6)}$$

where n represents the timestamps of behavioral observations within θ given the vector of model parameters Q=(φ_1, κ_1, λ, φ_2, κ_2).

All default fitting options and parameter value restrictions used by CircMLE v0.2 were kept unchanged. Accordingly, for bimodal models, the minimum angle difference between φ_1 and φ_2 was set to $\pi/4$ radians, corresponding to a minimum time difference of 3 hours. Moreover, the λ parameter being kept within a [0.25, 0.75] boundary minimized convergence to bimodal models whenever a large discrepancy in behavioral count peak occurred at a particular time compared to another one. To avoid cases in which κ may tend towards infinity, this parameter was restricted to restricted $0 < \kappa \leq 227$ (approximatively 14 min).

It is well known that the von Mises distribution can be approximated by a wrapped normal distribution with second trigonometric moments related by

$$\sigma \approx \frac{1}{\sqrt{\kappa}} \qquad \text{(Eq. 7)}$$

where σ is the standard deviation of the normal distribution. Detailed information about the mathematical foundation underlying (Eq. 7) can be found in Mardia and Jupp (2018). Given the above approximation, the time range containing 95 % of the behavioral observations falling under a single von Mises component m, and hereafter denominated bandwidth (β_m), was calculated as

$$\beta_m = 2 * 1.96\sigma_m \qquad \text{(Eq. 8)}$$

where σ_m value is computed from the κ_m fitted estimate of the von Mises component using (Eq. 7).

The relative performance of each fitted model within the set Ψ = {Ψ_i, i = M1, M2A, ..., M5B} was assessed using a model selection approach based on the Akaike information criterion (AIC; Akaike , 1974). given by

$$AIC_i = -2 \log L\left(\widehat{Q}\right)_i + 2\widehat{Q}_i \qquad \text{(Eq. 9)}$$

where $L\left(\widehat{Q}\right)_i$ is the log-likelihood function for the candidate model i and $\widehat{Q}$ is the vector of estimated parameter values obtained after the fitting procedure for a given model. Accordingly, the model with the lowest AIC value (best model) is asymptotically equivalent to choosing that with the lowest expected information loss as estimated by the Kullback-Leibler discrepancy. Detailed information about the theoretical concepts and mathematical formalisms underlying this information criterion are provided in Burnham and Anderson (2002). Because AIC values vary on a relative scale and are much affected by the data sample size, we reported their differences (Δ) to the minimal value min(AIC) under consideration, such that

$$\Delta AIC_i = AIC_i - min\,(AIC) \qquad \text{(Eq. 10)}$$

As a general rule, models with AICs differing from the best (ΔAIC)<2 are well supported, models with ΔAICs ranging from 2 to 7 should rarely be disregarded, and models with ΔAIC>9 are weakly supported.

Departure from uniformity was also assessed using the Rayleigh test. which compares the likelihood of a dataset, assuming a uniform von Misses distribution, with the likelihood of a non-uniform von Mises distribution.

Table 2.2 summarizes how each of the ten models proposed by Schnute and Groot (1992) may support, given certain parameter values, specific hypotheses of temporal niche categories, i.e., diurnal, nocturnal, crepuscular, and cathemeral. With regard to the first model (M1), activity is assumed to be spread out uniformly throughout a 24-hour cycle, as expected for cathemeral animals exclusively.

Table 2.2. Predicting temporal niche preferences according to ten models of animal orientation developed by Schnute and Groot (1992)

Model	Modes	Name	$Q=(\varphi_1, \kappa_1, \lambda, \varphi_2, \kappa_2)$	N_p	Most plausible hypothesis
M1	0	Uniform	(na, 0, 1, na, na)	0	**Cathemeral**
M2A	1	Unimodal	$(\varphi_1, \kappa_1, 1, \text{na}, \text{na})$	2	**Strictly diurnal**, given $\varphi_1 \pm 0.5\beta_1$ within daytime hours **Strictly nocturnal**, given $\varphi_1 \pm 0.5\beta_1$ within nighttime hours
M2B	1	Symmetric modified unimodal	$(\varphi_1, \kappa_1, 0.5, \text{na}, 0)$	2	**Diurnal** or **nocturnal** with 50% of residual activity uniformly distributed in the other temporal niche categories
M2C	1	Modified unimodal	$(\varphi_1, \kappa_1, \lambda, \text{na}, 0)$	3	**Diurnal** or **nocturnal** with some residual activity uniformly distributed in the other temporal niche categories
M3A	2	Homogenous symmetric bimodal	$(\varphi_1, \kappa_1, 0.5, \varphi_1 + \pi, \kappa_1)$	2	**Crepuscular,** given $\varphi_1 \pm 0.5\beta_1$ within twilight hours **Cathemeral**, given $\kappa_1 \to 0$
M3B	2	Symmetric bimodal	$(\varphi_1, \kappa_1, 0.5, \varphi_1 + \pi, \kappa_2)$	3	**Crepuscular** with matutinal or vespertine bias, given $\varphi_1 \pm 0.5\beta_{1,2}$ within twilight hours **Cathemeral**, given κ_1 and/or $\kappa_2 \to 0$
M4A	2	Homogenous axial bimodal	$(\varphi_1, \kappa_1, \lambda, \varphi_1 + \pi, \kappa_1)$	3	**Crepuscular** with matutinal or vespertine bias, given $\varphi_1 \pm 0.5\beta_1$ within twilight hours **Cathemeral**, given $\kappa_1 \to 0$
M4B	2	Axial bimodal	$(\varphi_1, \kappa_1, \lambda, \varphi_1 + \pi, \kappa_2)$	4	**Crepuscular** with matutinal or vespertine bias, given $\varphi_1 \pm 0.5\beta_{1,2}$within twilight hours **Cathemeral**, given κ_1 and/or $\kappa_2 \to 0$
M5A	2	Homogenous bimodal	$(\varphi_1, \kappa_1, \lambda, \varphi_2, \kappa_1)$	4	**Crepuscular** with matutinal or vespertine bias, given $\varphi_{1,2} \pm 0.5\beta_1$ within twilight hours **Cathemeral**, given $\kappa_1 \to 0$
M5B	2	Bimodal	$(\varphi_1, \kappa_1, \lambda, \varphi_2, \kappa_2)$	5	**Crepuscular** with matutinal or vespertine bias, given $\varphi_{1,2} \pm 0.5\beta_{1,2}$ within twilight hours **Cathemeral**, given κ_1 and/or $\kappa_2 \to 0$

N_p, number of free parameters; na, parameter not included in the model; β_m is the bandwidth (see eq. 8)

Unimodal models (M2A-C) should better fit any activity time budgets characterized by a single peak over a 24h period. This profile is typical of diurnal and nocturnal chronotypes due to the synchronizing influence of light upon their circadian system, which globally drives activity up and then down once a day. Note, however, that the best ranking of any three models is insufficient to provide definitive evidence for diurnality or nocturnality. The parameter values fitted to these models are equally important. On the premiss that the periodic light-dark cycle is the main synchronizer of diurnal or nocturnal chronotypes, a large bulk of the activity around their acrophase should be contained within daytime or nighttime hours, respectively. This concept may be reasonably quantified by setting a threshold criterion according to which at least 95% of the activity modeled by a single von Mises function ($\varphi_1 \pm 0.5\beta_1$) is required to occur during defined periods of lightness or darkness. The only difference between the three unimodal models rests on the value of the parameter λ which controls the amount of randomly distributed behavioral (residual) activities outside the von Mises component. M2A does not account for such residual activity; M2B and M2C do so.

All the remaining models are bimodal. As mentioned above, bimodal models have been constrained to have their activity peak times at least 3 hours apart. Models of equal distribution profile of activity around each mode ($\kappa_1 = \kappa_2$) are referred to as homogeneous. The axial property of a model refers to distribution means in opposite directions (φ_1, $\varphi_1 + \pi$). The difference between a cathemeral and crepuscular hypothesis can be inferred from the activity peak periods and the temporal spread of those peaks ($\varphi_m \pm 0.5\beta_m$), whereby a large dispersion (small κ_m) tends to cathemeral species and the opposite (large κ_m) suggest a predominantly crepuscular pattern of activity.

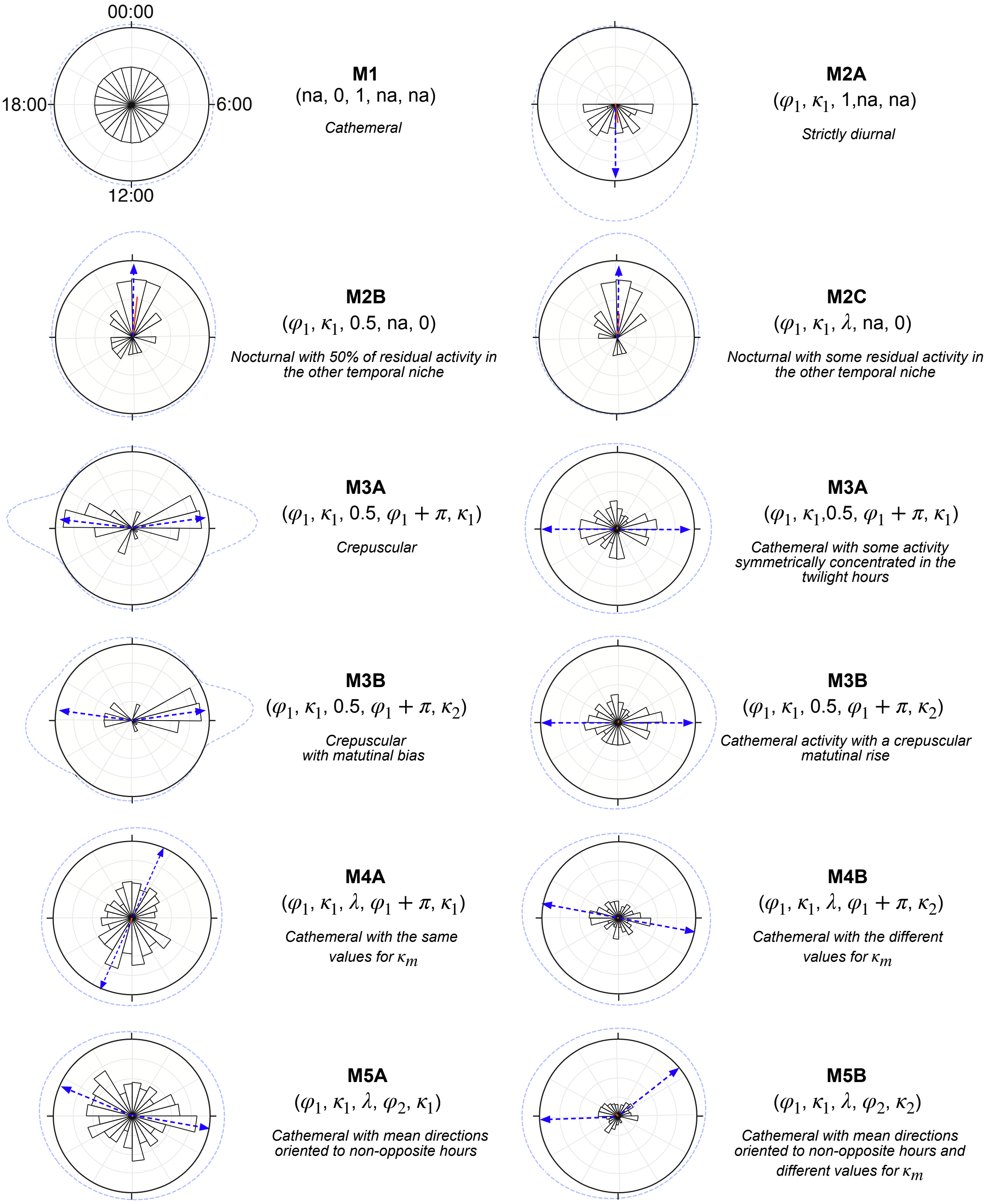


Figure 2.3. Examples of temporal niche preferences according to ten models of animal orientation. The circular histograms (gray bars) represent the quantification of behaviors in bins of 60 minutes. The density (dashed line) and mean direction(s) (dashed blue arrows). **(M1) Uniform** distribution; **(M2A) Unimodal** here represents a strictly diurnal activity; **(M2B) Symmetric modified unimodal** represents a nocturnal profile with 50% ($\lambda = 0.5$) of residual activity uniformly distributed in the other temporal niche; **(M2C) Modified unimodal** depicts nocturnal activity with some residual activity ($\lambda \neq 0.5$) uniformly distributed in the other temporal niche; **(M3A) Homogenous symmetric bimodal** can represent a crepuscular (left) or cathemeral profile (right); in the latter case, the activity is largely distributed around the clock, with some activity symmetrically concentrated in the twilight hours and 50 % ($\lambda = 0.5$) of the data distributed around each mean direction; **(M3B) Symmetric bimodal** resembles M3A**,** but the concentration parameters (κ_m) values differ, this example shows a crepuscular profile with matutinal bias (left) or a cathemeral activity with a crepuscular matutinal rise (right) and mean directions symmetrically oriented toward twilight hours; **(M4A) Homogenous axial bimodal** represents, in this case, a cathemeral activity with the same values for κ_m (same bandwidths), opposite mean directions and $\lambda \neq 0.5$; **(M4B) Axial bimodal** also illustrates a cathemeral chronotype with different values for κ_m (different bandwidths), **(M5A) Homogenous bimodal** represents a cathemeral activity similar to M4A**,** but the mean directions oriented to non-opposite hours; **(M5B) Bimodal** represents a cathemeral chronotype similar to M4B, except that the mean directions are not opposite.

2.7.3 Indices of diurnality and crepuscularity

Birdwatchers know that the twilight hours are one of the best times of the day to observe birds, regardless of whether they are diurnal or truly crepuscular. One of the reasons for increased activity during twilight is that ambient temperature is mild; also, there is still a good light level that allows various bird species to have the first or last feeding time, depending on their temporal niches. Here we used diurnality and crepuscularity indices described by Ensing and colleagues (2014) to measure diurnal and crepuscular preferences, respectively. The diurnality index ($I_{diurnality}$) is described as the relative difference between activity/hour during the day (act_{day}) and activity/hour in the night (act_{night}).

$$I_{diurnality} = \frac{act_{day} - act_{night}}{act_{day} + act_{night}} \qquad \text{(Eq. 11)}$$

Similarly, the crepuscularity index ($I_{crepuscularity}$) measures the relative contrast in activity between the astronomical twilight ($act_{twilight}$) and the non twilight times ($act_{nontwilight}$).

$$I_{crepuscularity} = \frac{act_{twilight} - act_{nontwilight}}{act_{twilight} + act_{nontwilight}} \qquad \text{(Eq. 12)}$$

The day length was determined by the time between the beginning of the astronomical dawn (when the sun is 18º below the horizon before sunrise) and the end of astronomical dusk (when the sun is 18º below the horizon after sunset). The night was defined as the period after astronomical dusk, and before astronomical dawn, so no indirect sunlight is visible during the astronomical night. The twilight times comprise the beginning of the astronomical dawn until the sunrise as well as the time of the sunset until the astronomical dusk. All the astronomical times were provided by Steffen Thorsen in © Time and Date AS 1995-2021.

Random integers uniformly sampled from 1 to 86400 (86400 s = 24 h) were generated to simulate the crepuscularity and diurnality index. After this, we bootstrap a hundred random distributions. The numbers of values (vector length) matched the counts of the overtly active behaviors of each owl, so we had 600 (6x100) different random with the same length of the owls' activity distributions. The index was calculated using the same methods, and average values were compared to establish a comparison with the real values.

Also, the diurnality ratio (DR) was calculated to compare our results with cathemeral lemurs. DR is given by the ratio of day to night activity reported in many lemurs species studies. Low values of DR suggest that activity is more evenly distributed in 24 hours (Kappeler & Erkert, 2003; Eppley *et al.*, 2015).

2.7.4 Doze and wake conditional probabilities

To quantify the temporal organization of sleep-wake patterns in terms of transition probabilities between periods of sleepiness and wakefulness, we adopted the procedure developed by Wiggin and colleagues (2020). This procedure was originally used to analyze the underlying processes driving sleep and wake states in drosophila on the basis of their binary locomotor behavior (active and inactive), and defines two conditional probabilities: (1) P(Wake), the probability of switching from an inactive state to an active state; and (2) P(Doze), the probability of switching from an active to an inactive state. Interestingly, Wiggin and colleagues (2020) showed that, in flies, P(Wake) and P(Doze) provide reliable and relatively independent indicators of sleep depth and sleep pressure, respectively. In the context of the present study, we computed P(Wake) and P(Doze) as in Wiggin *et al.* (2020). Thus, each raw behavioral record were transformed as a binary time-series $\left\{Y_t\right\}$ taking the values of 0 or 1 when the owl was *asleep* or a*wake*, respectively. $\left\{Y_t\right\}$ was sampled at an equally spaced, discrete set of time points $t = 1,2,\cdots,T$ such that $t_{i+1} - t_i = 1\,\mathrm{s}$. P(Wake) and P(Doze) were then calculated in adjacent, 1-min long, sliding windows of $\left\{Y_t\right\}$, denoted $\left\{y_t\right\}$. In more formal terms, P(Wake) and P(Doze) were conceived as conditional probabilities of a first-order, two-state, discrete Markov process, such that

$$P(Wake) = P\left(y_t > 0 \mid y_{t-1} = 0\right) \qquad \text{(Eq. 13)}$$

and

$$P(Doze) = P\left(y_t = 0 \mid y_{t-1} > 0\right) \qquad \text{(Eq. 14)}$$

In practical terms, P(WAKE) was computed by dividing the total number of instances when bins scored as *asleep* state were immediately followed by a bin of *awake* state by the total number of transitions from the *asleep* state. Note that, to calculate the denominator of this division, all *asleep*-state bins were counted without considering the last bin of the running window, since no transitions can be established for this bin. P(Doze) was calculated as P(WAKE), except that *awake* and *asleep* states were reversed. Figure 2.1 gives examples of P(Doze) and P(WAKE) calculations for different patterns of sleep/wake transitions.

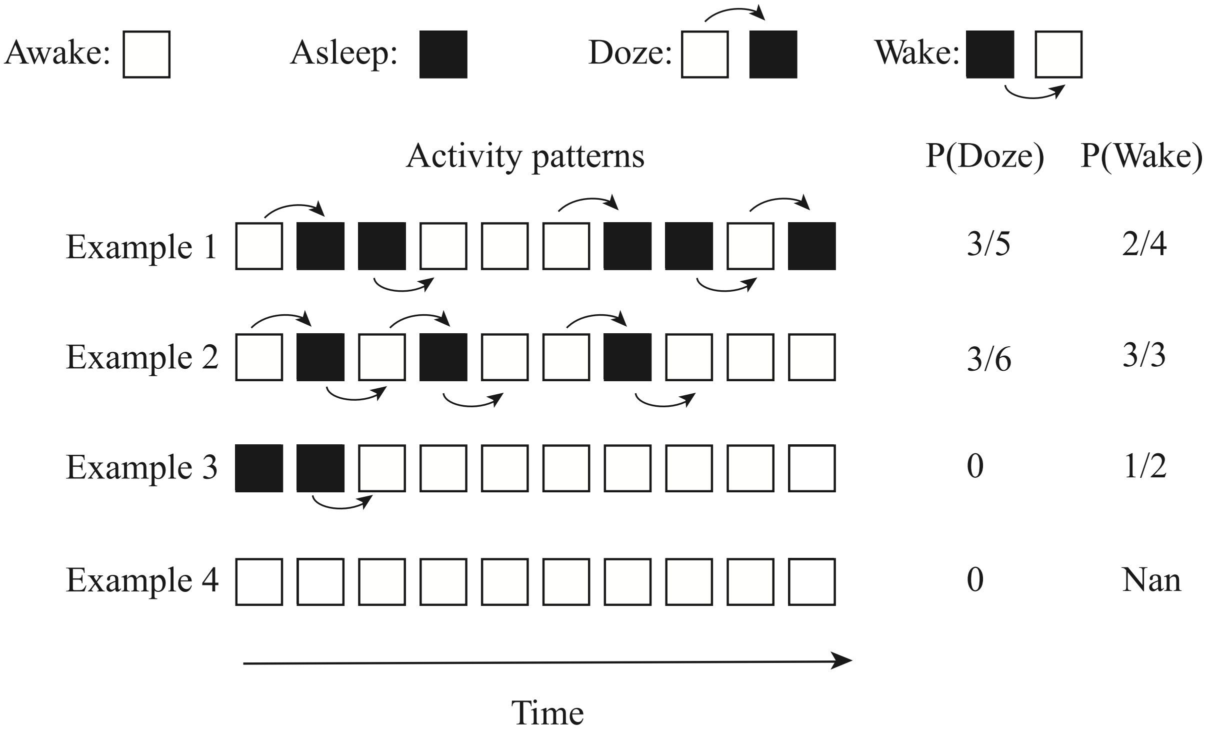


Figure 2.4. Illustrative examples of P(Doze) and P(Wake) calculations.
Transitions from wake to sleep states (Doze) are presented by curved arrows above the blocks. Arrows indicating transitions from sleep to wake states (Wake) are shown below. Calculations for each example of P(Doze) and P(Wake) are shown on the right. White squares: Awake, Black squares: Asleep, Nan: not a number.

2.7.5 Event-triggered analysis of sleep-related behavior

We examined how behavioral markers (e.g., isolated blink, rest pose, head saccades) vary with the elapsed time from binocular eye closure onset (“Before BEC”) and offset (“After” BEC), as described in Hillar and colleagues (2018). First, we aligned the onset of BECs and counted five minutes before BEC onset in a resolution of 1-second bin so that each row before BEC onset corresponds to 300 seconds. Each bin containing the behavior we want to evaluate was assigned as 1 and those without as 0. All rows were added together and divided by the number of BECs to calculate the probabilities. Each point plotted corresponded to the mean of 5 consecutive bins (5 seconds), resulting in 60 points per owl. As an example, we will consider an illustrative case where we had only three BEC occurrences and counted 10 bins (10 s) before and after each occurrence.

Probability of isolated blinks before BEC

Probability of isolated blinks after BEC

1	0	1	0	0	0	1	0	0	1	onset BEC offset	0	0	1	1	1	0	1	0	1	0
0	0	1	1	0	0	1	0	1	1	onset BEC offset	0	0	1	0	1	0	0	0	1	0
0	0	0	0	0	1	0	1	1	1	onset BEC offset	0	0	1	1	0	0	0	0	1	1
1/3	0/3	2/3	1/3	0/3	1/3	2/3	1/3	2/3	3/3		0/3	0/3	3/3	2/3	2/3	0/3	1/3	0/3	3/3	1/3

sum of aligned bins / # of BEC

sum of aligned bins / # of BEC

Figure 2.5. Illustrative examples of probabilities of isolated blinks before and after BEC.
Left: Binary score indicating occurrences of isolated blinks 10 seconds before BEC onset. **Right:** Binary scoring of isolated blinks after BEC offset for 10 consecutive seconds. Below each column, probabilities were calculated as the sum of the aligned bins divided by the number of BECs.

2.7.6 Burstiness and memory metrics of inter-saccade interval

The head saccades account for approximately 46% of all overtly active behaviors and continually occur throughout the day and night. As a first step in examining the temporal organization of sleep-wake patterns, we chose the most representative behavior of the awake state: the head saccades alone.

A wide range of mathematical methods exist to assess the temporal structure embedded within time series. Inspections of the distribution of inter-saccade intervals (ISI) systematically showed a skewed, heavy tailed profile, rejecting-the hypothesis that the behavioral dynamics under scrutiny can be modeled as an homogeneous process, that is, a process in which the events occur at an overall constant rate, but are independent of one another (i.e. Poisson process).

To quantify this irregular, non-homogeneous temporal structure of ISIs, we used a simple, yet powerful, method developed by Goh and Barabási (2008). This method was introduced to characterize the properties of complex intermittent systems such as fluctuations, earthquakes and e-mail communications. Thus, for a given sequence of n ISIs, denoted $\left\{\tau_i\right\}_{i=1,\ldots,n}$, we computed the burstiness index (B), given by

$$B = \frac{\sigma_i - \left\langle\tau_i\right\rangle}{\sigma_i + \left\langle\tau_i\right\rangle} \qquad \text{(Eq. 15)}$$

where $\left\langle\tau_i\right\rangle$ and σ_i are the mean and the standard deviation of $\left\{\tau_i\right\}_{i=1,\ldots,n}$, respectively. B can be conceived as the dispersion of $\left\{\tau_i\right\}_{i=1,\ldots,n}$ at time scale $\left\langle\tau_i\right\rangle$. It is defined within a finite range bounded between -1 and 1. A steady-state time series with equal inter-event times has a B of -1 (periodic system). A Poisson process has an intermediate $B \simeq 0$. The longer and fatter the ISI probability distribution, the higher are B values ($\sim$1).

On its own, B is not sufficient to fully understand the origin of heavy-tailed ISI distribution. The time ordering of ISI values also needs to be considered. Following the method developed by Goh and Barabási (2008), this temporal ordering dimension can be quantified by a memory coefficient (*M*) capable of detecting whether temporal correlations are present, even in the case of heterogeneous time series. M is a correlation-based measure defined as a linear (Pearson) correlation coefficient between $\left\{\tau_i\right\}_{i=1,\ldots,n}$ and the interevent time between two consecutive saccades $\left\{\tau_{i+1}\right\}_{i=1,\ldots,n-1}$, mathematically expressed as

$$M = \frac{1}{n-1}\sum_{i=1}^{n-1}\frac{\left\langle \tau_i\tau_{i+1}\right\rangle - \left\langle \tau_i\right\rangle \left\langle \tau_{i+1}\right\rangle}{\sigma_i\sigma_{i+1}} \qquad \text{(Eq. 16)}$$

where $\left\langle \tau_{i+1}\right\rangle$ and σ_{i+1} are the mean and standard deviation of $\left\{\tau_{i+1}\right\}_{i=1,\ldots,n-1}$, respectively. Similarly to the burstiness metrics, M is also bounded between -1 and 1. M values $\simeq 0$ indicate a memory-free, stationary (i.e.Poisson) process. $M >> 0$ means that short IBSs tend to be followed by short ones and long ones by long ones. $M << 0$ is found when when the length of the current IBI is inversely proportional to the length of the previous IBI.

Given that $\left\{\tau_i\right\}_{i=1,\ldots,n}$ series may have at least two different origins, we sometime represented these series in a (*M*-*B*)-space (fig 2.3). As pointed out by Goh and Barabási (2008), natural phenomena like recurrent seismic activities and weather patterns tend to be dispersed along the diagonal of this projection space, suggesting a strong link between burstiness and memory. Results presented in CHAPTER 3 and 5 corroborate this idea.

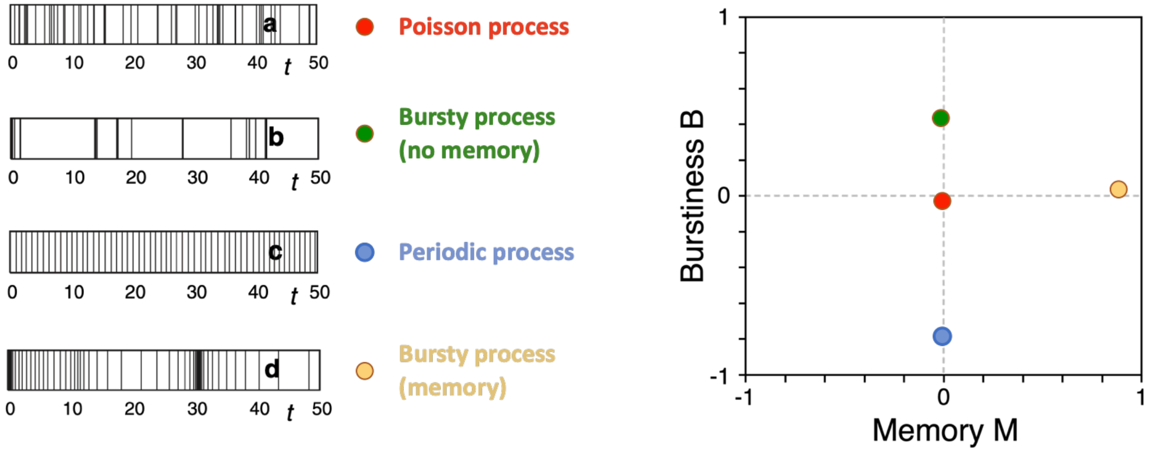


Figure 2.6. Illustrative examples *B* and *M* values for different generative processes. Raster plots on the left represent the timestamps of discrete events driven by a Poisson process **(a)**, a bursty process without first order temporal correlation, or memory **(b)**, a periodic process **(c)**, and a bursty process with a significant amount of memory, that is first order correlation in time **(d)**. Values of *B* and M obtained for these different time series are plotted on the right hand-side (adapted from Goh and Barabási, 2008).

To generate independent event sequences from real data, we randomly shuffled $\{\tau_i\}_{i=1,\ldots,n}$ vectors allowing to change the temporal order between saccade timestamps but keeping the original saccade number unchanged.

2.7.7 General statistics and statistical modeling

Several standard statistical tests were computed using Matlab (version R2016b, MathWorks, Natick, MA) or the JMP statistical software (version 14.3.0, SAS Institute, Cary, NC). For numerical variables, we used the Shapiro-Wilk test to check normality of specific data sets. If normality was verified, we applied a t-test to compare the means of two populations or an ANOVA test if comparisons were made between more than two populations. Otherwise, the Wilcoxon rank sum and Kruskal-Wallis tests were used, as nonparametric equivalents of the t-test and ANOVA tests, respectively. Pairwise comparisons were performed using the Wilcoxon Matched-Pair Signed Rank Test. The Pearson correlation test was used to evaluate the relationship between two numerical vectors. The significance of differences among categorical properties was assessed with the Fisher's exact test or the χ^2 test, depending on the sample size. The significance level used for all tests was $P < 0.05$.

To handle multivariate statistical problems based on count, probability, and duration data, we used a generalized linear mixed model (GLMM) framework. This approach has the advantage of matching the data according to its theoretically expected distribution (e.g., Poisson models for count data) and accounts for the boundaries of the data scale by applying an appropriate monotonic transformation, also known as link function (e.g. log link function for Poisson models, which ensures, in this example, positive fited values). To perform this modeling analysis, we used the GLMM Add-in platform available for JMP (https://JUMP GLMM Add-in).

Proportional data (e.g. % of time spent sleeping in a given time period) were modeled with a binomial distribution and logit (log odd) link function. Duration of behavioral bouts was assumed to be derived from a counting process of consecutive counts. This assumption matches the properties of a count variable since episode duration can only take non-negative integer values. Therefore, to model the duration of behavioral bouts as a function of covariates, we used a Poisson GLMM with a log link function.

Typical fixed GLMM factors (predictors) were *temporalNicheCategory* (categorical set with four levels, i.e. dawn, day, dusk, night) and *behavioral category* (categorical set with two levels, e.g. *overtly active* behaviors and *vigilant pose*). Other typical constituent factors of a GLMM were *sleepDeprivationIntervention* (categorical set with two levels, i.e. before sleep deprivation, after sleep deprivation) and observational block (categorical set with 4 levels, i.e. bock1, …, block4). Since all GLMMs were always constructed with only two fixed predictors, only one interaction term could be assed per model. Models were fitted using a maximum likelihood procedure. We relied on the F-Ratio to test the null hypothesis that a fitted model was unable to explain the variance of a given behavioral response (dependent) variable. The alpha level (type I error rate) was set at 0.05. Least square means (LS-mean) estimated from GLMMs, and also known as marginal means, were reported together with their 95% confidence interval (CI). Multiple comparisons with adjustment were carried out using the Tukey's honestly significant difference (HSD) test.

CHAPTER 3

AROUND THE CLOCK ACTIVITY IN BURROWING OWLS: ROOM FOR CATHEMERALITY?

3.1 INTRODUCTION

The rotational movement of the earth and its inclination towards the sun determine the succession between night and day. This periodic alternation of darkness and light has been a powerful driver in the emergence of the temporal niches of each species throughout evolution (Bennie *et al.*, 2014). Temporal niche is defined as the partition of time where individuals exhibit most locomotory activity and other 24 hour rhythms expressed by active behaviors (Refinetti, 2008; Smarr *et al.*, 2013).

Strictly diurnal and nocturnal temporal niches are at the very end on a continuum of time partitioning strategies that are undoubtedly insufficient to represent the multiple activity patterns displayed by the species (Refinetti, 2008). Apart from these two categories, other two temporal distributions are described in the literature, yet under-explored: the crepuscular and cathemeral niches. Crepuscular animals concentrate most of their total activities in the twilight hours (Halle, 2006). Cathemeral animals are active all day long, including during the twilight (Tattersall, 1987; Bartness and Albers, 2000). In this work, we will focus on the cathemerality, a behavioral trait that, albeit being scarcely investigated in birds, is quite likely to be the temporal niche that most closely portrays the activity pattern of the burrowing owls.

The term cathemeral comes from the Greek roots *kata* (through) and *ημέρα* (the day), meaning that the activity of an animal is distributed approximately evenly throughout the 24 hours or at least a considerable quantity of overt activity, like feeding, locomotion, and other body movements occur all day long (Tattersall, 1987, 2006).

Cathemerality was initially proposed to describe the activity of the Mayotte Lemur (*Eulemur fulvus fulvus*), erroneously categorized as diurnal. Using scan sampling methods, Tattersall observed the Mayotte Lemur during extended periods of day and night and then confirmed his suspicion about this species being nocturnal as well diurnal (Tattersall, 1977, 1979). Unfortunately, the reviewer of Tattersall's manuscript disapproved the introduction of a new terminology, but the evidence-based adherence of other primatologists to the concept led him to formally publish the definition of the term cathemerality (Tattersall, 1987). Since then, several species (in particular lemurs and rodents) have been classified as cathemeral; some of them include Lemuridae species, *Eulemur ssp.*, *Hapalemur ssp*., *Lemur catta,* (Borgognini-Tarli, 2006; Donati *et al.*, 2013; Eppley *et al.*, 2015); arvicoline rodents (voles) (Halle, 1995) and shrews (Merritt & Vessey, 2000), carnivores (Ikeda *et al.*, 2016; Kemna *et al.*, 2020; Rasphone *et al.*, 2020), and others as sloth species, for example (Giné *et al.*, 2015; Castro-Sa *et al.*, 2021). Thus, it is evident that, over the years, systematic studies about cathemerality concentrated mainly on mammal species (Tattersall, 1987, 2006; Halle, 2000).

Cathemerality is unique in two fundamental aspects. First, it involves the undertaking of adaptive awake behaviors in all light conditions. Second, it may accommodate flexible activity schedules, varying from random to rhythmic ones (Halle, 2006). Since a cathemeral animal may exhibit multiple activity patterns and rhythms, it only makes sense to talk about a cathemeral species or a cathemeral activity budget, as opposed to a cathemeral activity pattern or rhythm. Some animals change their pattern of activity along the year but still keep being cathemeral (e.g., cathemeral with peaks activity in the daytime and cathemeral with activity peaks in night-time); others can be cathemeral during only a season or still exhibit a similar cathemeral activity all year long (Halle, 1995; Curtis & Rasmussen, 2006; Tattersall, 2008). Rasmussen (1999) proposed three models of cathemerality: mode A, the species shift from nocturnality to diurnality according to the season; mode B, the species change from diurnal or nocturnal activity to an around the clock active pattern; mode C, the specie is 24 hours active throughout every season.

Considering the foregoing regards, it is clear that cathemerality is a highly plastic temporal niche. Cathemerality may be derived from non-excluded adaptive responses as the need to forage throughout the 24 hours due to energetic constraints or minimizing exposure to adverse conditions such as large open habitats, extreme temperature, and predation pressure (Curtis & Rasmussen, 2006; Donati & Borgognini-Tarli). Accordingly, Bennie and colleagues (2014) reported that cathemeral mammals' richness is greatest at mid-to-high latitudes in the Northern Hemisphere, emphasizing the role of light and temperature in niche partitioning strategies.

It is sound to assume that birds have to deal with similar ecological challenges met by mammals. Interestingly, the cathemeral niche has been proposed as a common ancestor of extant birds (Wu & Wang, 2019). However, there has been scarce systematic research about cathemeral activity in birds. Below, we explore some behavioral and ecological evidence of the burrowing owl that motivated us to assess whether or not the burrowing owl (*Athene cunicularia)* is a cathemeral bird.

Burrowing owls are broadly distributed throughout the American continent from the Western part of Canada to extreme South America, occupying open fields such as prairies, grasslands, as well as anthropic habitats (Burn, 1999). In Brazil, they live in tropical savannas (Cerrado Biome), grazing lands, as well as urban regions, including cemeteries, airports, and parks (Sigrist, 2009).

Burrowing owls in Canada and the US migrate to the south (e.g., Arizona, Oklahoma, New Mexico) during the non-breeding season. Still, non-migratory burrowing owls are also present in Florida and California (Poulin *et al.*, 2020). Burrowing owls living in South America seem to be permanent dwellers. Being a monogamous species, burrowing owls mate throughout the breeding season so that both participate in the incubation and fledging periods (Coulombe, 1971; Catlin *et al.*, 2005; Rodriguez-Martínez *et al.*, 2014). During this time, the female remains next to the burrow all day long, occasionally entering the burrow, while the male plays a role in keeping guard at the site burrow and supplying its partner and brood with food (Coulombe, 1971; Thomsen, 1971; Martin, 1973; Poulin & Todd, 2006).

The burrowing owl exhibits a conspicuous behavior of nesting in burrows excavated by themselves or by fossorial animals (Thomsen, 1971; Martin, 1973). As an opportunistic predator, they are frequently seen catching arthropods close to their burrows in less warm hours of the day (Coulombe, 1971; Marti, 1974; Haug & Oliphant, 1990; Green *et al.*,1993; Chipman *et al.*, 2008). Furthermore, foraging methods may be different across the day (Thomsen, 1971). In the daylight, owls perch near the burrows at a higher position from the ground, enabling them to scan the environment to detect prey (observation foraging) and potential threats (Poulin & Todd, 2006). At night, the owls hunt small mammals hovering or flying most extended distances (Haug & Oliphant, 1990; Motta-Junior & Bueno 2004; Poulin & Todd, 2006; Braga & Motta-Junior, 2009)

Several studies about burrowing owls' ecology are grounded in foraging behavior observations and diets-based partitioning. As a generalist predator, the burrowing owls' diet depends on the availability of the prey (Chandler *et al.*, 2016). The availability of preys is contingent on the collection site, human activities, weather, edaphic factors, seasonal and population fluctuations; therefore, data must be collected from several sites and at different times of the year (Silva Porto and Cerqueira, 1990; Silva *et al.*, 1995; Green, 1983; Green *et al.*, 1993; Teixeira, 2008; Andrade *et al.*, 2010; Chandler *et al.*, 2016). For example, Green (1993) showed a change for more Orthopetera to more coleopteran preys predominance in two successive years. Hall and colleagues (2009) found regional differences in the composition of pellet contents sampled from ecoregions due to a decline in the availability of invertebrates, especially scorpions and sun spiders (nocturnal prey) during the winter.

It has been reported that the most frequent prey items consist of invertebrates ˜ 60-90%, but vertebrates compose the primary biomass of burrowing diet (Gleason and Craig, 1979; Silva *et al.*, 1995; Marti, 1974; Poulin & Todd, 2006; Hall *et al.*, 2009; Chandler *et al.*, 2016). Another general finding the authors agree upon is that owls hunt insects during the day and vertebrates between dusk and dawn. Corroborating with those findings, Poulin & Todd (2006) videotaped massive prey delivered by burrowing owls during the breeding season. The authors reported that the timing of the insect deliveries might be crucial because, in the absence of diurnal insects, nestling burrowing owls would have to wait more than 16 hours between crepuscular feedings (Poulin & Todd, 2006).

It is not surprising that pellet contents may provide valued information about the prey ingested by owls (Errington, 1930, 1932; Marti, 1974). However, Thomsen (1971) reported that pellet contents of burrowing owls are very fragmented due to the way they break their prey before ingestion. Furthermore, they cache food for later consuming so pellets may be a poor indicator of a comprehensive study of burrowing owls' feeding ecology (Haug, 1985; Thomsen, 1971; Poulin & Todd, 2006) unless combining data from other methods are pooled to quantify better and qualify the owls' diet (Haug, 1985; Plumpton & Lutz 1993; Lewis *et al.*, 2004).

Since foraging for insects does not necessarily require large movements, the conclusions about the temporal niche of burrowing owls based on hunting behavior indicate that they are crepuscular-nocturnal owls (Haug & Oliphant, 1990). In the same study, however, the authors claim that burrowing owls are notable for their activity patterns because, although they do not fly long distances during the day, they opportunistically hunt diurnal prey while guarding their territory (Haug & Oliphant, 1990).

Ecology literature reveals a confusing picture concerning the temporal niche of burrowing. According to some studies, burrowing owls are nocturnal-crepuscular birds (Thomsen, 1971; Haug & Oliphant, 1990; Green *et al.*,1993; Valdez-Gómez *et al.*, 2018), while others consider them to be diurnal-crepuscular owls (Coulombe, 1971; Poulin & Todd, 2006; LaFever *et al.*, 2008). On the other hand, Marti (1974) mentioned that burrowing owls were active at almost all hours of the day but this information is not detailed.

Under laboratory-controlled conditions, Coulombe (1971) reported that burrowing owls were mainly diurnal-crepuscular at mild room temperature (35°C), switching to a nocturnal profile at temperatures above their thermoneutrality (41°C). Also, the 24-hour polysomnographic study revealed that the sleep-wake cycles of burrowing owls were spread widely across the day (Berger and Walker, 1972), a fact that suggests they could be cathemeral.

The lack of general agreement about the activity pattern of burrowing owls comes from the difficulty in obtaining reliable quantitative measures throughout 24 hours. Also, changes in the activity pattern of the burrowing owl occur as a function of breeding and non-breeding seasons, dry or rainy weather, snowy winter, high or low temperatures (Coulombe, 1971; Thomsen, 1971; LaFever *et al.*, 2008; Valdez-Gómez *et al.*, 2018). Another issue to consider is that several of ecological studies focused on how burrowing owls forage and what type of food they consume. This bias may lead to inconsistent assumptions about the temporal niche of this owl species. At last, part of the inconsistency concerning the activity mode of the burrowing owl presumably also comes from the fact that most of the methodological approaches were not designed to comprehensively tackle the problem, thus resulting in a lack of information that leaves room for different interpretations.

Furthermore, it is crucial to bear in mind that burrowing owls are motionless most of the time, surveying the environment like a sentinel (Coulombe, 1971; Haug & Oliphant, 1990; Mrykalo, 2005; Chipman *et al.*, 2008; LaFever *et al.*, 2008; Scobie *et al.*,

2014) or sleeping with their eyes open in an upright posture (Berger & Walker, 1972). On the field, these characteristics may confuse not only the prey or predators, but also the researchers who wish to ascertain whether burrowing owls are sleeping or awake.

With this in mind, our approach was straightforward, especially to establish whether burrowing owls are cathemeral birds. We use a 24-hour video scoring methodology to discern their rich behavioral repertoire observed during awake and sleep-like states. Due to the fine detail of our video observations, we were able to identify behaviors that were correlated to the animal's level of activity, with the benefit that the owls were outside the laboratory in a more natural context.

3.2 RESULTS

During the days our observations were made, the average time (hh:mm ± SD) of sunrise was 06:24 ± 00:06 while sunset occurred at 17:38 ± 00:04, resulting in a mean day-length of 11:14 ± 00:10. Using the astronomical reference of sun angular position, the average duration (hh:mm ± SD) of twilight was 01:16 ± 00:01, with dawn starting at 05:07 ± 00:04 and ending at 06:23 ± 00:06 and dusk beginning at 17: 38 ± 00:04 and ending at 18:54 ± 00:02. Owls were thus exposed to similar ambient light conditions.

Out-of-sight and *owl's eye-out-of-camera* coding represented only 0.153% and 6% of the total observational sample, respectively. In the analysis, this particular small part was not included, but this fact does not change the results since our observations were based on 518400 seconds video recording, scored at a resolution of 1 second.

3.2.1 Pose control analysis

As a starting point for this study, it is essential to consider that all inactive behaviors, i.e., motionless behaviors, constituted roughly 60% of the total 24 hours. However, our video recordings, which allowed us to observe the owls' behavior in great detail, revealed a clear difference between two characteristic and recurrent static postures, each presumably reflecting a distinct internal state. An erect upright posture with wide-opened eyes and a protruding neck that exposes the white ruff of the burrowing owl (Fig. 3.1 A, left-hand side picture). This posture, designated as *vigilant pose*, was concomitant with an increase in *overtly active* behaviors, head saccades being the most frequent of these behaviors. The *vigilant pose* contrasted with another prevalent static stance, herein referred to as *rest pose*, and identified by an overall body relaxation resulting in a diminution of the apparent height of the bird, a retracted head/ neck, partially or entirely hiding the white ruff, and feathers fluffed-up (Fig. 3.1.A, right-hand side picture). This resting posture was frequently accompanied by partial eyelid closure and was, de facto, quite reminiscent of the posture adopted by burrowing owls when in NREM and REM sleep (Berger and Walker, 1972).

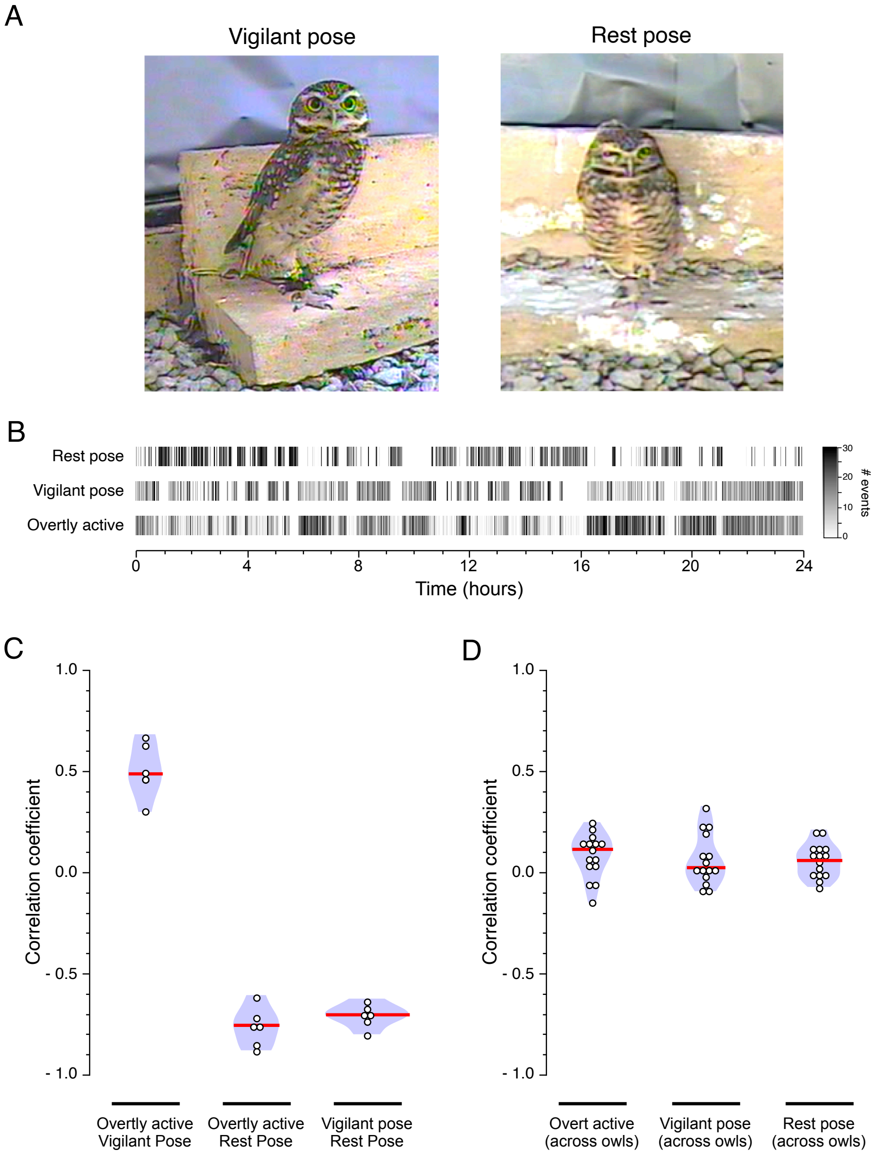


Figure 3.1. Strength of the relationship between *overtly active* events and *inactive pose* events. **(A)** Example of a vigilant pose and a rest pose**. (B)** Representative example (owl AC1408) of the number (#) of behavioral event occurrences (see scale bar) per unit of time (1-min binning) for behavioral category (overtly active, vigilant pose, rest pose) over 24h. All correlation data presented in (B) and (C) are based on this sampling strategy. **(C)** Within-owl Pearson correlation coefficients (abscissa) between behavioral variables (ordinate). **(D)** Across-owl Pearson correlation coefficients computed for each behavioral category. Blue shadings are violin representation of the distribution shape of correlation coefficient samples (white dots). Red horizontal lines indicate the population median of correlation coefficient samples.

As detailed further below, over a daily cycle, the *vigilant pose* was almost as prevalent as the *overtly active* behaviors grouped together, and represented about 30% of all the behavioral repertoire exhibited by the owls. Let us take for granted that this posture effectively represents a covert expression of tonic alertness or sustained attention states. Should this be the case, it is reasonable to predict that its diel fluctuation would go hand-in-hand with that exhibited by *overtly active* behaviors, and that no such pattern would be seen in relation to the the *rest pose*.

A close inspection of figure 3.1.B, which plots a representative example of the rate of o*vertly active* behaviors, *vigilant pose,* and *rest pose* in 1-min bins during a full daily cycle, suggests that this may indeed be the case. To quantify the relationship among these three behavioral variables, we computed the within-animal Pearson correlation of their time-dependent rate (15-min binning). We found that there was a significant positive correlation between *overtly active* behaviors and the *vigilant pose* (r_{575} = 0.49, $P < 0.0001$). The *rest pose*, on the other hand, was significantly anti-correlated with *overtly active* behaviors($r_{575} = -0.77$, $P < 0.0001$) and the *vigilant pose* ($r_{575} = -0.68$, $P < 0.0001$). There was no statistical difference between the two anti-correlated comparisons (Wilcoxon Signed-Rank test, $S_5 = 3.5$, $P = 0.56$). These results indicate a robust, dissociable profile of temporal interdependences, which reinforces the validity of our behavioral scoring scheme whereby the assignment of wake and sleep belonging states was in part based on postural criteria.

Figure 3.1 C shows the distribution of Pearson correlation values computed across birds for *overtly active* behaviors (median = 0.118, IQR = 0.12, n = 15), the *vigilant pose* (median = 0.02, IQR = 0.21, n = 15) and *rest pose* (median = 0.06, IQR =

0.12, n = 15). According to the Wilcoxon matched-pair signed-rank test, there was no statistical differences between correlation median values. Though close to 0, indicating absence of correlations, are these median values actually different from 0? Under the null hypothesis that the answer to this question is yes, the Wilcoxon signed-rank test revealed that this hypothesis could be rejected for *overtly active* behaviors ($S_{14} = 43$, $P = 0.012$) and the *rest pose* ($S_{14} = 37$, $P = 0.035$) but not for the *vigilant pose* ($S_{14} = 30$, $P = 0.094$). Thus, we conclude that at relatively fine grain resolution of 15 mins, owls exhibit a considerable individual dynamic in active and inactive behavioral patterns.

3.2.2 Circular distribution of awake, vigilant behaviors

The round-the-clock nature of our experimental independent variable (time) prompted us initially to characterize the daily distribution of owl's awake budget using a circular analytical approach.

Figure 3.2.A shows the distribution of raw counts of *overtly active* behaviors throughout a daily cycle for each owl. Model selection results for this dataset are reported in table 3.1.

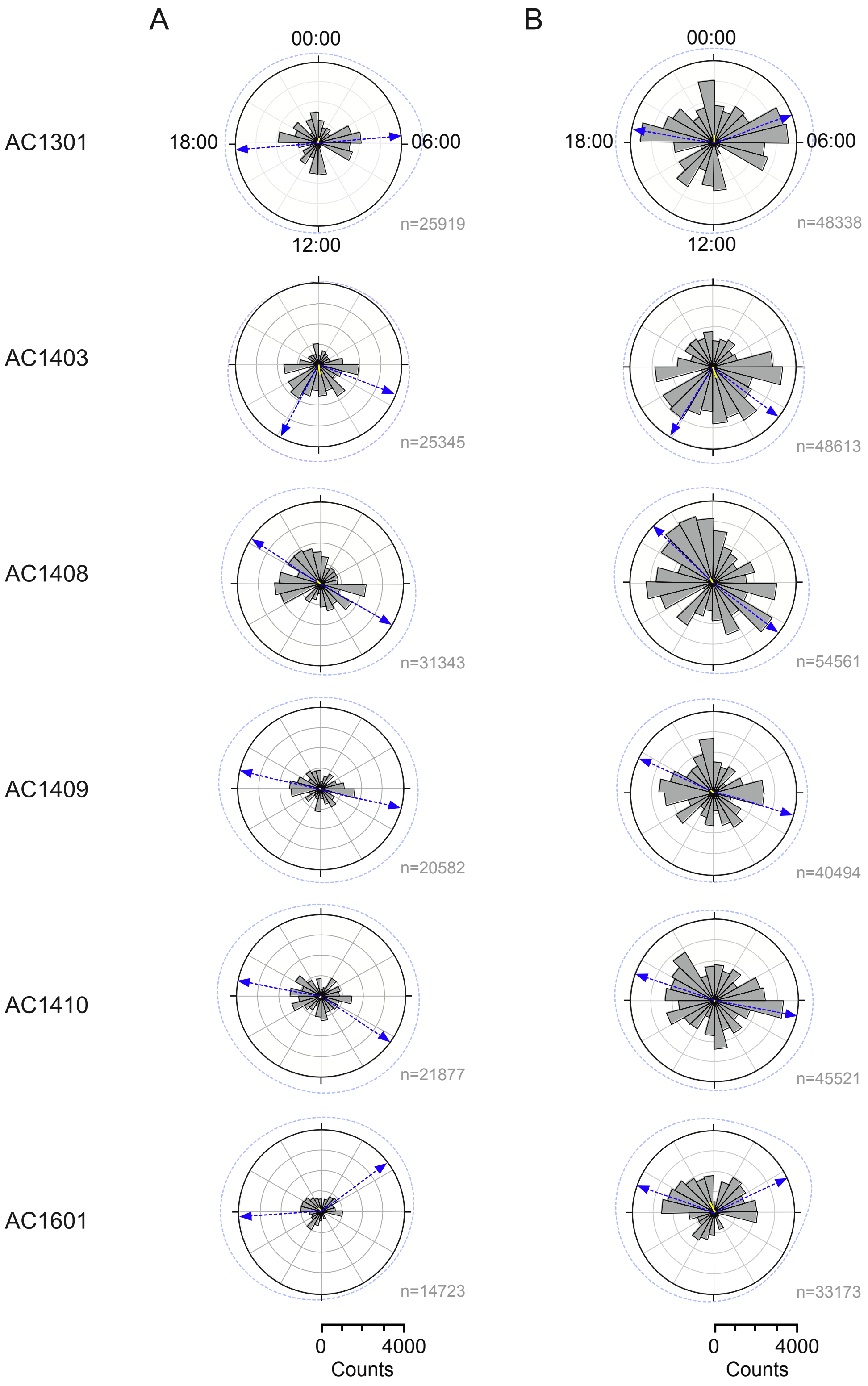


Figure 3.2. Circular distribution of overtly active behavior and the awake state.
The circular histograms (gray bars) represent the quantification of behaviors in bins of 60 minutes. The density (dashed line) and mean direction(s) (dashed blue arrows) of the best model are provided. The

yellow line represents mean vector of the circular histogram. **(A)** The overtly active behaviors show a bimodal distribution for all animals (AC1409, AC1301: M4B; AC1403, AC1601:M5A; AC1410, AC14081:M5B). The owls also present peaks of these behaviors close to the twilight hours. **(B)** Circular distribution of the awake state. The sum of all active behaviors was extensively spread out all over the 24 hours. All animals present as best fitting the bimodal model (M5B).

For all owls, the hypothesis of a random uniform distribution was rejected by the Rayleigh's test ($P << 0.05$). This finding was coherent with the fact that bimodal models prevailed over amodal and unimodal models in fitting the data. The von Mises modes of the best fits often occurred during or close to twilight hours. For two owls, an axial distribution peaking during at dawn and dusk was obtained (AC1409, AC1301: **M4B;** mean direction ($\varphi_{1,2}$): 5:41,17:41 and 6:52, 18:52). Yet, to infer that these two owls were crepuscular was not appropriate given that their activity was widespread outside twilight periods. This feature was indicated by the activity bandwidth parameters ($\beta_{1,2}$), which encompassed more than 6 hours for one von Mises distribution and 22 hours for the other, and was accentuated by the fact that, as determined by λ, the broader bandwidths represented 75% of the activity budget. For the four remaining owls, bimodal non-axial models were preferred (AC1403, AC1601 : **M5A**;$\varphi_{1,2}$: 7:25,13:50 and 03:44,17:52; AC1410, AC1408: **M5B;** $\varphi_{1,2}$: 7:57, 18:44 and 8:13, 20:09).

Table 3.1. Model selection summary results for *overtly active* behaviors

Analysis	Variable	Owl					
		AC1301	AC1403	AC1408	AC1409	AC1410	AC1601
Sample size	n	25919	25345	31343	20582	21877	14743
Rayleigh test	R	6,18E-02	0,247	1,08E-01	4,76E-02	8,38E-02	1,75E-01
	P	8,97E-44	0,0	2,05E-162	5,25E-21	1,64E-67	1,94E-197
Model ΔAIC	M1	1,61E+03	3,21E+03	2,69E+03	1,10E+03	1,22E+03	1,69E+03
	M2A	1,42E+03	62,3	1,95E+03	1,01E+03	1,22E+03	7,85E+02
	M2B	1,45E+03	2,27E+02	1,80E+03	9,94E+02	9,36E+02	8,57E+02
	M2C	1,51E+03	1,01E+02	6,39E+03	1,45E+03	9,91E+02	1,27E+03
	M3A	1,27E+03	3,18E+03	8,04E+02	1,57E+02	4,32E+02	1,08E+03
	M3B	1,22E+03	2,40E+02	2,64E+02	70,5	3,61E+02	8,79E+02
	M4A	1,25E+03	5,27E+02	1,13E+02	86,2	3,82E+02	7,67E+02
	M4B	**0,0**	3,06E+02	13,3	**0,0**	2,80E+02	4,48E+02
	M5A	1,08E+03	**0,0**	99	87,8	1,10E+02	**0,0**
	M5B	80,1	1,07E+03	**0,0**	1,17E+03	**0,0**	8,07E+02
BM angle	φ_1	5h41	13h50	7h57	6h52	18h44	17h52
	φ_2	17h41	7h25	20h09	18h52	8h13	3h44
BM width	β_1	6h20	17h03	9h44	22h32	9h01	6h48
	β_2	22h52	17h03	15h05	8h10	20h15	6h48
Lambda	λ	0,25	0,60	0,28	0,74	0,27	0,59

The sample size (n) refers to the count of behaviors for each owl. The Rayleigh test summary shows the mean vector magnitude (R) and p-value (P). ΔAIC refers to the difference model AIC value relative to the best model. Best models are indicated in bold. BM angle (φ_m) refers to the best model mean direction angle expressed in hours (h: min). BM width (β_m) is the best model bandwidth around the mean concentrating 95% of data, given in hours (h:min). Lambda (λ) represents the proportional size of the first distribution. See the Materials and Methods section for more detailed information.

In the case of owl AC1403, the two von Mises distribution modes occurred during daytime, indicating that this individual had a more diurnal activity profile. Note, however, that there was also a great deal of activity during the night, as indicated by $\beta_{1,2}$ = 17:05 and λ = 0.60. On the other hand, Owl AC1601 spent a great deal of its activity during the night, but again, also showed considerable activity during the day (a peak at the beginning of the morning and other after midday) plus the twilight period. Our interpretation of this pattern corresponds to a case of cathemerality because activity peaks almost 12 hours apart, and the data is largely dispersed around those peaks, in a proportion of 60/40 in terms of quantity ($\beta_{1,2}$= 6:48; λ = 0.59). Concerning owls AC1408 and AC1410, the M5B model was univocally associated with cathemerality since activity peaks occurred both at day and night, bandwidths were broad 20 hours (= 15:05 and 20:15), and encompassed around 70 % of the activity.

Altogether, the results described above corroborate the hypothesis that burrowing owls are cathemeral. The fact that owls show different bimodal distributions reflects the inter-individual variability and may also represent the inherent flexibility of cathemeral animals.

To get a more complete picture of how owls distribute their time while being effectively awake over a daily cycle, we combined o*vertly active* behaviors and *vigilant poses*, and submitted this grouped data, named *awake state*, to the same circular model selection procedure described above (see figure 3.2 B**,** Table 3.2). The latter yielded M5B as best model for all animals, with most activity peaks ranging between 4:00 and 8:00 or between 18:00 and 20:00. Note that, though adding *vigilant pose* occurrences to *overtly active* behaviors expectedly increased the magnitude of the circular histograms, the shape of their distribution remained pretty similar. Bandwidths around activity peaks for the *awake state* were usually even more widespread than those of *overly active* behaviors. Moreover, the broadest dispersions around peak time contained around 70% of the sample size. As expected, Rayleigh's test rejected the null hypothesis of uniform distribution (P < 0.01) for all animals.

Table 3.2. Model selection summary results for *awake state*

Analysis	Variable	Owl					
		AC1301	AC1403	AC1408	AC1409	AC1410	AC1601
Sample size	n	48338	48610	54561	40494	45521	33192
Rayleigh test	R	1,04E-01	0,168	7,82E-02	7,39E-02	2,74E-02	0,217
	P	1,21E-229	0,0	1,08E-145	7,50E-97	1,18E-15	0,0
Model ΔAIC	M1	3,52E+03	2,82E+03	2,40E+03	1,16E+03	1,05E+03	5,66E+03
	M2A	2,46E+03	49,3	1,74E+03	7,22E+02	9,94E+02	2,51E+03
	M2B	2,56E+03	68,9	1,66E+03	6,96E+02	9,95E+02	2,90E+03
	M2C	2,98E+03	50,6	1,42E+03	6,85E+02	1,42E+03	3,42E+03
	M3A	2,14E+03	2,75E+03	6,69E+02	4,89E+02	1,43E+02	4,60E+03
	M3B	1,93E+03	74,3	1,47E+02	1,57E+02	1,45E+02	2,90E+03
	M4A	2,05E+03	1,50E+02	1,01E+02	1,26E+02	1,45E+02	3,51E+03
	M4B	7,29E+02	54,5	77	75,8	72,7	3,62E+03
	M5A	1,04E+03	19,6	35	52,8	71	8,97E+02
	M5B	**0,0**	**0,0**	**0,0**	**0,0**	**0,0**	**0,0**
BM angle	φ_1	4h42	14h09	20h55	7h03	19h19	4h22
	φ_2	18h36	8h25	8h30	19h38	6h44	19h17
BM width	β_1	9h05	27h54	16h24	11h42	10h19	5h45
	β_2	19h09	13h24	11h26	18h41	21h14	15h36
Lambda	λ	0,25	0,74	0,69	0,25	0,25	0,25

Legend, same as Table 3.1.

Over 24 hours, the total count of *overtly active* and *vigilante pose* behaviors varied across owls (Table 3.1), indicating that some birds were more active than others. To investigate the effect of this variability, we normalized the activity budget of each owl by summing up all behavioral scores of interest into one-hour bins and dividing each hour bin data by the value of the bin that had the greatest number of counts. Figure 3.3 shows the circular normalized distribution of owl population for *overtly active* behaviors (fig. 3.3 A) and *awake state* behaviors (fig. 3.3 B). Neither of those distributions was statistically uniform (Rayleigh's test, *overtly active* behaviors: R_{23} = 3.31, P = 0.0365; *awake state* behaviors: R = 3.24, P = 0.0391) at the 0.05 significance level. But note that the Rayleigh's test P-values of both distributions were close to the rejection zone of the alternative hypothesis of deviation from uniformity. Comparatively, when considering each owl separately, *P*-values were much further away from this rejection zone (P<<0.0001). Thus, removing individual differences in activity level, the mean circadian profile of *overtly active* behaviors and *awake state* behaviors tends to diminish its non-uniform characteristic. Having said that, the relative increase during the twilight periods was still visible. A drop in activity level after midnight and until dawn also became more evident.

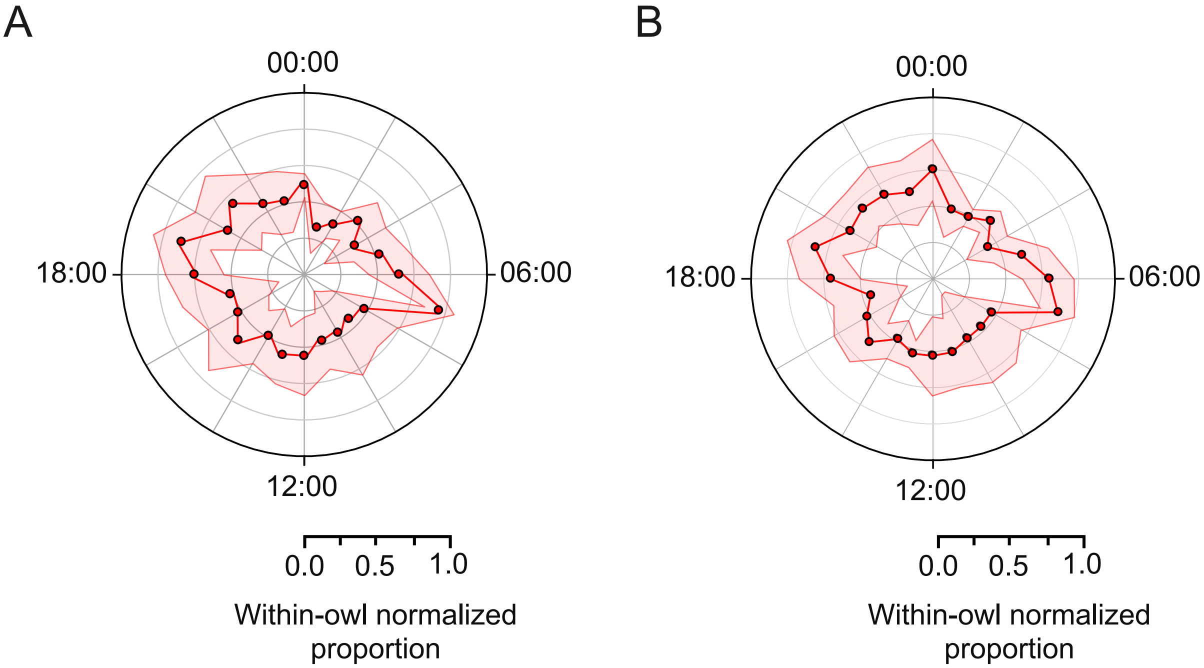


Figure 3.3. Circular distribution of the overtly active behaviors and the awake state.
(A) *Overtly active* behavior proportion: the red dots represent the mean(n=6) in bins of 60 minutes and shadow area, the standard deviation. **(B)** *Awake state* (overtly active behaviors + vigilant pose) proportion: the red dots represent the mean(n=6) in bins of 60 minutes and the shadow area, the standard deviation.

3.2.3 Allocation of wake behaviors into temporal niche categories

Next, we reanalyzed the diel activity patterns of burrowing owls by dividing a daily cycle into four canonical categories: dawn, day, dusk, and night. For some analysis, we also defined four additional sub-categories: morning, afternoon, first half of the night (end of the dusk period until midnight), and second half of night (midnight until the beginning of dawn). Temporal niche categories (and sub-categories) vary in duration. Therefore, for each owl and each *awake state* sub-categories (*overtly active behaviors*, *vigilant pose*), we calculated the proportion of behavioral scores per temporal niche sub-category by dividing the time spent within a given sub-category by the total number of seconds contained in this sub-category.

Figure 3.4 A shows, for each animal, the proportion of *overtly awake* and *vigilant pose* behaviors in six different periods of a daily cycle and hour by hour. Despite some differences among temporal niche sub-categories and some inter-animal variability, it is clear that every owl was awake during a considerable amount of time in all periods of the day. To get a statistical assessment of this dataset, a GLMM analysis was carried out, with the fixed effects being temporal niche category and *awake state*. The LS-mean estimates obtained from this analysis are shown in figure 3.4 B. Temporal niche significantly affected a*wake state* proportions (GLMM, $F_{1,3}$ = 8.28, $P < 0.001$), but not more for *overtly active* behaviors than for *vigilant pose* episodes (GLMM, *awake state* * temporal niche category: $F_{1,3}$ = 1.15, P = 0.33), probably so because no group effect was found for the *awake state* (GLMM, $F_{1,3}$ = 0.74, P = 0.39). This result reinforces the tight temporal association between *overtly active* and *vigilant pose* behaviors described in section 3.3.1. Owls spent 26.7% roughly an equal amount of time being active between day and night.

As shown in figure 3.4 D, the variation in diel activity level described above was explained, at least in part, by changes in the probability of *awake state* episode occurrences across the dawn, day, dusk, and night periods (GLMM, $F_{1,3}$ = 13.6, $P <$ 0.0001). This effect exerted its influence with equal intensity on *overtly active* and *vigilant pose* behaviors (GLMM *awake state* * temporal niche category: $F_{1,3}$ = 0.41, P = 0.60). Although a significant group effect was found for the *awake state* (GLMM, $F_{1,3}$ = 15.1, P = 0.0004), the tendency of *overtly active* episode rate to be higher than that of *vigilant pose* episodes was not supported by our statistical analysis.

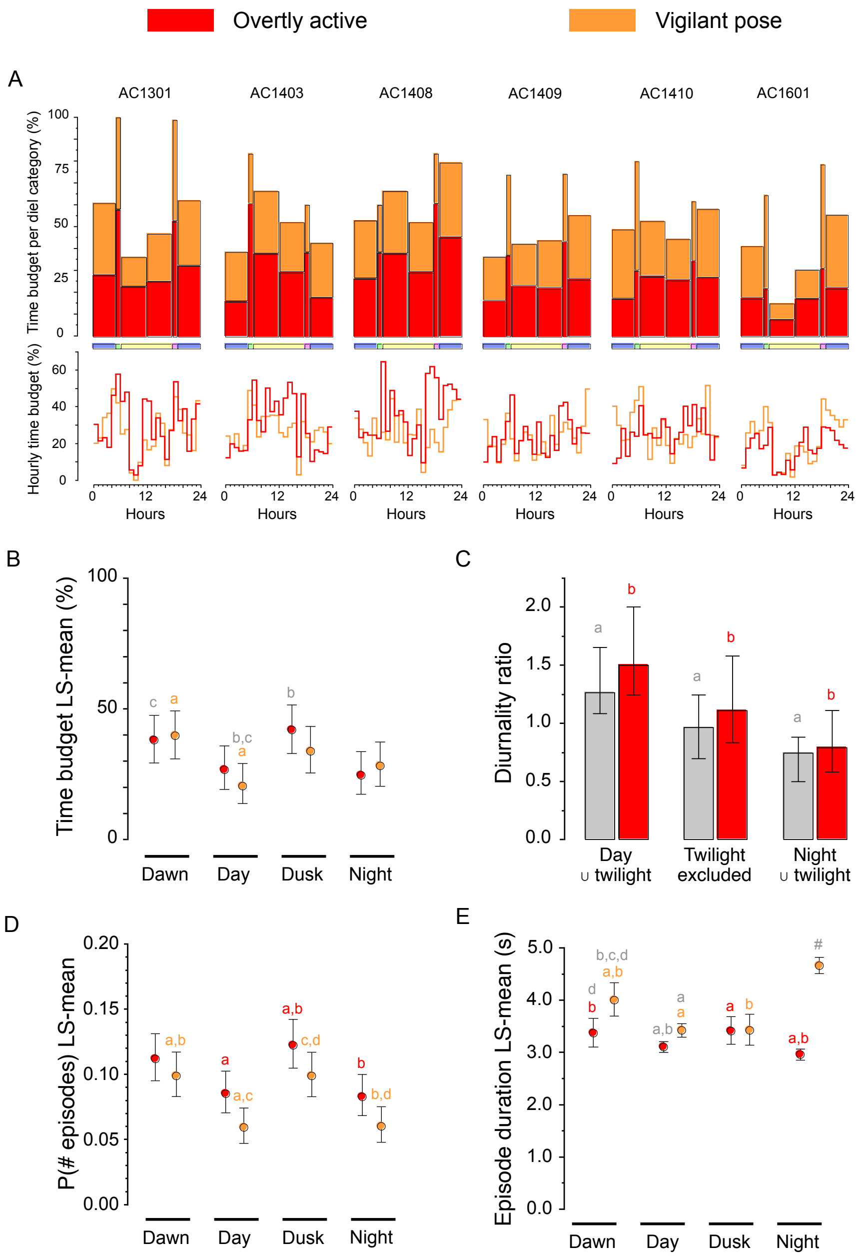


Figure 3.4. Summary of overtly active and vigilante posing states as function of diel category
(A) Proportion distribution of overtly active behavior and vigilant posture by hour (counting the activity of six owls by hour, n=6). The colored horizontal bar between the graphs indicate the periods of night (blue), dawn (green), day (yellow), and dusk (violet). **(B)** Overtly awake behaviors (red) and vigilant posture (orange) proportions for each owl. **(C)** Median (interquartile range) of the diurnality ratio (see materials and methods). **(D)** Mean probability (P) of episode occurrence (#) as a function of diel category. **(E)** Mean duration of episodes in seconds. All central tendency estimates are reported as least-squared means LS-mean). Letters indicates results of Tukey's HSD post hoc comparison. Ls-means connected by the same letter of the same color are significantly different at the 0.05% level. Colored letters denote significance between LS-means estimated for the same behavior following the color code used for this effect; grey letters indicate significance across behavioral categories. # indicates significance between all other means estimates.

In general, a*wake state* episodes lasted a few units of seconds (Fig. 3.4 E). The longest *overtly active* and *vigilant pose* episodes had a duration of 9.5 and 13.8 min, respectively. Only 0.11% of *overtly active* episodes lasted longer than 1 min. For *vigilant pose* bouts, this proportion was even lower (0.03%). The distribution of episode durations (not shown) largely deviated from normality (Kolmogorov-Smirnov test, *overtly active*: D_{45323} = 0.38, P < 0.01; *vigilant pose*: D_{45323} = 0.34, P < 0.01). This distribution was more positively skewed and leptokurtic for *vigilant pose* (skewness = 63.1, excess kurtosis = 6291.7) than for *overtly active* behaviors (skewness = 34.5, excess kurtosis = 1962.6).

Episode duration was significantly altered by temporal niche category (GLMM, $F_{1,3}$ = 19.4, $P < 0.0001$) and depended on *awake state* type (GLMM, $F_{1,3}$ = 65.0, $P < 0.0001$), but there was no consistent pattern among predictor variables (GLMM *awake state* * temporal niche category: $F_{1,3}$ = 41.2, $P < 0.0001$). Differences in episode duration between *vigilant pose* and *overtly active* behaviors were small but statistically significant during daytime (LS-mean difference = 0.31s, lower 95% CI = 0.29s, upper 95% CI = 0.34s; Tukey's HSD, P = 0.0032) and at night (LS-mean difference = 1.70s, lower 95% CI = 1.65, upper 95% CI = 1.75; Tukey's HSD, $P < 0.0001$), but not at dawn and at dusk. At night, the marginal mean of *overtly active* episode duration decreased in relation to that estimated at dusk (Tukey's HSD, $P < 0.0225$), but was not significantly different to that estimated during daytime (Tukey's HSD, $P = 0.52$) and at dawn (Tukey's HSD, $P = 0.08$). Also, there was no significant difference in probability of *overtly active* episodes between night and day (Tukey's HSD, $P = 1.00$). Therefore, it is unlikely that the nocturnal increase in *vigilant pose* bout duration was simply due to a fragmentation of overtly active behaviors, but rather, a self-driven process of consolidation of alert static poses.

A diurnality ratio (DR) was calculated to be able to compare our results directly with those of several studies on cathemeral lemur species. DR was given by the ratio of day to night activity. Thus, values close to 1 indicate more evenly distributed activity in 24 hours (Kappeler & Erkert, 2003; Eppley *et al.*, 2015). As shown in figure 3.4 C, owls' DR medians for *overtly active* behaviors only were lower than those calculated the *awake state*. Using the matched-pair Wilcoxon signed-rank test, we verified that this difference was statistically significant when twilight hours were considered as part of the day ($S_5 = 10.5$, $P = 0.0313$) or excluded from the analysis ($S_5 = 10.5$, $P = 0.0313$), but not when they were deemed constituents of the night ($S_5 = 9.5$, $P = 0.0625$). DR medians also changed considerably depending on whether the matutinal and vespertine twilight were considered as part of the night or part of the day. Some authors have included twilight time into nighttime, others as part of the day given that some form of lower atmosphere illumination does occur. The problem is that, tough lasting only about 1 hour and 16 min, twilight periods carry a considerable amount of activity level, biasing

DR values. To circumvent this problem, we removed the twilight periods from the analysis. In this case, the proportion of activity between day and night was found to be almost the same, resulting in a DR median close to, and not significantly different from one, for both *overtly active* behaviors (median = 1.11, Wilcoxon signed-rank test, H_0: median $\neq$ 1, $S_5 = 3.0$, $P = 0.62$) and *awake state* (median = 0.96, Wilcoxon signed-rank test, H_0: median $\neq$ 1, $S_5 = -1.5$, $P = 0.84$). These results reinforce the idea that burrowing owls are indeed cathemeral birds (see Discussion for comparisons with the Lemur data)

We also assessed the preference of burrowing owls for crepuscular, diurnal, and nocturnal temporal niches employing two additional indices: the crepuscularity index ($I_{crepuscularity}$) and the diurnality index ($I_{diurnality}$). Both indices range from 0 to 1 (see Materials and Methods, section 2.7.3). They indicate a preference to be more active during crepuscular or diurnal hours. If the values are close to zero, the trend is weak, and corroborates the cathemeral nature of the animal. We compared these indices to simulated index values generated from a random uniform distribution (see Materiais and Methods). Results for $I_{crepuscularity}$ and $I_{diurnality}$ are discussed below, but were not plotted in any figure.

The diurnality index of one owl (AC1403) indicates a tendency to be more diurnal ($I_{diurnality}$= 0.38), as also is pointed out by the circular model parameters. However, for the majority of owls, $I_{diurnality}$ is next to zero, resulting in a mean, close to zero (n=6; mean=0.07 ± 0.17). There was no difference between the experimental values and the shuffled ones (Paired-Ttest = 1.02; P = 0.329), which indicates that owls are neither predominantly diurnal nor nocturnal. The average crepuscularity index for all owls was positive at about 0.2 (mean= 0.22 ± 0.09), which is not surprising since, on average, they are 60% more active during twilight hours. The shuffled $I_{crepuscularity}$ was close to zero (mean = 0.0004 ± 0.0002) and was statistically different from the experimental values. This can be explained by the fact that each random distribution was flat, i.e., they do not exhibit any peak as the distribution with twilight peaks (Paired-Ttest= 5.60; P=0.0002). The fact that we did not find $I_{crepuscularity}$ index values too far from zero is a good indication that burrowing owls are cathemeral with heightened activity levels during twilight, a pattern exhibited by many cathemeral animals.

3.2.4 Detailing overtly active behaviors

On average, the overtly active behaviors, i.e., locomotor and other body movements, occupied 27.1 ± 6.6 % of the 24 hours (n=6). Head movements (saccades alone + saccades with blink + other head movements) are the most frequent of behaviors representing about 49% of the total duration of all *overtly awake* behaviors. In comparison, locomotor behaviors occupy 10 % of a daily cycle.

Figure 3.5 shows the LS-mean % occupation of *overtly active* behaviors in function of diel categories. Although some behaviors seem to occur more during the daylight, such as saccade with blink and cleaning behavior, or during the night and twilight such as the feeding, none varied significantly across diel categories. In actual fact, this predictor had no significant influence on the variance of behavioral responses (GLMM, $F_{6,3} = 0.50$, $P = 0.68$). On the other hand, a significant effect was found for the behavior type category (GLMM, $F_{6,3} = 42.3$, $P < 0.0001$), but in a non systematic manner across diel categories (GLMM, behavioral type * temporal niche category: $F_{6,3} = 41.2$, $P =$

0.029). Multiple comparisons using Tukey's HSD test showed that owls dedicated significantly more time performing head saccades than any other behaviors, irrespective of the daily period.

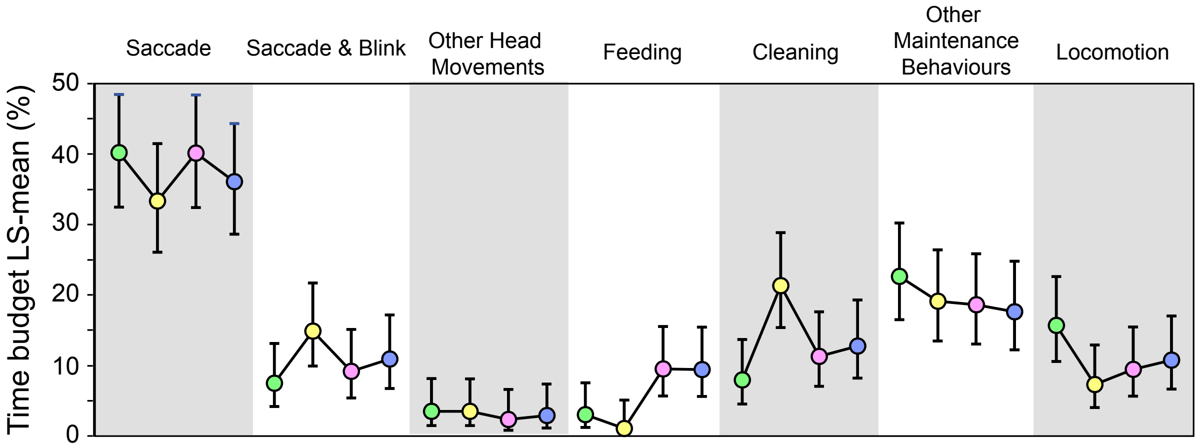


Figure 3.5. Percentage of overtly active behaviors in different periods of the day.
Dawn (green circle) , day (yellow circle), dusk (rose circle) and night (blue circle). The graph illustrates the percentage of total time spent in each diel category for different active behaviors. All the central multiple comparisons of least square means estimated were performed using post hoc Tukey's HSD test.

From a statistical perspective, the success rate for detecting reliable differences across predictor levels may be reduced due the limited number of owls participating in this study, the lack of repeated measures, and the variability of behavioral types among individuals. This variability is likely to increase as one proceeds with a more fine-grained decomposition of behavioral types, but is a *sine qua non* dimension to assess animals' personality and plasticity. In the analysis described below, we eliminated this inter-individual variability by lumping the data from each owl together. Comparisons between specific categorical levels were performed using the χ^2 test. Thus, the inferences drawn from this analysis may be thought as a way to characterize variations in behavioral traits that may be subsequently useful for making comparisons at an inter-specific level.

Figure 3.6 depicts the raw counts of each *overtly active* behavior ranked in decreasing order, and the cumulative proportion of these counts, for relevant pairs of diel categories. Considering first the most relevant day-night pair (fig. 3.6 A), it is interesting to observe that, even in captivity, the time budget of locomotor and feeding behaviors significantly increases during the night (*locomotion*: χ^2 = 317.7, P < 0.0001; *feeding*: χ^2 = 3850.4, P < 0.0001). In consonance with the intensification of *vigilant pose* at night described earlier (fig. 3.4 E), a small but significant increase in the proportion of head movements without blinks (*saccades alone*) was found when compared to daytime (χ^2 = 44.89, P < 0.0001). Quite the opposite is seen when considering the proportion of saccades associated with blinks (referred below as *blink-saccade synkinesis)*. There was also an increase in the proportion of *blink-saccade synkinesis* in the afternoon as opposed to morning (fig. 3.6.B; χ^2 = 219.3, P < 0.0001). During the day, mainly in the morning, owls spend more time exhibiting cleaning behaviors (day vs night, χ^2= 1010.3, P < 0.0001; morning vs afternoon, χ^2= 991.9, P < 0.0001).

Comparing the two twilight periods (Fig. 3.6. C), there was no significant difference in proportion of saccade alone (χ^2= 0.57, P = 0.57). There was a small but significant difference in *blink-saccade synkinesis* (χ^2= 22.85, P < 0.0001). The proportion of feeding behavior was greater during dusk, suggesting that owls started to feed during the transition of day to night. It is necessary to keep in mind that, in its natural setting, the major feeding behavior, consumption of small mammals, occurs at night. The proportion of locomotor behaviors was significantly greater at dawn (χ^2= 227.27, $P <$ 0.0001).

During the first half of the night, *locomotion* and *feeding* occurred more frequently than in the second part (fig. 3.6. D; *locomotion*, χ^2= 49.63, $P < 0.0001$; *feeding*, χ^2= 19.94, $P < 0.0001$). On the other hand, differences in the proportion of *saccade alone* and *blink-saccade synkinesis* were significant during the second part of the night compared to the first part (*saccade alone*, χ^2= 4.09, $P = 0.0429$; *blink-saccade synkinesis*, χ^2= 132.43, $P < 0.0001$).

It is crucial to bear in mind that the differences reported in figure 3.6 are minor, of the order of 5%. Still, overall, they are consistent with the idea that burrowing owls are more or less equally active across all diel categories. At the same time, it points to some preferences that match what is reported in the wild. For example, owls are frequently seen foraging (i.e., behaviors related to locomotion and feeding) during the twilight hours and the night.

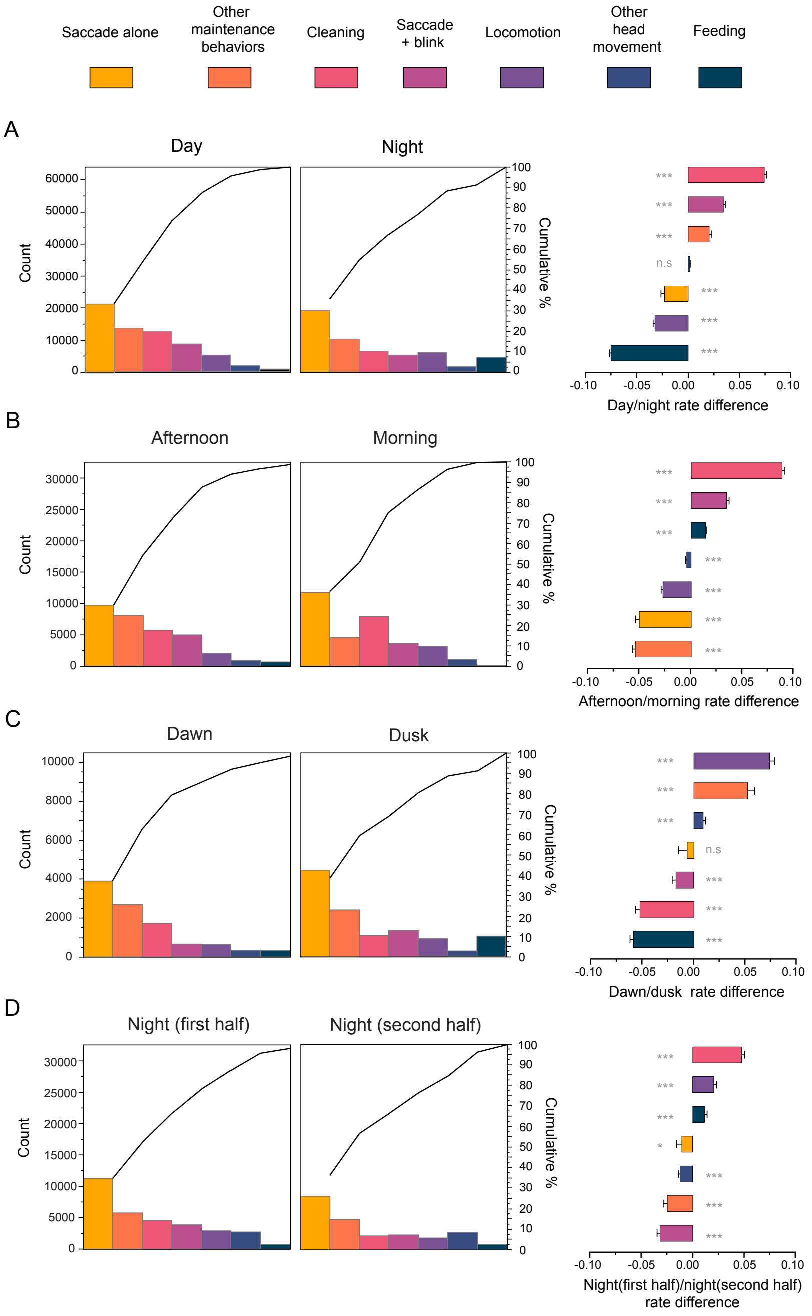


Figure 3.6. Overtly active behavior in details.

Left: Pareto chart details the count and cumulative proportion of overtly active behaviors within each diel category. **Right**: Graphs show the difference (rate difference) between the proportion of distinct types of *overtly active* behavior. Comparisons of raw counts and cumulative proportion of overtly active behaviors between **(A)** day and night; **(B)** morning and afternoon; **(C)** dawn and dusk; **(D)** first half half of the night (night period before midnight) and the second half (night period after midnight). *** $P < 0.0001$, n.s., not significant at the 5% level.

3.2.5 Head saccades as a proxy for characterizing the temporal dynamics of awake states

There is no question from the above results that head saccades represent the bulk of all *overtly active* behaviors. Moreover, over a 15 min resolution timescale, the rate of *head saccade alone* was significantly correlated with the rate of *vigilant pose* ($r_{575} = 0.64$, $P < 0.0001$), but negatively related to the rate of *rest pose* ($r_{575} = -0.648$, $P < 0.0001$). These considerations enticed us to choose this behavior as an event marker for investigating the temporal organization of a state variable, namely the *awake state*. For this analysis, we treated head saccades as a point process, and calculated, for each owl, two relevant metrics from the probability distribution of inter-saccade intervals (ISIs), namely the burstiness index and the memory coefficient (see Materials and Methods, section 2.7.6).

A representative example of a ISI sequence over 24 hours is shown in Figure 3.7 A. Observe that relatively short ISIs are abundant while long ISIs are intermittently observed. Note also that this pattern appears even more pronounced at night. The distribution of ISI was found to for each owl (not shown), we witnessed a positively skewed, heavy-tailed profile suggesting that saccades are generated by a mechanism that can not be explained by a homogeneous process.

In line with the heavy-tail distribution of ISIs, median values of burstiness index and memory coefficient over a daily cycle were robustly positive (Fig. 3.7 C,D), and independent of the number of head saccade counts (fig. 3.7 B). This finding indicates that the temporal ordering of head saccades is not governed neither by random

(Poissonian) process, nor by a regular (periodic) process. Head saccades bursts', i.e., the short period of increased activity followed by a relatively prolonged period of inactivity, seem to enhance during the night, although the total number of head saccades is only slightly different between the day and night (fig. 3.7 B). The Burstiness index (B) is positive for all-day periods, which attests to the bursty nature of head saccades. Interestingly, the B index shows a striking increase at night, which is indeed close to 1 and highly conservative across owls. Moreover, the Memory coefficient (M) exhibits a positive value, which remains nearly the same for all diel categories.

Since both B and M were positive, we can say that the bursty nature of head saccades results from a short inter-event time interspersed with a long inter-event time (given by B). Additionally, the temporal organization of head saccades carries the same memory effect throughout the day since the ISIs show substantial positive correlation between them (given by M). This evidence is consistent with a cathemeral species since

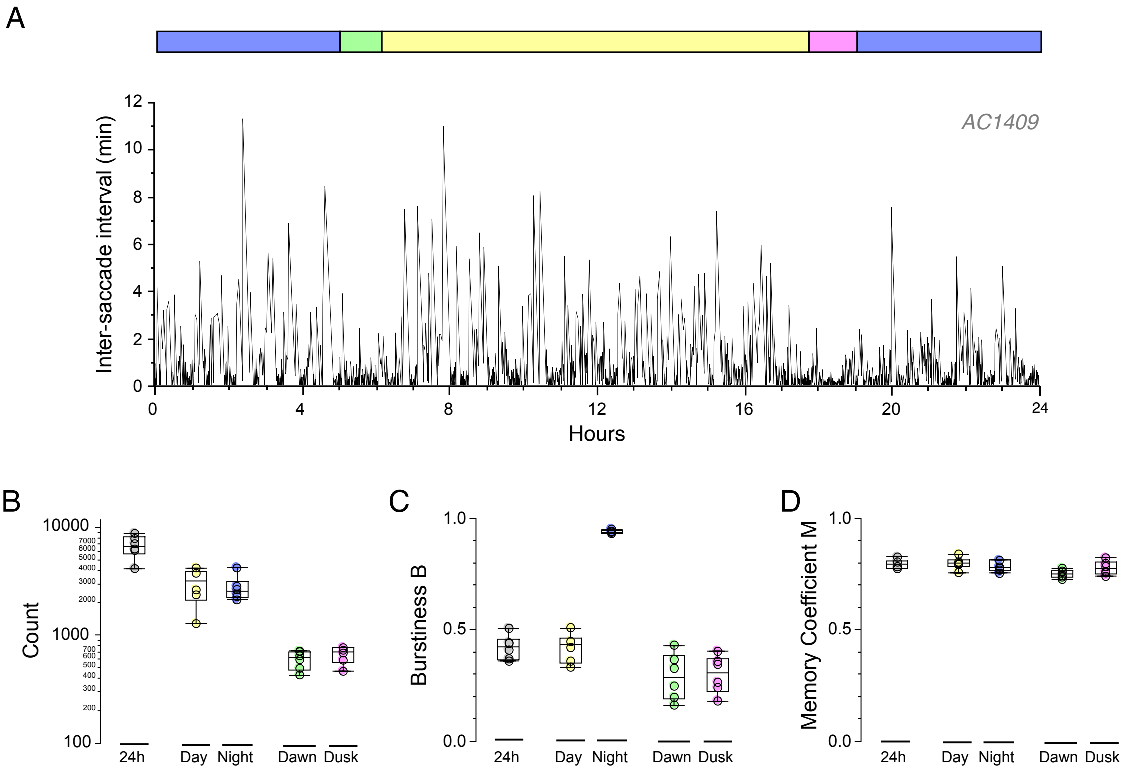


Figure 3.7. Temporal organization of head saccades as a function of diel categories. **(A)** Representative example (owl AC1409) of the minute-based (min) inter-saccade intervals over 24h. The colored horizontal bar above the graph indicates the periods of night (blue), dawn (green), day (yellow), and dusk (violet). **(B)** Number of saccadic events over 24h and for each diel category .Comparisons are **(C)** Burstiness index. Note the **(D)** Memory coefficient. Box plots .

they exhibit bouts of enhanced activity (expressed by short ISIs, so short intervals following short ones) interspersed by bouts of inactivity where the ISIs are lengthy, i.e., long intervals following long intervals. This pattern remains unchanged during the night, as demonstrated by M, but the bursty nature of head saccades is enhanced (B ≈ 1). We wonder why this happens since the total number of head saccades is not as distinct night and day. First, the duration of the bouts of the vigilant pose is more significant at night. Furthermore, monitoring the environment at night is more challenging than at day, although owls have a fully adapted sight for hunting in the dark (Lisney *et al.*, 2012; Vieira *et al.*, 2016). As a result, they should depend more heavily on auditory cues typical of the night to spatially locate themselves (Marti, 1974), which would require a more intense burst of saccades during the periods of vigilance.

3.2.6 Influence of local ambient temperature and humidity

Figure 3.8 summarizes ambient temperature and relative humidity throughout the during the days of video recording. On average, ambient temperature was 19.6°C (max: 24°C, min: 15.7°C) and relative humidity fluctuated around 71% (max: 84%, min: 52.7%). Day and dusk show higher temperatures and lower relative humidities (fig. 3.8 B, E). It is important to note that the major variations in temperature and relative humidity occur during the day (fig. 3.8 C, F).

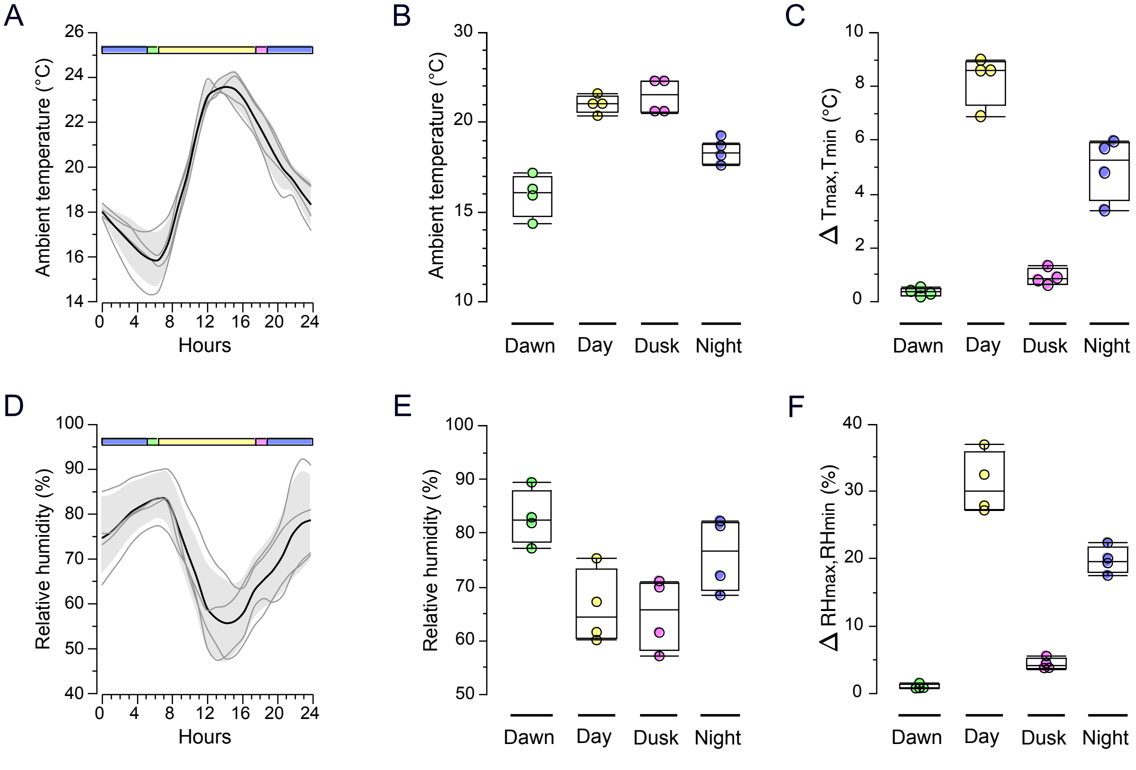


Figure 3.8. Summary of local ambient temperature and relative humidity throughout the 24 hours of video recording.

(A) Mean of ambient temperature for 24 h, interpolation data of 3 minutes resolution, shadow area represent the standard error mean, and gray lines the measures for each recording day (n=4). **(B)** Box plot values of ambient temperature in function of diel categories: dawn (green), day (yellow), dusk (pink) and night (blue). The colored circle of each diel category correspond to the mean value for each day of recording (n=4). **(C)** Difference (Δ) between maximal and minimal temperature in function of diel categories (n=4). **(D)** Mean of local relative humidity during 24 h, interpolation data of 3 minutes resolution, shadow area represent the standard error mean, and gray lines the measures for each recording day (n=4). **(E)** Box plot values of relative humidity in function of diel categories. The colored circle of each diel category correspond to the mean value for each day of recording (n=4). **(F)** Difference (Δ) between maximal and minimal relative humidity in function of diel categories (n=4)

In figure 3.9, the overall picture of the heat maps indicates that owls' *overtly active* behaviors rise with increasing environmental temperature, and declines with increase of relative humidity (RH%). However, *overtly active* behaviors and the awake state show a very weak correlation with environmental temperature and RH% throughout 24 hours (RhoT°C= 0.04 and RhoRH%= -0.04 respectively, P = 0.65).

Looking at the diel categories, we could find some interesting results which explain those poor correlations. During the daylight hours, temperature increments are followed by declines in activity, while for relative humidity, this relationship is positive, but neither is significant. During the night, these trends were inverted. The frequency of *overtly active* behaviors tend to decline with temperature' falls and humidity' raises (RhoT°C = 0.51, P << 0.001; RhoRH% = -0.49, P << 0.001). Similarly, the awake state presents the same tendency. This inversion (see figure 3.9) can be explained by the fact that the temperature attains its minimum value, dropping almost linearly while relative humidity increases (ΔT°C =5.8; Δ RH%= 18.6). On the other hand, the temperature goes high during the day and peaks the maximum value, so the owls tend to be less active. In contrast, the relative humidity reaches the minimum value, maybe inducing the animal toward a sleep-like state.

Remarkably, also there is an inversion in the relationship of the environmental variables and the active behaviors when we look at the night period in detail. There is a positive correlation with temperature during the first half of the night, and the opposite occurs during the second half of the night (Overtly awake: $Rho_{T°C} = 0.56$, $Rho_{T°C} = -0.65$; $P \ll 0.001$). A reciprocal inversion happens to RH% correlations, but in the case with the negative correlation at the first half of night (Overtly active: $Rho_{RH\%} = -0.54$, $Rho_{RH\%} = 0.63$, $P \ll 0.001$, see figure 3.9 B). In the first half night, despite the activity level being slightly higher than the second half night, the owls' activity tends to fall, anticipating the drowsiness state exhibited in the earliest hours of the second part of the night. Inversely, there is an increase of activity at the last hours of the second half of the night due to the twilight hours, so the overall tendency is an increase of activity after 4:00 o'clock. At the same time, the temperature and the humidity fall and increase linearly in both parts of the night.

Morning and afternoon time are negatively associated with temperature and positively with RH%, yet the values are weak. A possible interpretation is that during the morning, the activity decreases extensively between 6:00 and 8:00 o'clock. In the following hours, there is an increase in the active state while temperature and RH undergo significant changes almost linearly ($\Delta T°C = 7.3$; $\Delta RH\% = 24.6$). In the afternoon, the temperature and RH% vary relatively short ($\Delta T°C = 1.6$; $\Delta RH\% = 9$); at the same time, the activity level shows a trend that appears to decline, but at the very end, it raises driving by the twilight hour.

To better understand these correlations, we choose the most characteristic behaviors of the awake state: the locomotor behavior and the head saccades. The locomotor behavior presented the same relationships of the awake state, being significantly correlated during the day and second half night (RhoT°C = - 0.48, RhoT°C =-0.69, $P << 0.001$; RhoRH% = 0.47, RhoRH% = 0.68, $P << 0.001$). The analysis for the head saccades was separated into two types: saccades alone and blink with saccades; because we predict that saccades that occurred concomitant by blinks, although active, indicate the approximation of the inactive state. Saccades alone follow the same tendency aforementioned, but the correlations are strongest. The afternoon is the only period where correlation is weak, maybe because the saccades rates tend to rise until 15 o'clock, followed by a sudden fall after this time while the curve of the environmental variables is flattening out. The saccades accompanied by blinks follow the rise and fall of temperature and RH%, almost of the time, resulting in higher correlations values. It is interesting to remark that among these behaviors, the saccades accompanied by blinks were the only ones that did not show a great peak during the twilights. Dawn and dusk are imperative times that drive the animal to be highly active almost independent of the temperature and RH%, which is an important factor in explaining the poor correlation presented in specific day periods.

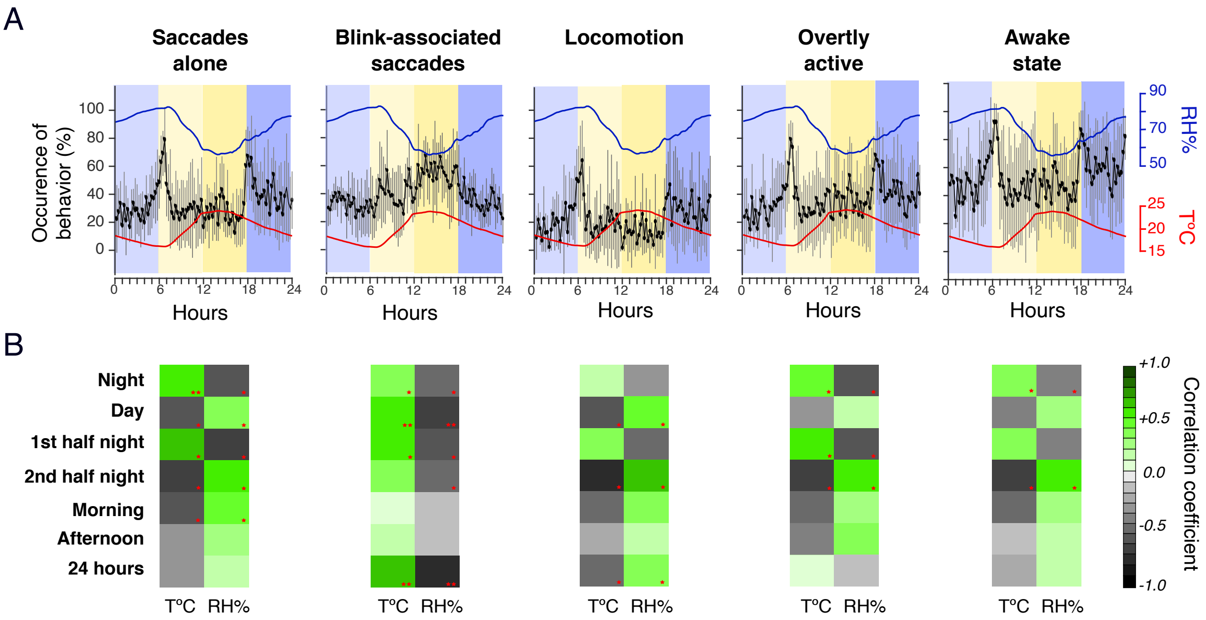


Figure 3.9. Pearson correlation between active behaviors, temperature and relative humidity according to sub-diel categories.

(A) Ratio of overtly active behavior (some in detail) and awake state. The back ground colors represent first half (violet) and second half of the night (light violet), morning (light yellow) and afternoon (yellow). Left ordinate refers to mean and standard deviation percentage in 15 minutes rate resolution (n= 6 owls). Right ordinate refers to mean of local temperature (red) and relative humidity (blue). **(B)** Heat map representation of correlation strength between temperature (T°C, first column of each heat map) and the correspondent behavior in the above graph, the same for relative humidity (RH%, second column). Green scale represent positive correlations and gray scale negative correlation. One red star for p-value < 0.05 , two red stars for p-value << 0.01.

3.3 DISCUSSION

In this study, we quantified six burrowing owl most relevant awake behaviors throughout 24 hours in a semi-natural setting, aiming to overcome some difficulties found in both field observations and experimental laboratory studies. We found that overtly active behaviors and vigilant poses occur in every hour of the day with a relative increase during twilight hours.

3.3.1.On the pros and cons of the methodological approach employed in this study

Different methodologies used for assessing the activity budget of an animal may result in diverging conclusions (Poulin & Todd, 2006). Binoculars, pellet identification, and radio telemetry have been the principal methodologies employed to infer the time partitioning of burrowing owls' activity (Coulombe, 1971; Thomsen, 1971; Martin, 1973; Haug & Oliphant, 1990; Poulin & Todd, 2006; LaFever *et al.*, 2008). All these techniques furnish valuable information. However, they carry some limitations (Halle and Weinert, 2000). Spotting by binoculars is not continuously scored and is often confined to daylight hours; pellet content may bias findings; radio-telemetry provides good monitoring of traveling behavior and the habitat range of raptors. But when the animal is immobile, determining its state is not possible. Because burrowing owls spend a considerable amount of time immobile (vigilant or resting), the latter approach would miss a significant portion of its activity. A major limitation of these methods lies in the fact that they do not provide information gathering for a full-detailed round 24 h hours (Poulin & Todd, 2006).

The video recording approach has the advantage of accessing the animal's behavior for 24 hours continuously, thus allowing the scoring of behavior to be highly precise (Gilestro, 2012). However, it also presents some caveats (Rattenborg *et al.*, 2017); in the wild, the animal's range is restricted by the shooting plane, for example, nests or burrows. Consequently, when the animal is out of sight, the information about its activity will be missed. One way to minimize this problem would be to videotape the animal in captivity. However, a captive animal does not have to deal with the ecological demands, so its activity pattern can be biased. Despite this, we have chosen the latter approach in a more natural setting because the video recording technique allows us to study the owl's behavior in detail, including the distinction of two motionless poses: the vigilant pose and the rest pose. Furthermore, we could survey the owls' eyes by applying this method, which provided precious knowledge about these highly visual animals.

Another methodological issue concerns the studies conducted inside the laboratory; even if the experimental animal is acclimated to the artificial conditions, its activity can be different from that of natural environments (Campbell & Tobler, 1984; Traber & Müller, 2006; Gattermann *et al.*, 2008). One incontestable explanation for those differences is that the animal does not have to confront dangerous situations inside a laboratory, such as a predator menace or starvation due to lack of food (Rattenborg *et al.*, 2017). The study of the sleep-wake cycle in burrowing owls by Berger and Walker (1972) showed that burrowing owls' sleep is extensively distributed throughout the day, but the laboratory conditions could influence their results. Indeed some overt behaviors as body relaxations during sleep were not observed in their work, which emphasizes the importance of regarding behaviors of an animal in detail since polysomnographic recordings cannot represent all the features of a particular state (sleep or wakefulness). It is noteworthy that despite being attached by a falconry leash, our owls displayed locomotor behavior all day long, which increased throughout the night. Despite access to dead mice *ad libitum*, our owls mainly fed during the night, concealing a bit of it to consume later. These observations suggest that, although being captive, they had become well-acclimatized to the experimental aviaries. Our experimental environment provided a natural context wherein their behavior expression could be more closely related to what is found in the field.

3.3.2 To what extent do our results match the ecological literature ?

The prevalence of bimodal fits in circular model selection supports the idea that like other cathemeral animals (Curtis *et al.*, 1999; Donati & Borgognini-Tarli, 2006; Eppley *et al.*, 2015), burrowing owls exhibit a typical bimodal activity pattern peaking around at least in one of the twilight periods (dusk or dawn).

It was possible to derive some notion of the temporal niches of the burrowing owls from the model selection analysis since the parameters showed pertinent information. For example, despite the fit activity of the best model being bimodal for all six owls, one of them displayed a tendency toward the diurnality, which was reinforced by the two mean directions oriented towards the daytime. Even though both means being oriented to the diurnal period, we observe that a large amount of activity was concentrated during the night. Moreover, considering the vigilant pose, the difference between day and night amounts is lessened. Schnute and Groot (1992) emphasized the importance of considering the ten models selection as a method to identify plausible biological scenarios with the potential to be explored than a definitive assessment. The other analyses were therefore essential to concluding about the owl's temporal niche.

Regarding this bimodal behavior and some ecological observations, one could wrongly classify the burrowing owls as crepuscular species. However, a substantial amount of activity is distributed throughout the rest of the day (overtly awake behavior occupying about 15-25% of each hour). LS-mean analysis indicates that time spent in overtly active behaviors does not significantly differ between diel categories.

The proportion of overt activity and the awake state is not significantly different between day and night. As the diurnality index indicates, $I_{diurnality}$, the overtly active behavior is close to an even distribution since the values are not significantly different from the $I_{diurnality}$ values derived from the uniform distribution simulation. Possibly some differences arise from the circadian entrainment that drives the activity to its maximum during sunrise and sunset, a common characteristic of cathemeral animals (Donati & Borgognini-Tarli, 2006). Also, our results show that burrowing owls presented one of the lowest diunarlity ratio (DR index) compared to other cathemeral primates in literature: *Eulemur collaris* (3.5:1), *E. rufifrons hybrids* (2.4:1) (Donati *et al.*, 2009), *Hapalemur meridionalis* (3.98:1) (Eppley *et al.*, 2015), *E. rufifrons* (3.4:1) (Kappeler & Erkert, 2003), *E. mongoz* (1.96:1) and *Varecia variegata variegata* (1.72:1), *Lemur catta* (2.00:1) (Rea *et al.*, 2014), *Lemur catta* (4.8:1) (Donati et al., 2013).

It is relevant to highlight that Donati and colleagues (2013) found DR values to *Lemur catta* activity varied throughout the year from 1.78:1 (about equal amounts of diurnal and nocturnal activity) to 9.56:1 (fully diurnal), demonstrating the ability of cathemeral animals to adjust their behavior in response to environmental and seasonal changes. Our measurements occurred during the winter, a season that does not require as much activity as the breeding season; therefore, we suspect that during the spring/ summer, the burrowing owls may still be more equally active during night and day. In fact, Poulin and Todd (2006) have shown that burrowing owls deliver prey to nestlings 24 hours a day during Canada's breeding season.

3.3.3 Visual adaptation to light

The morphology of the eyes is one critical feature to consider when studying cathemeral species since those animals have to deal with extreme light conditions (Halle, 2006; Tattersall, 2008). For example, nocturnal birds and mammals have corneal diameters larger than their axial lengths (CD/Al ratio); the opposite appears in diurnal birds and mammals: larger axial lengths relative to their corneal diameters (Kirk, 2006; Hall & Ross, 2007). There is a tendency for cathemeral mammals and birds to have the eye shape intermediate (CD/Al ratio) between those of diurnal and nocturnal relatives (Kirk, 2006; Hall & Ross, 2007). Similarly, burrowing owls present a CD/Al ratio that is intermediate; however, it is closer to that of nocturnal species (Lisney *et al.*, 2012). This is not surprising given that this species mainly hunt small vertebrates in low light intensities (Haug & Oliphant, 1990).

Furthermore, Lisney and colleagues (2012) demonstrate an interesting association between the owl's temporal niche, habitat type (open or enclosed), and retinal ganglion cell topography (RGC). Diurnal and open habitat owls present an area of increased RGC density in the horizontal retinal meridian, called the visual streak. On the other hand, nocturnal or more enclosed habitats owls have a poorly-defined visual streak. They observed that the burrowing owl has a distinctly defined visual streak with an intermediate isodensity contour map, while the barn owls, a nocturnal species that also occupies open habitat, present a poorly defined visual streak. In contrast to *Tyto* *alba*, which presents an *area centralis*, burrowing owls have a true fovea pit, an adaptation to diurnal vision (Bravo & Pettigrew, 1981). Accordingly, burrowing owl visual neurons prefer, on average, higher spatial frequencies than neurons in the mammalian striate cortex, which is also a good indicator of visual acuity (Pinto & Baron, 2009). These characteristics enable burrowing owls to balance the need for visual acuity during the daytime and visual sensitivity during the night, consistent with the fact that they occupy both diurnal and nocturnal niches, as typical cathemeral animals.

The ambient light level is one of the critical factors that impact the activity of cathemeral animals. The presence of a source illumination, such as the full moon, at night, increases the likelihood of their nocturnal activity (Donati & Borgognini-Tarli, 2006; Donati *et al.*, 2013; Eppley *et al.*, 2015). Interestingly, burrowing owls are more responsive to the sound of playback at full moon nights (Braga & Motta-Junior, 2009). In our experimental area, indirect artificial light was present at night. However, it does not seem to shed light upon the experimental aviaries since attempts to directly measure nocturnal luminosity was unsuccessful due to a lack of luxometers that could measure differences between shallow levels of luminosity with sufficient accuracy inside the experimental place.

3.3.4 The influence of temperature and relative humidity on owl activity

Considering other abiotic factors that could influence owls' activity, we investigate the correlations between the environmental variables (local temperature; relative humidity) and the overtly active behaviors. A significant factor to consider is that burrowing owls become hyperthermic and may die when ambient temperatures exceed 40°C (Coulombe, 1970). Consequently, it is reasonable to presume that temperatures rise during the day may result in a decrease in animal activity. Coulombe (1970) also reported that burrowing owls' thermoneutrality zone is between 25°C - 37°C and, inside the laboratory, they show a lowered body temperature during the dark period as a result of an endogenous process. This may explain why activity tends to decrease with temperature in the first part of the night, reaching lower levels after midnight until 5:00 o'clock.

Furthermore, regardless of habitat, the burrowing owls' activity seems to be circadian-entrained by sunset and sunrise time, a force that exerts a more substantial influence than other abiotic factors. Accordingly, the combination of climatic factors like temperature and relative humidity affects cathemeral lemurs activity when regarded in conjunction with photoperiod and illumination. Considered by themselves, temperature and relative humidity act as masking factors only in environments with extreme variations (Curtis & Rasmussen, 2002; Fernandez-Duque, 2003; Donati & Borgognini-Tarli, 2006).

A particular point to be highlighted is that some behaviors, such as blinking with saccades, seem to be strongly modulated by relative humidity and ambient temperature. During afternoon hours, blink with saccades likely increase as temperature and relative humidity reach their maximums and minimums, respectively. We wonder if this can be related to an increase of eye-blink reflex in the function of the driest hours of the day since the head movements associated with eye-blink may cause corneal dehydration (Rand, 2021). Additionally, burrowing owls' ocular movement is practically inexistent (Steinbach & Money, 1973); thus, eye-blinks may play a role in corneal hydration beyond the nictitating membrane. Another issue to consider is that the diurnal species are reported to blink more frequently than the nocturnal species, suggesting that blinking may be reduced in low light conditions (Kirsten & Kirsten, 1983; Rand, 2021).

3.3.5 Monitoring surroundings: vigilant pose and head saccades

In this context, it is important to bear in mind that head saccades contribute substantially to activity quantification since these movements are the most frequent among overtly active behaviors. The head saccades alone are positively correlated with the vigilant posture and even with locomotor activity. Interestingly, research on deer populations comparing head movements and GPS locomotion data showed that both can be used to measure daily their activity patterns (Ensing *et al.*, 2014), supporting the validity of head movements as a good indicator of activity.

In studies conducted on the field, burrowing owls have already been described as very vigilant animals (Coulombe, 1971; Haug & Oliphant, 1990; Mrykalo, 2005; Chipman *et al.,* 2008; LaFever *et al.*, 2008; Scobie *et al.*, 2014). Considering this, we point out that the vigilant pose is a good indicator of the awake state since it is closely associated with overtly active behaviors and negatively correlated with overt inactive behaviors.

When the vigilant posture is viewed independently of the other behaviors, it is only different from dawn to daytime, maybe because the duration of the vigilant pose is longer in the dawn following the same night's trend. Since the bout of vigilant poses is longer at night, we suggest that due to low luminosity, burrowing owls have extended their bout of fixations (vigilant pose), using the foveal area to gain more accurate information about their surroundings. Furthermore, the vigilant pose helped us interpret the abrupt changes in head saccades temporal organization during the night. As head saccades play an integral part in vigilant behavior, the increase in the burstiness nature of head saccades during the night must provide them with an advantage in detecting dangers and food successfully. We recognize, however, that overt signs of vigilance, such as a change in posture, may not necessarily reflect an internal state of vigilance, and an internal state of vigilance may not always be readily discernible (Beauchamp, 2015).

Interestingly, it was demonstrated that house finches increased vigilance under low light conditions by increasing scan bout duration (Fernández-Juricic et al., 2007). Also, Fernández and colleagues (2011) demonstrated that, in a group setting, cowbirds located at the edges make more head movements than the birds positioned in the center of the group. The separated cowbirds exhibit longer fixations on specific aspects of their environment in the function of a decrease in head movements. According to their hypothesis, individuals might use specialized parts of the retina to obtain more precise information during vigilance.

Cathemerality may play a role in minimizing exposure to adverse conditions as large open habitats, extreme temperature, and risk of predation (Colquhoun, 2006; Curtis & Rasmussen, 2006). Interestingly, burrowing owls are small-sized birds that inhabit open fields areas and nest inside burrows, making them particularly vulnerable to predators (Coulombe, 1971; Burn, 1999; Motta-Junior & Bueno, 2004; König and Weick, 2008); consequently, they should always be in the vigilant state.

3.3.6 Final considerations

Although the cathemeral niche has been noted as a possible candidate to describe the pattern activities of birds, it has not been widely considered. A phylogenetic study has already suggested cathemerality as a plausible ancestor condition among living birds (Wu & Wang, 2019).

Still today, the concept of species temporal niches is strongly oriented towards the diurnal-nocturnal dichotomy, but there is a range of activity rhythms not explored between these two extremes. This dichotomy may come from the need for systematization by researchers than a natural temporal organization among species (Refinetti, 2006). Many studies are based on temporal niches previously attributed to a particular species to interpret their findings in light of evolution; consequently, a flawed classification can bias those interpretations. Apart from the knowledge on some species which exhibit a well-defined pattern of activity, it is necessary to conduct more comprehensive studies on the niches of bird species.

The present work fully quantified the burrowing owl activity patterns, demonstrating that the latent notion of burrowing owls being a cathemeral bird is accurate.

CHAPTER 4

BEHAVIORAL SLEEP IN THE BURROWING OWL

4.1 INTRODUCTION

Brain patterns characterizing NREM and REM sleep share similar characteristics in both mammals and birds: low-frequency, high-amplitude waves in NREM sleep, and high-frequency, low-amplitude waves occur during REM sleep or waking state. Accordingly, avian REM sleep is characterized by typically rapid eye movements, binocular eye closure, twitching behavior, reduced thermoregulatory responses, and partially muscle atonia (Lesku, Martinez-Gonzalez, *et al.*, 2009). However, in contrast to mammals, birds do not exhibit hippocampal theta rhythm during REM episodes. Moreover, thalamocortical spindles appear absent in NREM sleep (Meij *et al.*, 2018).

In response to ecological demands, a variety of birds species (e.g., mallard duck, gull, pigeon, blackbirds) exhibit unihemispheric or asymmetrical sleep with one eye closed, as do some aquatic mammals (Ball *et al.*, 1988; Rattenborg *et al.*, 2000; Lesku *et al.*, 2012; Rattenborg *et al.*, 2019). Thus, sleeping birds express a diversity of sleeping traits, such as flying in mid-sleep over the ocean without landing for up to two months (Rattenborg *et al.*, 2016).

The fact that slow-wave amplitude depends on prior awake or asleep time budgets, has provided evidence that birds' sleep is homeostatically regulated (Rattenborg *et al.*, 2012). It has been proposed that the dense interconnectivity characterizing neural networks within the mammalian neocortex and the avian hyperpallium is an essential condition for generating slow-wave activity (Butler & Hodos, 2005; Rattenborg, 2006). The sparse interconnectivity between the three layers in the dorsal cortex of reptiles endorses this view, since most studies have only identified a partial resemblance to REM and SWS sleep of mammals and reptiles (Shein-Idelson *et al.*, 2016; Libourel *et al.,* 2018). Despite its nuclear and pseudo-layer organization, the avian 'cortex' shares strikingly similar structural and functional features analogous to the neocortex of mammals, as manifested by the cognitive ability and elaborate behaviors across bird species (Jarvis *et al.*, 2005).

In general, birds display recognizable, albeit variable, sleeping behaviors, such as sitting or upright posture (sometimes on one leg), one or both eyes closed, neck withdrawn, lowered head, and bill on the back or buried into the wings (Goodman, 1974; Amlaner & Ball, 1983). Some birds can display behaviors that might seem, at first sight, incompatible with sleep. Ostriches, for example, sleep in an upright position with both eyes open (Lesku *et al.*, 2011). While flying, great frigatebirds enter into NREM and REM sleeping patterns (Rattenborg *et al.*, 2016).

There are about 250 living species of owls today (König & Weick, 2008). Yet, sleep in owls has been scarcely studied. More specifically, only four species of owls have received the attention of sleep researchers, namely: the snowy owl (diurnal, Karmanova and Churnosov, 1974), tawny owl (nocturnal; Šušić & Kovačević, 1973; Karmanova and Churnosov, 1974), barn owl (nocturnal; Scriba *et al.*, 2013) and the burrowing owl (Berger and Walker, 1972), focus of the present study. Most of these sleep studies have been studied in laboratory settings.

Despite close similarity in total time budget dedicated to sleep and wakefulness (see Table 1 in Scriba *et al.*, 2013), owls differ with respect to their daily sleep architecture and behavioral phenotype. Berger and Walker (1972) showed that burrowing owls' sleep is characterized by slow wave voltage traces of 200μV amplitude

(SWS sleep) and short episodes of desynchronized sleep (REM sleep), equivalent to REM sleep in mammals, yet without eye movements. Like burrowing owls, other birds also present shorter REM sleep episodes, about 10 seconds (Blumberg *et al.*, 2020). Berger and Walker (1972) further described that burrowing owls sleep in an upright posture with eyes open, half closed, or completely closed, and retracted head, along with cessation of body movements. Again, other avian species, including owls, are known to sleep with opened eyes (Šušić & Kovačević, 1973; Lesku *et al.*, 2011; Blumberg *et al.*, 2020). Unlike other bird species, owls lack rapid eye movements during REM sleep due to the enormous size of their eyes, which is encompassed by a rigid ringbone (Berger & Walker, 1972; Steinbach & Money, 1973; Mahecha & Oliveira, 1998).

In 1973, Šušić and Kovačević conducted a similar polysomnographic study in the tawny owl (*Strix aluco*). Although it is well known that tawny owls are predominantly nocturnal, the authors have shown that inside the laboratory, this species exhibits a fragmented pattern of sleep (REM and SWS sleep) distributed across 24 hours, which is very similar to burrowing owls' sleep. Šušić and Kovačević (1973) further described body and head nods as well as a decrease in heart rate and breathing during SWS and REM sleep.

In order to conduct sleep studies in a more natural context, Scriba and colleagues (2013) performed a wireless EEG recording in barn owls (*Tyto alba*). In the light phase, barn owls spent most of their time in SWS sleep (63 % of total sleep time in SWS and 7.5% in REM sleep). During the dark phase, they remained awake for longer periods (85%). Moreover, Scriba and colleagues (2013) reported that barn owls sleep with their eyes closed most of the time. The findings reported by Scriba and colleagues (2013) are in line with those found in natural settings, i.e., barn owls are predominantly nocturnal.

The basic EEG pattern recorded from avian the wulst during sleep staging has been characterized in burrowing owls by Berger & Walker (1972). The authors have also correlated EEG data with behavioral traits. Capitalizing on this previous study, we set out to analyze burrowing owls' sleep-related behaviors in a more natural, albeit controlled, environment.

4.2 RESULTS

4.2.1 Circular model selection to assess the daily distribution of sleep state

Binocular and monocular eye closure are univocal markers to identify overtly sleeping behavior. As shown in the rose graph (figure 4.1), these behaviors occur in all hours of the day, but do not show a uniform 24-hour distribution (Rayleigh's test, $P << 0.05$, for all animals, n=6). Figure 4.1 A shows the distribution of *overtly sleeping* behaviors throughout 24 hours. Bimodal models offer the most robust fittings for all animals, with mean peak times (φ_m) concentrated during daylight hours or during both day and night (see Table 4.1). In addition to the peak times, it is crucial to consider the range of hours within the 95% bandwidth (β_m) of each von Mises function and the proportional size of the first distribution (λ).

In contrast to *overtly active* behaviors, the distributions that include a wide range of hours do not necessarily concentrate most of the data. This result can be explained by the fact that *overtly sleeping* behaviors, most commonly associated with REM sleep according to Berger and Walker (1972), tend to occur more frequently during the day.

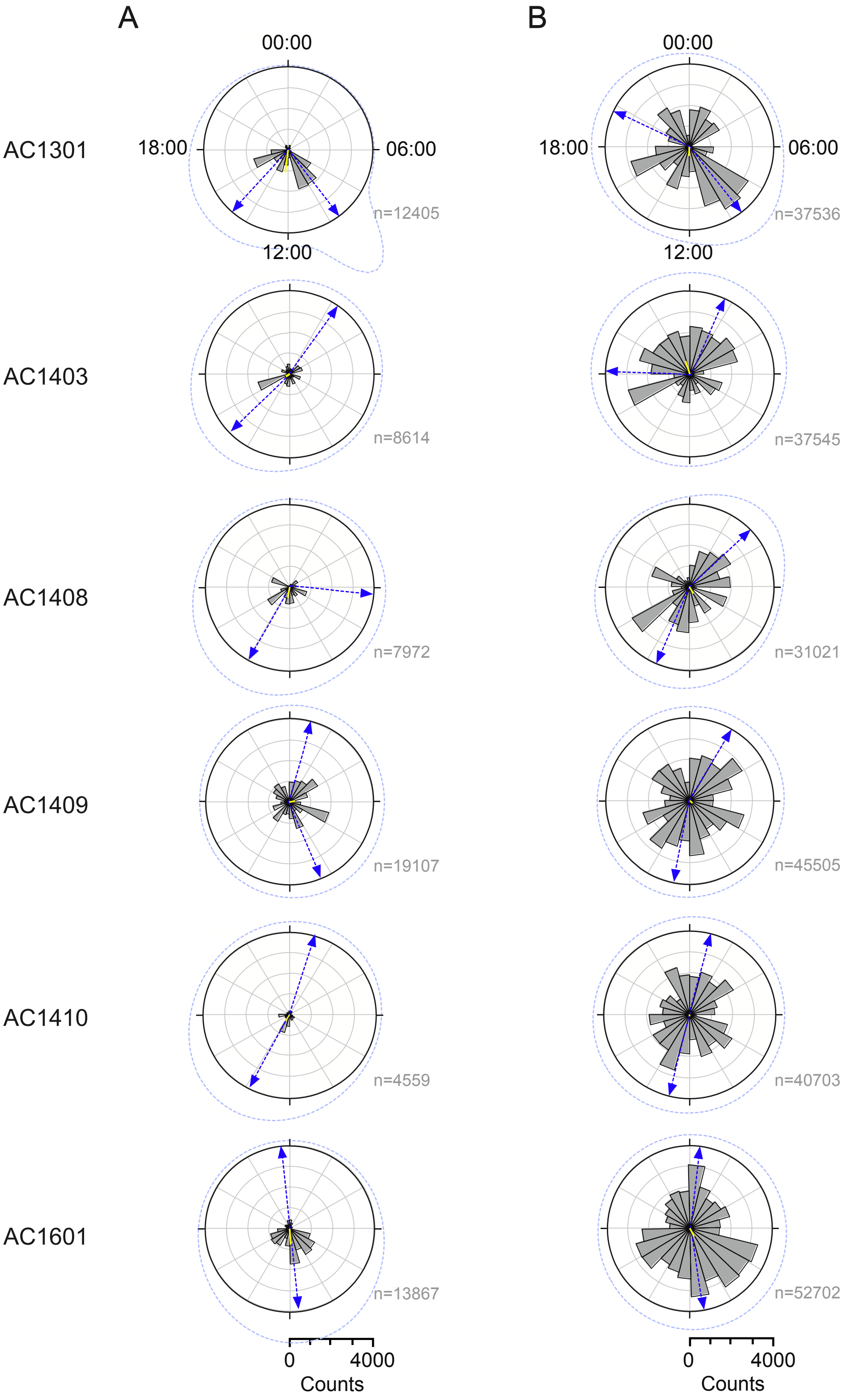


Figure 4.1.Circular distribution of overtly sleeping behavior and sleep state.
The Circular histograms (gray bars) represent the quantification of the behaviors in bins of 60 minutes. The density (dashed line) and mean direction(s) (dashed arrows) of the best model are provided. **(A)** Circular distribution of overtly sleeping state, the best fits follows a bimodal distribution (AC1403, AC1301:M5B; AC1408, AC1409, AC1410: M5A; AC1601: M4B. **(B)** Sleep state exhibit a bimodal distribution (AC1403, AC1409, AC1601: M5A; AC1408, AC1410: M5B; AC1301: M4B).

In general, the mean direction vectors pointing towards daylight hours show greater values of λ. For example, owls AC1408, AC1410, and AC1601 exhibit approximately 75% of the overtly sleeping behavior distributed around diurnal mean directions ($\lambda \sim 0.75$), with φ_1= 13:59; 13:49; 11:35 h, respectively (see Table 4.1). Thus, it may be that our owls engaged more in REM sleep during the day, contrary to what Berger and Walker (1972) reported (see Table 1).

Table 4.1. Model selection summary for *overtly sleeping* behaviors

Analysis	Variable	Owl					
		AC1301	AC1403	AC1408	AC1409	AC1410	AC1601
Sample size	n	12405	8614	7972	19107	4559	13867
Rayleigh test	R	0,564	1,39E-01	0,428	1,37E-01	3,95E-01	0,446
	P	0,0	2,94E-73	0,0	6,3E-157	9,4E-210	0,0
Model ΔAIC	M1	1,29E+04	7,12E+02	3,25E+03	9,38E+02	1,80E+03	6,07E+03
	M2A	4,28E+03	3,81E+02	1,72E+02	2,19E+02	3,24E+02	2,59E+02
	M2B	6,20E+03	3,27E+02	4,71E+02	2,70E+02	28,6	3,55E+02
	M2C	5,17E+03	1,58E+02	2,42E+02	1,96E+03	30,1	47
	M3A	1,24E+03	3,03E+02	3,07E+03	7,78E+02	1,20E+03	4,98E+03
	M3B	6,37E+03	22,3	5,26E+02	2,76E+02	27,7	3,90E+02
	M4A	6,88E+03	42,4	9,22E+02	3,64E+02	26,8	8,94E+02
	M4B	5,02E+03	14,8	3,66E+02	7,79E+02	46,6	**0,0**
	M5A	3,53E+03	18,9	**0,0**	**0,0**	**0,0**	5,22E+02
	M5B	**0,0**	**0,0**	55	2,54E+02	2,37	1,48E+02
BM angle	φ_1	9h35	15h00	13h59	10h23	13h49	11h35
	φ_2	14h49	2h20	6h20	00h59	1h10	23h35
BM width	β_1	2h48	10h11	11h06	15h40	11h06	11h10
	β_2	12h16	19h48	11h06	15h40	11h06	25h13
Lambda	λ	0,34	0,41	0,74	0,48	0,74	0,75

The sample size (n) refers to the count of behaviors for each owl. The Rayleigh test summary shows the mean vector magnitude (R) and p-value (P). The ΔAIC refers to the differences relative to the best model. The best models are in bold and indicated by an ΔAIC<2. BM angle (φ_m) refers to the best model mean direction angle expressed in hours (hr:min). BM width (β_m) is bandwidth of the best model that concentrates 95% of data within each distribution, given in hours (h:min). Lambda (λ) represents the proportional size of the first distribution.

The *sleep state*, i.e, *overtly sleeping* behaviors and *rest pose*, also shows a bimodal distribution being M5B, the best model for most of animals (AC1301, AC1408, AC1409, AC1410), and M5A, for owls AC1403 and AC1601 (Table 4.2). Owl AC1410 activity distribution admits two plausible models (M5B or M4B) as the ΔAIC < 2. For most owls, sleeping episodes concentrate close to midday and midnight (see $\varphi_{1,2}$ in figure 4.1 B and Table 4.2). Thus, beyond the sleep period during the daylight, owls appear to engage heavily in the sleep state during the late night after the great wave of activity in the first half of the night. As shown in Table 4.2, the bandwidths of all von Mises distributions are extensively large and include the major proportion of the sample size, indicating that the *sleep state* is well distributed within a daily cycle.

Table 4.2. *Sleep state m*odel selection summary

Analysis	Variable	Owl					
		AC1301	AC1403	AC1408	AC1409	AC1410	AC1601
Sample size	n	37536	37542	31021	45505	40703	52700
Rayleigh test	R	1,29E-01	0,217	1,23E-01	6,38E-02	2,88E-02	0,134
	P	4,9E-275	0,0	6,25E-204	3,85E-81	1,96E-15	0,0
Model ΔAIC	M1	4,42E+03	3,76E+03	5,05E+03	1,01E+03	1,31E+03	2,56E+03
	M2A	3,15E+03	1,83E+02	4,12E+03	6,47E+02	1,25E+03	6,73E+02
	M2B	2,98E+03	3,92E+02	4,26E+03	6,65E+02	1,24E+03	5,66E+02
	M2C	2,03E+03	1,18E+03	4,35E+03	9,05E+02	2,75E+03	8,73E+02
	M3A	2,60E+03	3,68E+03	2,06E+03	4,07E+02	2,84E+02	1,90E+03
	M3B	1,60E+03	4,06E+02	2,05E+03	3,24E+02	2,37E+02	2,74E+02
	M4A	1,51E+03	7,59E+02	1,95E+03	3,33E+02	2,03E+02	2,07E+02
	M4B	3,41E+02	2,99E+02	1,13E+03	3,10E+02	**0,9**	1,69E+02
	M5A	1,10E+03	**0,0**	8,37E+02	7,30E+02	2,05E+02	**0,0**
	M5B	**0,0**	5,07E+03	**0,0**	**0,0**	**0,0**	8,28E+02

BM angle	φ_1	19h46	1h38	13h33	2h01	00h56	00h21
	φ_2	9h32	18h13	3h08	12h44	12h54	11h20
BM width	β_1	23h25	16h39	15h37	21h25	9h59	15h48
	β_2	6h04	16h39	6h47	12h01	19h16	15h48
Lambda	λ	0,75	0,57	0,72	0,69	0,25	0,344

Legend, same as Table 3.1.

Around the clock, the normalized population mean of *overtly sleeping* behavior did not show a uniform distribution (Rayleigh's test, R = 56.8, P <<0.0001), but a clear bias for daytime hours (figure 4.2 A). This result reinforce our hypothesis that burrowing owls in general prefer to engage in REM sleep when solar light levels are sufficiently high. On the other hand, the normalized mean of *sleep state* proportions for the six owls is uniformly distributed according to the Rayleigh's test (R = 1.82, P = 0.1611). Thus, the normalized measures should be taken into consideration.

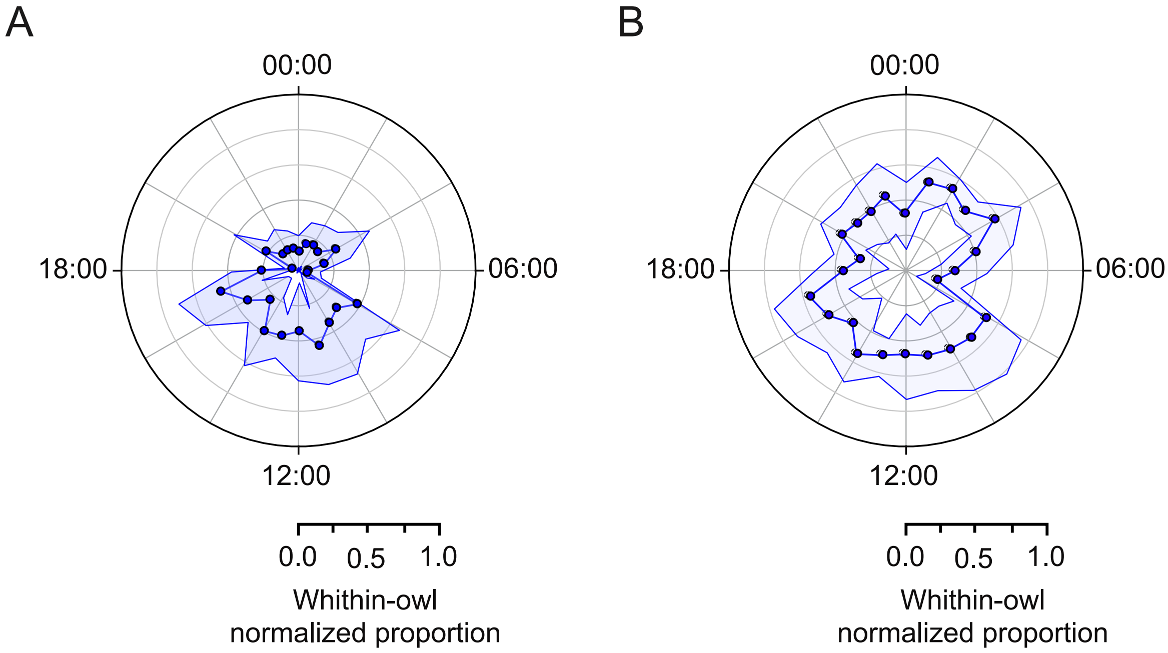


Figure 4.2. Circular distribution of overtly sleeping behavior and the sleep state.

(A) Hourly proportion of overtly sleeping behavior in 24 hour (mean of 6 animals, hr-ratio normalized counts, standard deviation is represented by the shadow are around the dots). **(B)** Hourly proportion of sleep state (overtly sleeping behavior + rest pose).

4.2.2 Allocation of sleep episodes into temporal niche categories

Figure 4.3 A displays the proportion of *overtly sleeping* behaviors and *rest pose*, as well as the distribution of these behaviors hourly for each animal. Remember that binocular eye closure is closely related to REM sleep in burrowing owls.

The LS-mean estimates obtained for our owl sample are shown in figure 4.3 B. *Temporal niche category* significantly affected the behavioral response covariates computed by the GLMM model (GLMM, $F_{1,3} = 7.05$, $P = 0.0006$). The same was true for the *sleep state* predictor (GLMM, $F_{1,3} = 37.85$, $P < 0.0001$), consistent with the fact that *overtly sleeping* episodes occur more sporadically than *rest pose* episodes. Interaction between factors was not significant (GLMM, *sleep state* * temporal niche category: $F1,3 = 2.76$, $P = 0.0545$), indicating that sleep-related behaviors were equally affected by daily period changes.

Figure 4.3 C shows that, globally, the mean amount of *sleep state* (*overtly sleeping* behaviors + *rest posture*) is practically equal between day and night periods (53.0% vs. 47.4%, Tukey's HSD test, P = 0.999). During these two periods, the difference in mean amount between *sleep state and wake state* is not significant (day: 5.9%, night: — 5.20%; 95% CI = 0.0 %; Tukey's HSD, $P = 0.999$). As described in the previous chapter, wakefulness largely predominates at twilight, but owls still indulge themselves with a 22.4% (95% CI: 0.13 - 0.35%) and 24.4 % (95% CI: 0.15 - 0.37%) amount of sleep at dawn and dusk, respectively.

As shown in figure 4.3 D, the probability of bout count was significantly affected by temporal niche category (GLMM, *F*1,3 = 6.40, *P* = 0.0012) and *sleep state* type (GLMM, $F_{1,3}$ = 65.03, *P* < 0.0001). There was no need to include an extra factorial product to explain the variance of covariates in the GLMM (GLMM, *sleep state* * temporal niche category: *F*1,3 = 2.13, *P* = 0.11). Overall, *rest pose* episodes were 6 times more frequent than *overtly active* behaviors, and the former remained always significantly more numerous than the latter, irrespective of the period of the day.

Figure 4.3 E shows that episode durations depend on *sleep state* type (GLMM, *F*1,3 = 30.46, *P* < 0.0001), and temporal niche category (GLMM, *F*1,3 = 19.96, *P* < 0.0001), but in a none-systematic manner (GLMM, *sleep state* * temporal niche category: *F*1,3 = 53.60, *P* < 0.0001). The most striking aspect of this data is that duration LS-mean estimates for *overtly sleeping* behaviors are considerably higher for *overtly sleeping* behaviors than for the *rest pose.* During the day, this difference is more pronounced (7.91s, 95% CI = 7.57 - 8.25 s; Tukey's HSD, *P* < 0.0001) than at night (2.80 s, 95% CI = 2.35 - 3.29 s; Tukey's HSD, *P* < 0.0001). During both periods, the relative difference between *overtly sleeping* behaviors *rest pose* was significantly inverted when considering the probability of bout occurrence (fig 4.3 D, day: −0.028, 95% CI = − 0.023 - −0.032 s; Tukey's HSD, *P* < 0.0001; night: − 0.026, 95% CI = − 0.021 - − 0.0361; Tukey's HSD, *P* < 0.001). Thus, it appears that a reduction in episode count is compensated by an increase in episode duration, and *vice versa*. This compensatory interaction also seems to extend to dusk.

The r*est pose* may reflect drowsiness or a transitional state between wakefulness and sleep. *Rest pose* episodes are expected to occur more frequently than *overtly* *sleep* episodes. There are at least two reasons for this: first, *rest pose* is predominantly associated with NREM sleep, which constitutes the greatest portion of total sleep time; second, many *rest pose* episodes tend to be short due to frequent, isolated head movements during these episodes. Furthermore, the *overtly sleeping* behavior tends to be slightly higher during the daylight, but without statistical significance. In contrast, the *rest pose* tends to occupy a more even proportion of all diel categories.

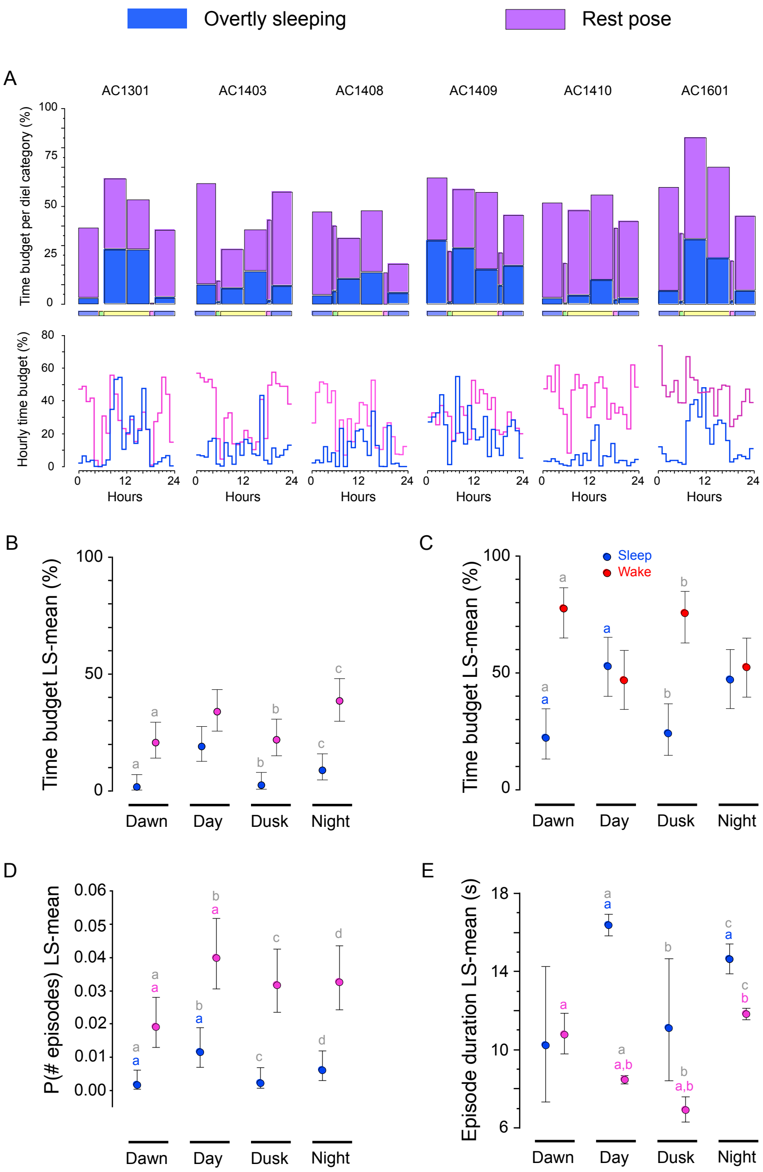


Figure 4.3. Summary of overtly sleeping and rest posing states as function of diel category. **(A)** *Overtly sleeping* behaviors (blue) and *rest pose* (violet) proportions for each owl according to diel categories and proportion distribution of *overtly sleeping* behavior and *rest pose* by hour (counting the activity of six owls by hour, n=6). **(B)** LS-mean of *overtly sleeping* behaviors (blue) and *rest pose* (violet) proportions according to diel categories. **(C)** LS-mean of the sleep state (*overtly sleepin*g behaviors + *rest pose* - blue) and awake state (*overtly active behaviors + vigilant pose* - red) proportions into diel categories. **(D)** LS-mean probability (P) of episode occurrence (#) as a function of diel category. **(E)** LS-mean duration of episodes in seconds. All central tendency estimates are reported as least-squared means, LS-mean. Letters indicates results of Tukey's HSD pot hoc comparison. LS-means connected by the same letter of the same color are significantly different at the 0.05% level. Colored letters denote significance between LS-means estimated for the same behavior following the color code used for this effect; grey letters indicate significance across behavioral categories.

4.2.3 Binocular and monocular eye closure during sleep

Resting with one or both eyes closed are overt sleeping behaviors that can be thus straightforwardly recognized as a *sleep state*. Figure 4.4 A,B shows, for each owl, the total number of bout counts for these two types of sleep-related behaviors. On average, owls spent 12.9%± SD 5.9% of their time in *overtly sleeping* behavior (monocular eye closure + binocular eye closure). In contrast, the *sleep state* (overtly sleeping behavior + rest posture) amounts to 47.5% of a daily cycle. Binocular eyes closure occupies 9.6% ± SD 4.2 of the 24 hours, and monocular eyes closure takes up around 3.1% of the time. Therefore, on average, our owls spend around 2 hours 19 minutes with both eyes closed. As discussed above, in most cases, they are therefore likely to engage in REM sleep during this period.

A brief glance at the raw data showed that the number of monocular eye closure (MEC) bouts was less frequent relative to the number of binocular eye closure (BEC) bouts at night than during the day. Therefore, we examined the relative occurrences of

BEC and MEC. As shown in figure 4.4 D, the probability of displaying either binocular eye closure (BEC) or monocular eye closure (MEC) was significantly greater at night. Interestingly, the number of bouts of BEC relative to MEC (bout gain) is more significant at night (see figure 4.4 C, Wilcoxon Matched-Pairs Signed Rank Test, $S_5 = 10.5$, $P = 0.031$). Also, the duration of BEC is significantly longer at night, despite the close-restricted p-value for all comparisons (see figure 4.4 E) while, there is no difference in the duration of monocular eye closure. Thus the increase of the probability occurrence of BEC relative to MEC is due to a higher number of BEC episodes and an increase in BEC duration.

Here is important to mention that, when observing the bouts of sleep state, we could observe the owls firstly adopted a *rest pose*, then closed one eye, remained in monocular eye closure for some seconds, and then closed both eyes. This sequence was recurrent across the 24 hours. However, the contribution of MEC reduces during the night, maybe because the need for sleeping with one eye closed is lessened at nighttime since diurnal predators, like raptors, are sleeping. Furthermore, this partially explains why the duration of the *rest pose* is greater during the night (see figure 4.3 E).

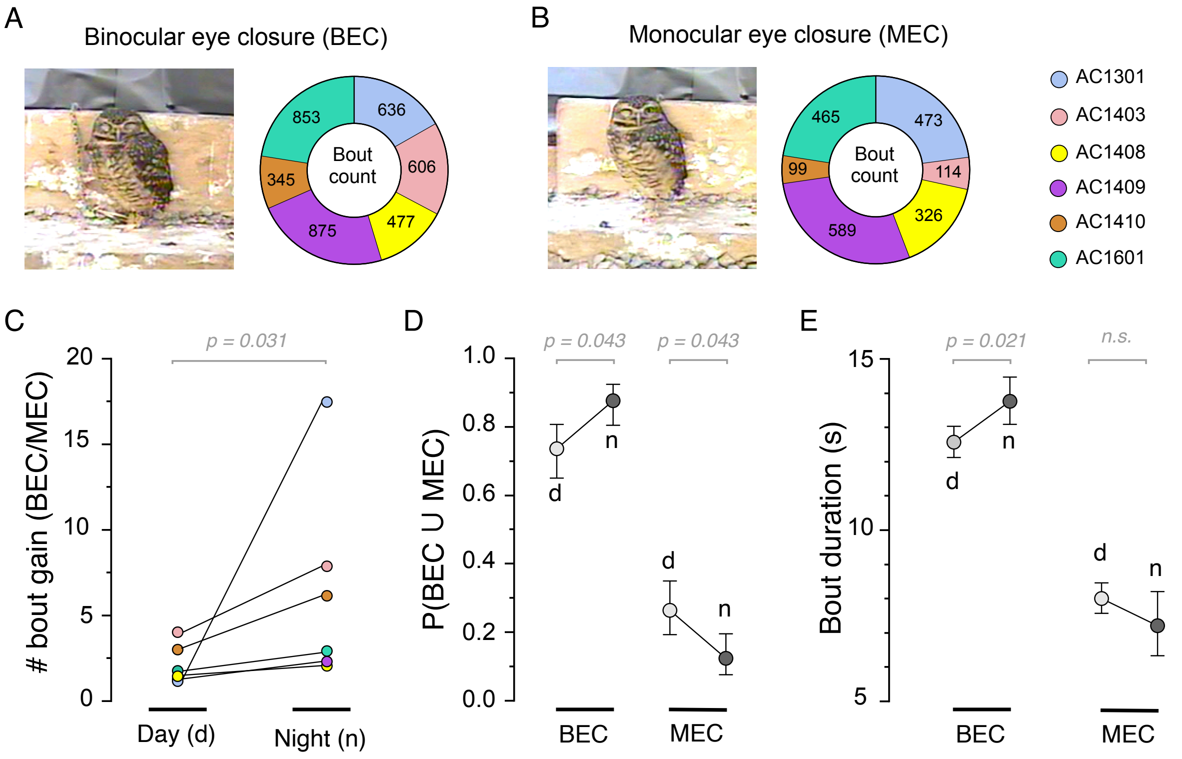


Figure 4.4. Summary of binocular eye closure and monocular eye closure during the day and night. (A) Number of bouts of binocular eye closure (BEC) for each owl throughout 24 h (n=6). **(B)** Number of bouts of monocular eye closure (MEC) for each owl (n=6). **(C)** BEC/MEC ratio in day and night, the line connects day and night ratio for the same owl (n=6). **(D)** Probability of BEC U MEC during the day (d) and at night (n). **(E)** Duration of BEC and MEC episodes during the day and at night. Statistical comparison in C, Wilcoxon Matched-Pairs Signed Ranks Test and; in D, E, Tukey's HSD test. Error bars represent 95%CI.

4.2.4 Hypotonia episodes during binocular eye closure sleep

Observing the behaviors of burrowing owls throughout 24 hours, we noted that many sleeping episodes with both eyes closed were accompanied by overt behaviors associated with body muscle hypotonia, referred to collectively here as body relaxation episodes. These episodes were relatively brief and included neck relaxation events expressed by downward jerks or bobbles of the head and drops of wing muscle tone sometimes followed by a momentary loss of balance (drop off). Several body relaxation events could occur during a single BEC episode. After grouping these events as body relaxation, we found their average number throughout the 24 hours to be 414 (mean 414 ± SD 104). Figure 4.5, shows the counts of body relaxations by owl and compares the marginal mean (LS-mean) between day and night (twilight periods were not considered for this analysis). The LS-mean of body relaxations counts was significantly higher during the day than at night. We hypothesized that this result could simply reflect the fact that the proportion of binocular eye closures tended to be higher during the day (median = 13.1 %, IQR = 7.9 %) than during the night (median = 4.25 %, IQR = 7.6 %). Nevertheless, this difference was almost but not quite significant (Wilcoxon Matched-Pairs Signed Ranks Test, $S_5 = -10.5$, $P = 0.0625$).

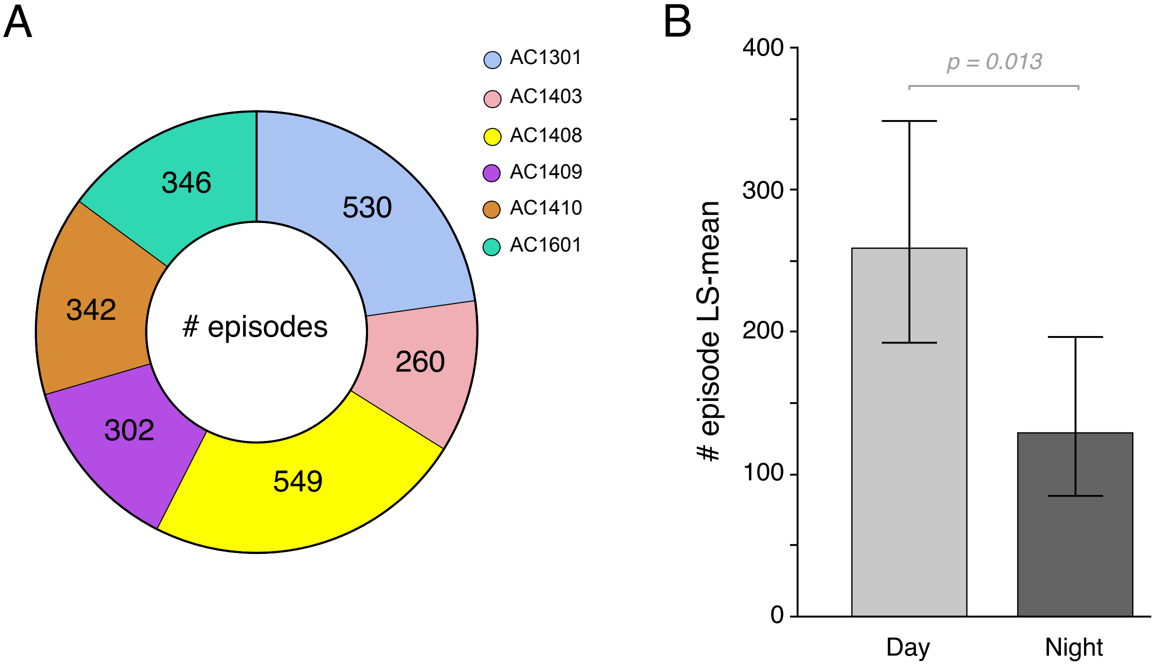


Figure 4.5. Counts of body relaxation episodes displayed during the sleeping behavior: binocular eye closure.

(A) The ring plot shows the total number (#) of body relaxation episodes for each owl over 24 hours (n=6). **(B)** Diel-dependent LS-mean of body relaxations counts (day vs night). Statistical comparison in B, Tukey's HSD test. Error bars represent 95% CI.

4.2.5 Getting ready for sleep: probabilities of behavioral markers occurring before and after binocular eye closure episodes

Figure 4.6 A reveals that within five minutes preceding and following a BEC episode, the probability of BEC remained relatively high, indicating the occurrence of other episodes of BEC in both intervals before and after BEC. Intriguingly, the chances of BEC episodes decreases 15 seconds before and after BEC. As shown in Figures 4.6 B and C, the probability of monocular eye closure (MEC) and *rest pose* responses increased 15 seconds before and after BEC, which may explain the sharp decline in BEC probabilities. Thus, BEC is often accompanied by those behavioral markers that we associated with sleep state.

Furthermore, the probability of isolated eye blink increases shortly before BEC (~ 5 s), whereas it decreases after BEC (fig. 4.6 D). Consistent with the fact that saccades alone provide a good index of *awake state*, their likelihood significantly decreases as BEC approaches (fig. 4.6 E). Interestingly, the peak in saccade alone probability immediately following a BEC was also indicative of brief awakenings or a transition to a sustained *awake state*.

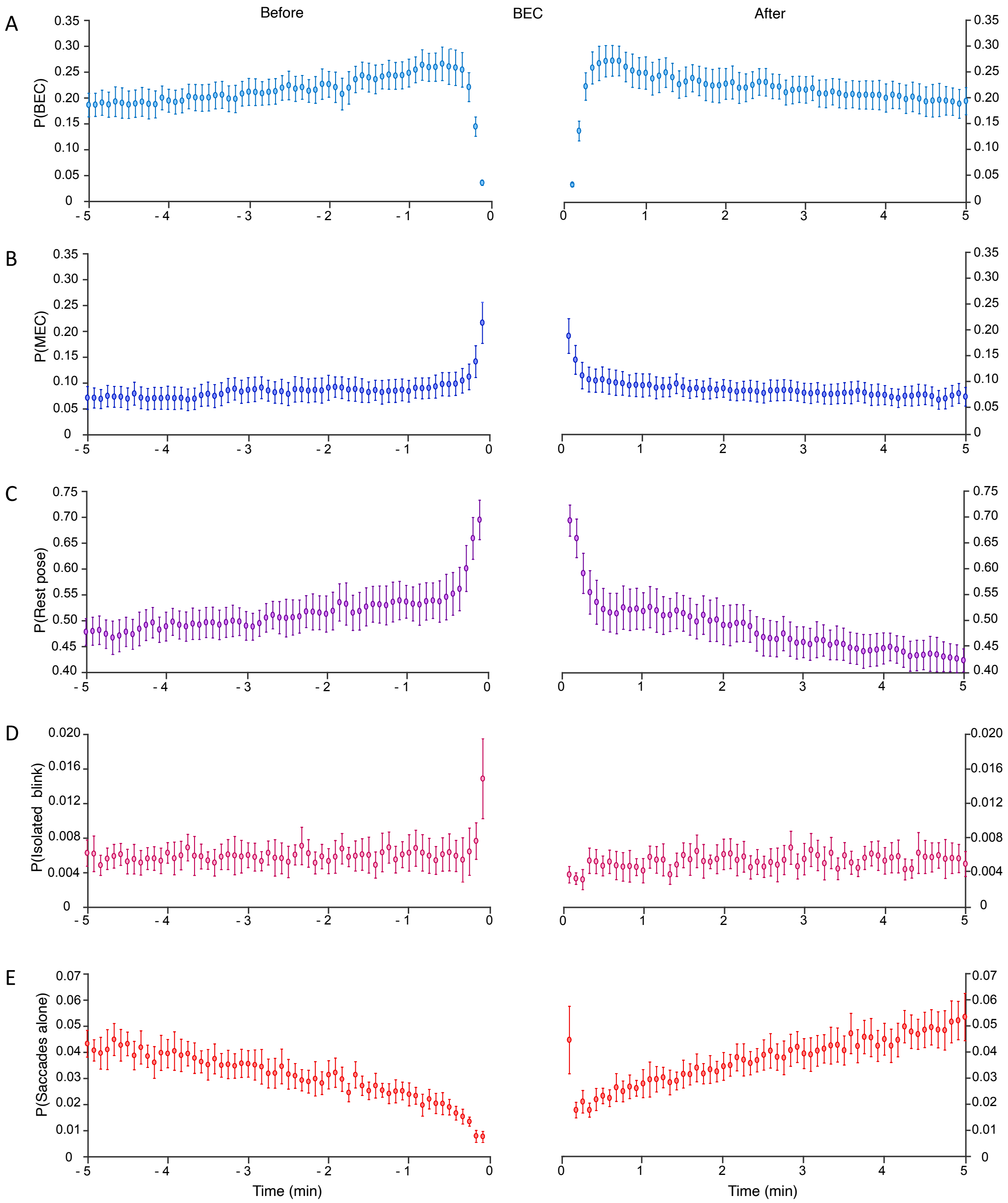


Figure 4.6. Probabilities of behavioral markers occurring before and after binocular eye closure episodes.

In the abscissas axis, the time lapses until the onset of a binocular eye closure is displayed in minutes (left graphs). On the right graphs, the time passes after both eyes open (time after BEC). A minute is divided into bins of five seconds, where each point is the mean for the six owls and dispersion bar is the standard error. The coordinates axis represent the probabilities of a behavior marker before (left) and after (right) binocular eye closure (BEC). **(A)** Probabilities of binocular eye closure before and after BEC episodes. **(B)** Probabilities of monocular eye closure before and after BEC episodes. **(C)** Probabilities of rest pose before and after BEC episodes. **(D)** Probabilities of isolated eye blinks before and after BEC episodes. **(E)** Probabilities saccades alone before and after BEC episodes.

4.3 DISCUSSION

In-depth observation of burrowing owls' behavior throughout 24 hours gave us crucial information for understanding their sleep-wake cycle behavior. Berger and Walker (1972) emphasized that they did not observe any neck hypotonia in EMG recording and that even when watching the owls sleeping, neck relaxations expressed by heads' downward jerks did not happen.

It has been observed that, during REM sleep, birds maintain muscle tone so that they can stay upright and keep their balance, but the muscles supporting the head may show modest reductions in tone (hypotonia) as opposed to muscles that control the legs (Rattenborg *et al.*, 2019). In contrast to the EEG study in burrowing owls, we could undoubtedly observe that they exhibit partial neck and body relaxation when sleeping with their eyes closed, a solid indication that they present hypotonia during REM sleep.

We are aware that eye closure does not always correspond to a REM sleep state. The owls could have been in REM sleep when their eyes were open or in NREM sleep when their eyes were closed (Berger & Walker, 1972). Without EEG correlates, some inferences about the effective brain state of the animals we studied need caution. Notwithstanding, overall, we consider that binocular eye closure is a reliable indicator that burrowing owls are entering into REM sleep since the temporal organization of behavioral indicators seems to match what it was reported: their REM sleep always follows SWS, often beginning with the closure of the eyes and often ending with eyes being partially or wholly opened accompanied by a head movement (Berger & Walker, 1972). Furthermore, the mean duration of binocular eye closure bouts was 12.3 seconds, and it occupied about 9% of the sleep state. Berger and Walker (1972) reported similar results: mean duration of REM sleep, 11 seconds, total amount of time spent in REM sleep, ~6%. The close match between our behavioral data and the previous EEG study is also interesting considering that, unlike the latter, our study was performed in a semi-natural setting, with which owls seemed entirely comfortable. Within their spacious enclosure owls are typically seen to dig burrows, engage in social interactions, vocalize, store food, eat and walk following circadian cues, sunbathe and even mate successfully. Despite that owls were leashed during the video recordings, we used falconry techniques to ensure a more natural acclimatization.

Interestingly, animals with small body sizes and high metabolic rates tend to present a sleep more fragmented and bouts of REM sleep with a shorter duration (Savage & West, 2007). Accordingly, the pressure of smaller mammals' metabolic requirements is a compelling explanation for why some of them successfully adapted to the niche cathemeral (Halle, 2006). One possible explanation for the highly fragmented sleep of some endothermic animals comes from the energy allocation hypothesis, which claims that cessation of thermoregulation during REM sleep allows the body to save or allocate energy, then serving other biological functions (Schmidt, 2014).

Concerning the constant vigilant behavior of burrowing owls, we hypothesize that copious amount of NREM-REM sleep cycle throughout 24 hours, and short bouts of

REM sleep, could result from an adjustment for optimum energy efficiency between the expensive thermoregulation and a constant need for vigilance, beyond other biological benefits gained by REM sleep. Accordingly, a prolonged period of REM sleep increases the likelihood of the core temperature to deviate toward the ambient temperature. Thus, in the end, the organism might require more energy to defense or maintain its core temperature (Schmidt, 2014). This push and pull to maintain body functions could be inclined to the economy of energy provided by the REM sleep when environmental temperature increases, mainly in the thermoneutrality zone range. We found that binocular eye closure duration was slightly longer at night but that owls spent more time closing both eyes during the day than at night. Moreover, although not demonstrated in the results, binocular eye closure occurrences are positively correlated with ambient temperature. If thermoregulation is abolished during REM sleep in burrowing owls, the energy-saving expected from REM sleep is maximized during these times of the day when thermoneutrality is prevalent. In contrast, Coulombe (1970) reported that the burrowing owl's body temperature decreased during the dark period. However, during his experiments, the owls were inside the laboratory and seemed to be highly inactive at night (Coulombe, 1971).

Burrowing owls present higher arousal thresholds during REM sleep (Berger & Walker, 1972). It is not difficult to imagine that for a small semi-colonial animal living in an open environment on the ground, spending a long time in continuous REM sleep may pose a serious risk, even though brain activity during REM sleep is similar to that of wakefulness (Lima *et al.*, 2005). Thus, it is possible that burrowing owls are better at detecting predators promptly because their brains alter between states of wakefulness and sleep more frequently. Shorter sleep cycles (alternating bouts of non-REM and REM sleep) may be related to a reduction in predation since they promote multiple bouts of shorter awakenings (Lesku *et al.*, 2008; Lesku, Roth, *et al.*, 2009). Accordingly, Scriba and colleagues (2014) showed that nestlings of barn owls presenting a more fragmented sleep exhibit an increased vigilant behavior. Interestingly, during the night, the switch between wakefulness and sleep states was most often, allowing owlets to detect faster the return of parents with food (Scriba *et al.,* 2014).

If sleep fragmentation seems to confer advantageous capabilities to burrowing owls, it worth emphasizing that this trait probably requires specific adaptive mechanisms yet to be discovered. Remember that sleep fragmentation can be harmful in species not physiologically prepared for it, like humans. As discussed by Stampi (1992b), humans with polyphasic sleep schedules need to maintain proper dynamics of biological rhythms such as sleep propensity (low sleep latency), sleep efficiency (enough amount of restorative sleep), and sleep inertia (no drowsiness or impairment in executing tasks just after waking).

Berger and Walker (1972) noted that electrophysiological brain changes, in burrowing owls, were conspicuously abrupt from one stage to the next. Behaviorally, we observed that burrowing could easily fall into a sleep-like state, but we did not perform any experiment to confirm if they have relatively short sleep latencies. Considering the ability of burrowing owls to cope with various biotic and abiotic challenges in nature, we suspect that they present high sleep efficiency and sleep inertia. In any case, it would be important to investigate their performance immediately following waking up.

In light of the above, we know that burrowing owls go through slow-wave sleep with their eyes open or partially opened and with both eyes closed just before entering REM sleep (Berger & Walker, 1972). We could clearly note the gradual changes in posture (rest pose) and the eye states (eyelid begins to droop, eye blinks increased), indicate that the owl entered in a sleep state and probably would close both eyes after some minutes. The analysis of behaviors exhibited by burrowing owls enabled us to identify behavioral indicators of sleep or drowsiness state. Among the main behaviors observed were the rest pose, isolated eye blinks, monocular and binocular eye closures accompanied or not by body relaxations. Observing the videos, we could see the frequency of these behaviors increase until they closed both eyes. The probability curve before and after binocular eye closures episodes for these behaviors corroborates with our observations. As we watched the videos, we could see that the occurrence of these behaviors increased progressively while the active behaviors decreased, resulting in recurring cycles in which the eyes remained closed for a few seconds and then opened, usually accompanied by a head movement.

The duration of *rest pose* was around 10 s, which is very different from the mean duration of slow-wave sleep duration, 148 s, reported by Berger and Walker (1972). This substantial difference may be due to head saccades, which often interrupted the rest posture. For example, three head saccades with fifty-second intervals breaks up a time sequence of rest pose in three. Thus, to compare our data adequately with those from Berger and Walker (1972), it would have been necessary to merge *rest pose* sequences when intercalated by isolated head saccades.

We also noted that owls spent several seconds in a *rest pose* with only one eye closed. Although electrophysiological data would be needed to confirm if burrowing owls present asymmetric sleep, we suggest that resting with one eye closed in burrowing owls is indicative of unihemispheric sleep. Having such a capability would provide the owl with the ability to cope with ecological challenges, such as predators. Note that animals such as cats, opossums, tegus, and falcons frequently visited the area surrounding the experimental aviary, and those visibly elicited predatory risk responses in the owls. Thus, our experimental conditions were conducive to natural predation pressures. Often accompanied by the closure of one eye, unihemispheric sleep is considered a functional adaptation that allows the animal to visually survey their environment while sleeping (Rattenborg *et al.*, 1999, 2019). During the night, the proportion of monocular eye closure (MEC) relative to binocular eye closure (BEC) was smaller than during the day,. maybe because, in nature, the presence of predators, mainly diurnal raptors, make them engage in monocular eye closure sleep more frequently to monitor this kind of threats. Also, burrowing owls are adapted to urban areas, so they may use this monocular eye closure strategy to deal with urban disturbances during sleep.

It is worth mentioning that Amlaner and McFarland (1981) found an association between sleeping postures and herring gulls' eye states. Besides presenting a higher arousal threshold during sleep postures. Those higher thresholds were also associated with an increased eye blink rate, suggesting that both eye closure and eye blinking are good indictors of sleep or drowsiness (Amlaner and McFarland, 1981). We did not determine the arousal thresholds for sleep, but our fine-grain behavioral scoring led us to a similar conclusion. A study of our team has shown that vigilant burrowing owls blink less and display more head saccades in a visual task context (Dias *et al.*, 2014). Peafowls decrease their blink rates when faced with a predator (Yorzinski, 2016). Interestingly, Yorzinski (2020) showed that great-tailed grackles adjust their blinking behavior depending on their flight stage: before, during, and after a flight, their blink rate was similar, but at takeoff and landing, their blink rate was higher. The risk of collisions can be the reason grackles blink less. On the other hand, shortly before (0.1s) touching the ground, the flight challenging phase is superseded so that the bird can become less alert, leading to an increased blink rate.

By having a continuous overview of the animal's behavior, we could better estimate when the animal was probably falling into NREM sleep (rest pose + blinks, the other indicators) and REM sleep (binocular eye closure). An interesting observation we have made is that burrowing owls close their eyes to sleep with the inferior eyelid, while they blink with the superior eyelid. In addition, the *vigilant pose*, an indicative of active state, has given us more cues towards the upcoming state change. Finally, highly fragmented sleep presented by burrowing is distributed along day and night, which also corroborates the cathemerality nature of these birds.

CHAPTER 5

EFFECT OF 24h SLEEP DEPRIVATION IN THE BURROWING OWL

5.1 INTRODUCTION

In 1980, Alexander Borbély coined the term sleep homeostasis based on the assumption that sleep loss is expected to trigger compensatory mechanisms. Considering sleep as a regulatory system, Borbély (1982b) proposed a two-process model that turned out to serve as a valuable conceptual framework for sleep research. In its essence, the model propounds that sleep regulation depends on the interaction between a homeostatic process (Process S), which regulates sleep intensity, sleep duration, as well as the amount of sleep, and a circadian process (Process C), which determines sleep timing. In general terms, the homeostatic mechanism depends on the time spent awake. In contrast, the circadian mechanism is independent from the time spent awake or asleep (Borbély, 1982a). However, sleep and alertness consolidation are promoted when both systems work together.

During birds' sleep, slow wave activity is highest in the first hours and gradually declines in the course of sleeping, suggesting a correspondence with sleep homeostasis observed in mammals (Szymczak *et al.*, 1996; Jones *et al.*, 2008; Martinez-Gonzalez *et al.*, 2008). Furthermore, some birds species exhibit an increase in sleep intensity, expressed primarily by a rise in SWA-EEG power during NREM sleep, but the relation between the intensity of SWS and prior wakefulness is not well understood in bird sleep research (Martinez-Gonzalez *et al.*, 2008; Hasselt *et al.*, 2019). The first study in pigeons that examined whether there was an increase in SWA during sleep found no difference in SWA prior to and after 24 hours of sleep deprivation, but REM sleep rebound was exhibited (Tobler & Borbély, 1988). In contrast, Martinez-Gonzalez and colleagues (2008) performed a short-term sleep deprivation in pigeons demonstrating, for the first time in birds, a significant rise in SWS and an increase in time spent in REM sleep during the recovery night. White-crowned sparrows also exhibits an increase in SWS after 6 hours of sleep deprivation, but they present no significant differences to REM sleep (Jones *et al.*, 2008).

Hasselt and colleagues (2019) demonstrated that, in captive starlings, REM sleep is not compensated for after sleep deprivation. Moreover, the intensity of NREM sleep during recovery was not different from the control condition. Hasselt and colleagues (2019) also showed a spectral power decrease in frequencies between 0 and 0.78 Hz and demonstrated that 4 or 6 hours of sleep deprivation did not modify the sleep recovery, indicating that sleep homeostasis in this species is perhaps not dose-dependent.

The above considerations outline how sleep regulation in birds still puzzles and challenges our understanding of sleep in the animal kingdom (Rattenborg *et al.*, 2009). Moreover, we cannot ignore the fact that, in the wild, birds and mammals have to cope with similar situations where sleeping is almost prohibitive (Siegel, 2008). This is especially true for migrating birds and aquatic mammals that engage mainly in unihemispheric sleep (US), limiting the bihemispheric sleep (BS) to a few minutes or even seconds (Lyamin *et al.*, 2008; Rattenborg, 2017). During non-stop flights over the ocean, frigate birds sleep less than 1 hour per day. Surprisingly, they also engage in few minutes of BWS and REM sleep for some seconds despite the risk of doing so in mid-flight, which suggests that these two brain states are critical for the survival of frigate birds. However, when on land, frigate birds exhibit deeper and longer sleep. While land-based sleep may be associated with sleep homeostasis, it is still unclear if frigate birds acquire a full sleep rebound (Rattenborg *et al.*, 2016).

It is fascinating that after spending several days almost without sleeping, birds migratory and aquatic mammals do not show the need for sleep recovery. In fact, apart from bottlenose dolphins (Oleksenko *et al.*, 1992), the existence of unihemispheric sleep rebound is still an open issue (Rattenborg *et al.*, 2019). Notwithstanding, Lesku and colleagues (2011) found evidence of local homeostatic sleep in pigeons that remained 8 hours awake while watching *Life of Birds* videos with only one eye open. Their results show that, during sleep, SWA modulation amplitude was greater in the contralateral visual wulst, the brain area responsible for processing visual input through the stimulated eye.

Sleep regulation appears to be ubiquitous in the animal kingdom. However, species-specific demands, sleep homeostasis, and interaction with the circadian process are manifold. Thus, in-depth research on different species is still needed (Rattenborg *et al.*, 2009). Capitalizing on our knowledge of behavioral sleep indicators for burrowing owls, we aimed to probe the effect of 24 hours of sleep deprivation in this singular owl species. This is the first study examining the impact of sleep deprivation in owls.

5.2 RESULTS

Owls lost approximately 5% of their weight after sleep deprivation (SD), and this loss diminishes to 1.7% after the sleep recovery phase. Thus, by the end of the experiment, owls almost regained the weight they had prior experimentation (mean weight 24 hours before SD: 176.6 g ± 12 g; just after SD: 165 g ± 8.6 g; after the recovery phase: 173.8 g ± 11.7 g). The next day after the sleep deprivation experiments, we observed them behaving naturally, and their weight was in a healthy range. Thus, we considered that the owls fit to return to their original aviary.

An example of a representative result of the first four hours following SD is shown in Figure 5.1. In general, after SD, there was a reduction in the frequency of behaviors associated with wakefulness and an increase in behaviors related to sleepiness. In the previous 24-hour observations study, isolated blinks (without concomitance with head-saccade) occasionally occurred during awake states, but it was more associated with sleep behavior (see fig. 4.7 D). However, due to its low prevalence, and the uncertainty of what state it represented, it was treated as a neutral behavior and not much explored. Here, it is striking that isolated blinks are strongly enhanced after SD, thus supporting the notion that they may be indicators of the sleep pressure.

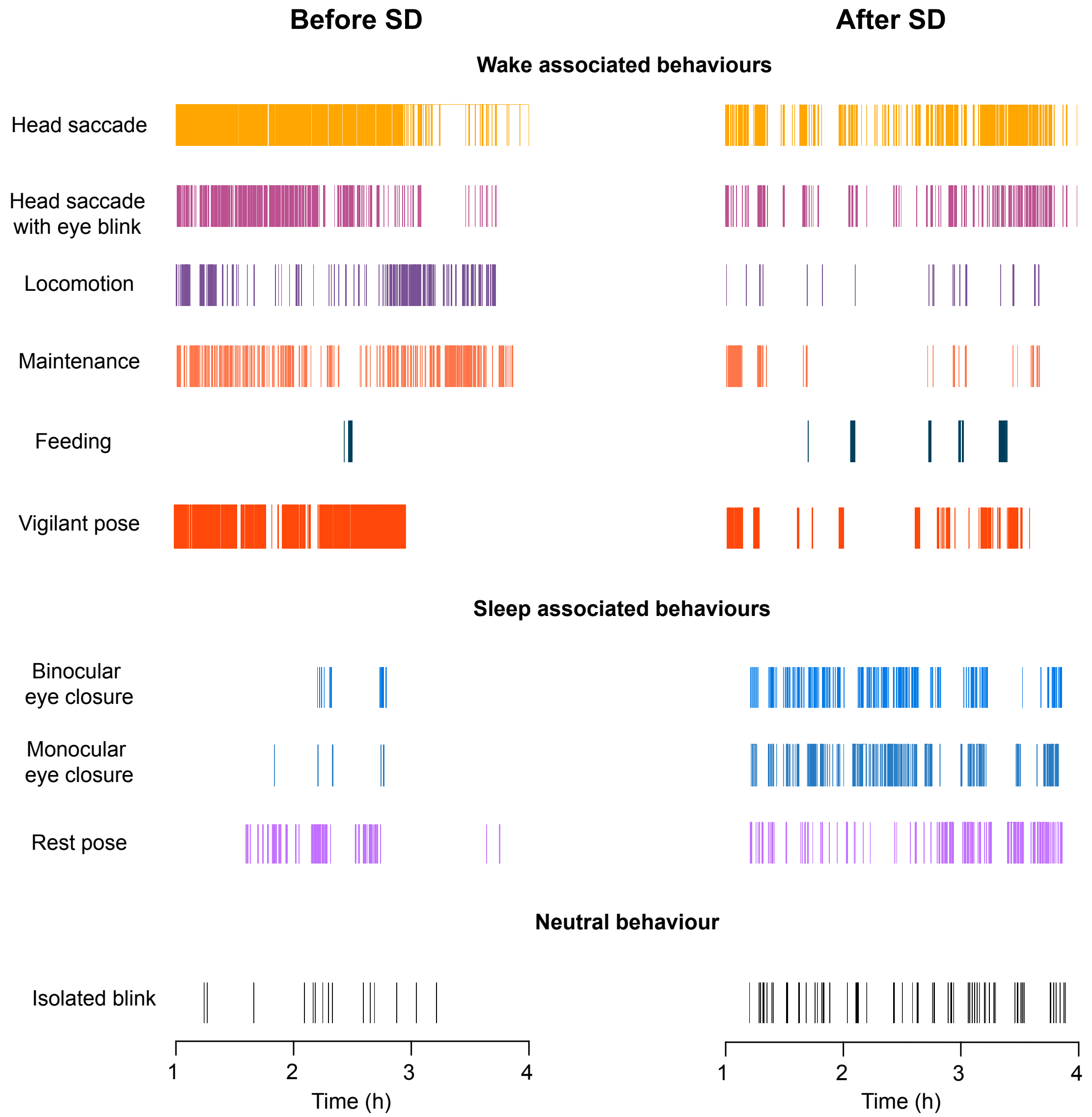


Figure 5.1. Frequency of the most representative behaviors before and after sleep deprivation.

Each trace represent a second occurrence. **(Before SD)** First four hours before sleep deprivation (start recording at 16 h 01min). **(After SD)** First four hours after sleep deprivation (start recording at 16 h 01min).

5.2.1 Sleep amount and transition probabilities before and after sleep deprivation

The following results have been organized into four time-window, referred to as Block # before and after SD. Block 1 stands for the first 4 hours of 24h observations before and after SD (n= 5 owls). A two-hour non-scoring interval separates blocks 1 and 2. Between blocks 2 and 3, there is a four-hour non-scoring interval, as between blocks 3 and 4. In contrast to block 1, which encompasses a time-window lasting four hours, each of the remaining blocks (n=4 owls) comprises a time-window lasting two hours.

For technical reasons, the SD protocol was started at two different times of the day. For owls AC1601 and AC1301, SD began at 18:03 and 18:09, respectively. For the three other owls (AC1408, AC1409, AC1804), the SD protocol began at 16:01, 16:27, and 16:34, respectively. Interestingly, the two owls that did not present a clear sleep rebound were those that the sleep deprivation started around 18 o'clock. This finding may be explained because burrowing owls show a peak in activity during this time, so perhaps this endogenous force compromised (or masked) their sleep in the first hours after SD.

Accordingly, it can be observed in Figure 5.2 A-B that three owls (AC1408, AC1409, and AC1804) exhibited a considerable increase in the amount of time spent asleep after sleep deprivation. Also, the *sleep state* remained more sustained for a prolonged time after SD. For owls AC1301 and AC1601, sleep rebound was less apparent, and a more fragmented sleep persisted even after SD.

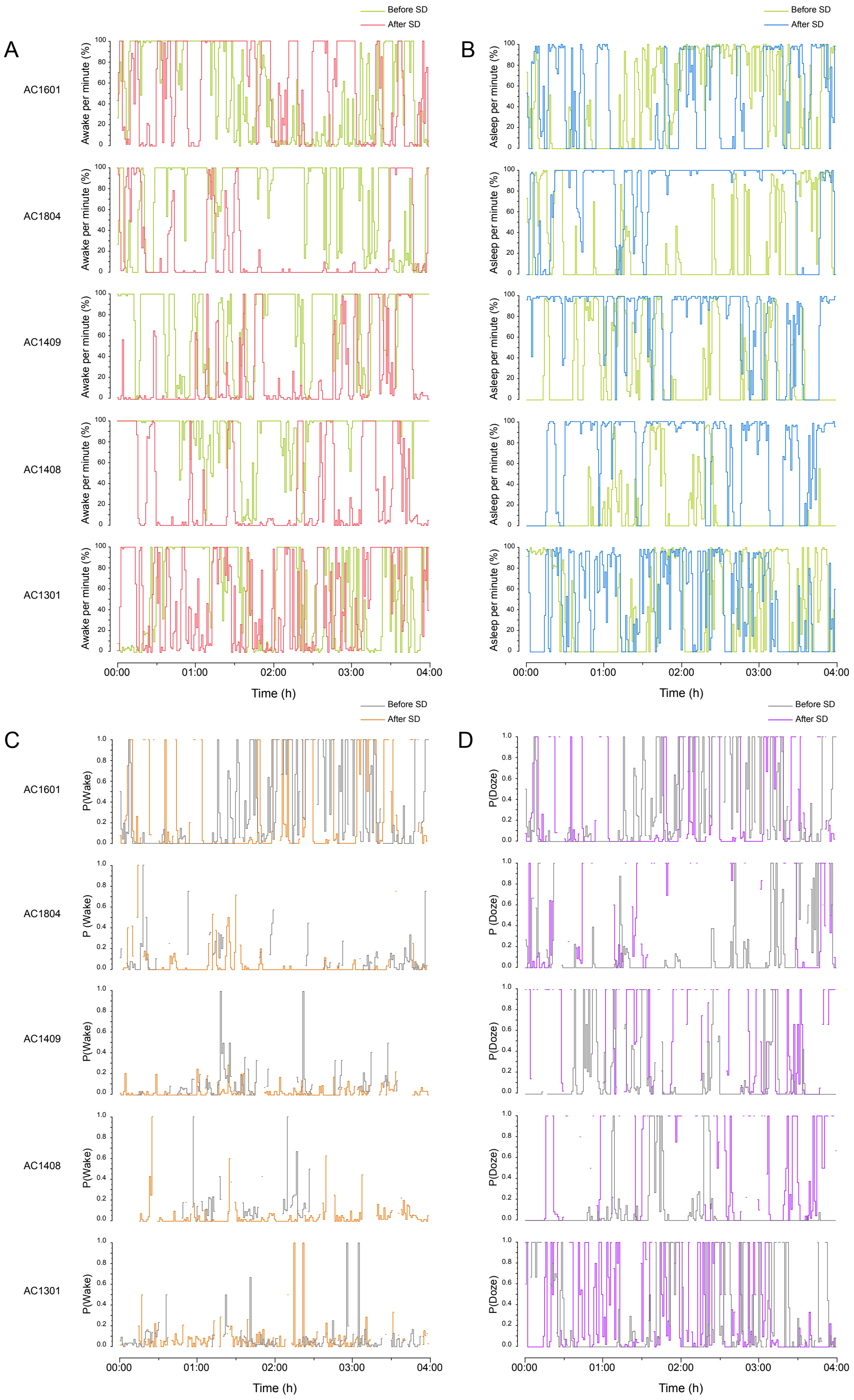


Figure 5.2. Transitions probabilities to awake and asleep state.

(A) First 4 hours of time spent awake state by minute before SD (green) and after SD (red) for each animal (n=5)**.** **(B)** First 4 hours of sleep state by minute before SD (green) and after SD (blue) for each animal (n=5)**.** **(C)** Probability transition to awake state by minute P(Wake) before SD (gray) and after SD (orange). **(D)** Probability transitions to sleep state by minute P (Doze) before SD (gray) and after SD (violet). The line breaks represent the analysis windows with no transitions, so the probability was computed as not a number.

Figure 5.2 C,D shows the values of P(Wake) and P(Doze) per minute in the first 4 hours before and after SD (block 1). The lines' breaks corresponds to the analysis windows where no transition could be computed, so the probability was assigned as not a number (see Materials and Methods, section 2.7.4). Because P(Doze) represents the probability of switching from an *awake state* to a *sleep state*, its increase may reflect sleep pressure, but it can also be a function of the degree of sleep fragmentation. For the three owls that showed a clear sleep rebound, P(Doze) after SD (median = 0.42, IQR = 0.150) were more than three times as high as before SD (median = 0.14, IQR = 0.073), while the P(Doze) of the other two owls were nearly the same before (median = 0.30, IQR = 0.0004) and after SD (median = 0.28, IQR = 0.0803). It is conceivable that before SD, those three owls spent more time in a continuous *wake state*, which reduced transitions to a *sleep state*. After SD, they displayed short periods of awake state followed by long bouts of sleep state, so the number of transitions to *sleep state* increased. On the other hand, the owls AC1601 and AC1301, frequently switched between the wake and sleep states before and after SD, resulting in very close P(Doze) values across treatment. Note that considering our owl population as a whole, the coefficient of variation (CV) calculated for P(Doze) before SD (CV = 52.0) was about twice that after SD (CV = 27.6). Thus, SD seems to regularize inherent, natural individual variations, indicating some limitation of behavioral plasticity in the face of previous sleep debt, at least concerning P(Doze). Therefore, the a priori assumption that SD effect needs to be assessed on an owl-by-owl basis may be relaxed. Grounding our analysis in line with this argument, we found that the P(Doze) population median significantly increased after SD, whether using the Wilcoxon Signed Rank Test (S_1 = 17, P = 0.0367) or the Fisher's Exact Test (S_1 = 17, P = 0.0159) to account for our sparse sample. Note that when using an owl-by-owl nonparametric comparison, this increase in P(Doze) was not significant (Wilcoxon Matched-Pair Signed Rank Test, S_4 = 5.5, P = 0.187).

P(Wake) may be conceived as a measure for sleep depth since fewer sleep to wake transitions means that the animal presents a more consolidated sleep state. Accordingly, for the three owls that showed a clear sleep rebound, P(Wake) after SD (median = 0.046, IQR = 0.03) showed a ~2.5 drop in relation to before SD (median = 0.13, IQR = 0.058). In relation to the two owls seemingly refractory to treatment, P(Wake) median values were nearly the same before (median = 0.08, IQR = 0.023) and after SD (median = 0.06, IQR = 0.017). On the premiss that, the CV of P(Wake) values before and after SD were 31.7 and 33.8, respectively, P(Wake) seems insensitive to the regularizing effect of SD inferred for P(doze). Thus, in coherence with the rational mentioned above, the Wilcoxon Signed Ranks Test for matched samples appear to be more appropriate to assess the decrease of P(Wake) after SD statistically. Accordingly, this decrease was found to be at the margin of statistical significance (S_4 = -7.5, P = 0.0625). Using a non-pairing assumption, the effect was statistically significant (Wilcoxon Signed Ranks Test, S_1 = 40, P = 0.0122; Fisher's Exact Test, S_1 = 40, P = 0.004).

Figure 5.3 A shows results from a GLMM analysis designed to assess the impact of SD across all experimental blocks and incorporating all the owls. Using this multivariate approach, the amount (%) of sleep time increased significantly during a 4h period just after SD (block 1, Tukey's HSD test, $P = 0341$). An overall significant effect was confirmed for treatment (GLMM, $F_{1,3} = 7.43$, $P = 0.0113$), but not for experimental block (GLMM, $F_{1,3} = 0.00$, $P = 1.00$). This result may be interpreted as validating the hypothesis of a rapid sleep recovery occurring only during block 1, and statistically accounted for by a third interactional variable in the linear model (GLMM, treatment * block: $F_{1,3} = 3.60$, $P = 0.0305$). Note that, there was a small across-block trend for LS-mean estimates to increase before SD, and to decrease after SD. This trend would be compatible with the idea of a homeostatic sleep process, had only our GLMM analysis confirmed it. Moreover, there was no statistically significant effect of treatment and block for P(doze) and P(wake). Thus, according to this modeling framework, the tendencies for LS-mean estimates to increase after SD, for P(doze), and decrease after SD for P(Wake), do not have statistical support. Convinced that this lack of support was due to the confounding effect of owls AC1601 and AC1301, we reanalyzed the data excluding these to owls. Similar lack of effects were found, but this time probably due sample size (n =3). We conclude that the only way to resolve the issue of whether SD truly has an impact on P(Doze) and P(Wake), and to clarify the potential circadian dependency of SD induction, is to increase experimentally our group sample size, testing at least two SD induction time points.

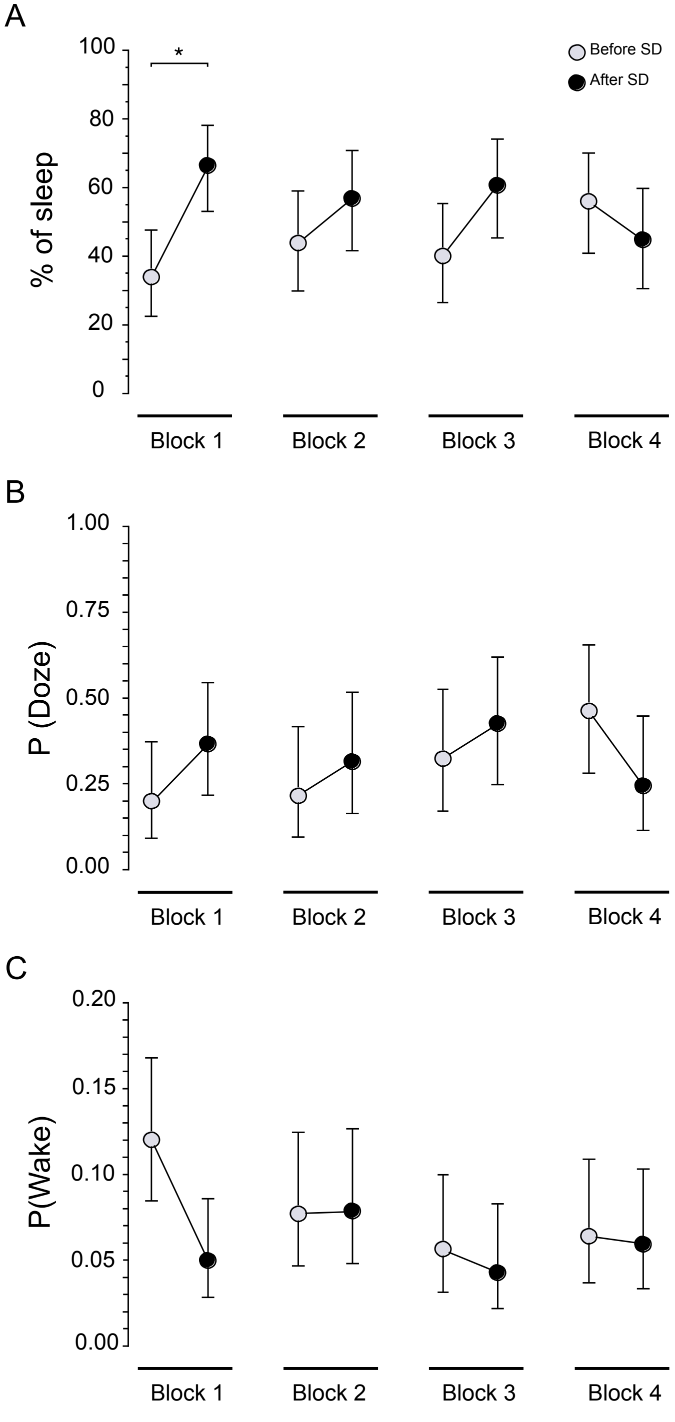


Figure 5.3. Sleep amount and transition probabilities before and after sleep deprivation (SD). **(A)** LS-mean percentage of sleep before SD (gray circle - mean) and after SD (black circle - mean). **(B)** Mean probability transition to doze state (P(Doze)) before SD (gray circle) and after SD (black circle). **(C)** Mean probability transition to awake state P(Wake) before SD (gray circle) and after SD (black circle). The colored circles referred to the values of each animal and vertical bars, the dispersion. For block 1 (n=5), for the other blocks (n=4). * p-value<0.05.

The effect of SD on the number and duration of binocular eye-closure episodes is presented in figure 5.4. For this behavioral trait, closely related to REM sleep, the relative number of episodes expressed as a difference (Δ # episodes, after SD minus before SD) was not a reliable indicator of an SD effect across blocks (figure 5.4 A). Changes were highly variable among owls, and this variability was not significantly modulated across blocks (Kruskal-Wallis test, $\chi^2 = 0.60$, $P = 0.896$). Although the duration of binocular eye-closure episodes was also not significantly influenced by block (GLMM, $F_{1,3} = 1.11$, $P = 0.34$), treatment had a significant impact on this variable (GLMM, $F_{1,3} = 28.65$, $P < 0.0001$), but not in a consistent way (GLMM, treatment * block: $F_{1,3} = 4.32$, $P = 0.0047$). Again, this finding suggests that homeostatic compensation of sleep deprivation occurs only during a short period after this intervention. It also suggests that REM sleep may be a regulated variable in burrowing owls.

In light of the fact that we did not conduct EEG recordings, we cannot be absolutely sure that the various SD effects described above are due to an increase in SWS, REM sleep or both. Nonetheless, our findings provide a good indication that burrowing owls exhibit at least some kind of short-lived sleep rebound.

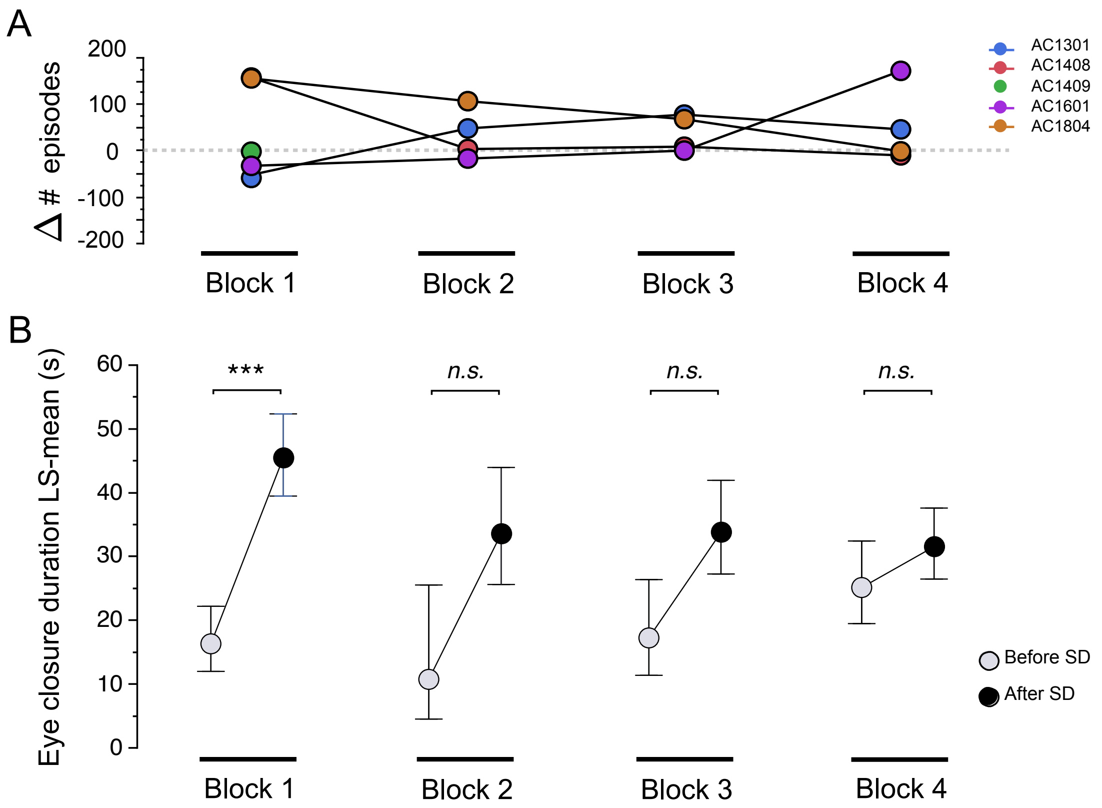


Figure 5.4. Effect of 24-h sleep deprivation on the number and duration of eye closure episodes. **(A)** Difference of the number episodes before and after SD across blocks. Block 1 shows results for 5 owls and the other blocks for 4 owls. **(B)** LS-mean binocular eye closure duration along blocks before (gray) and after SD (black). *** p-value < 0.0001, Tukey's HSD test.

5.2.2 Effect of SD on the temporal organization of head saccades

To investigate if SD impacted the temporal organization of wake behaviors, we calculated the burstiness index (B) and the memory coefficient (M) of head saccade events before and after sleep deprivation. Figure 5.5 A shows the values plotted in a phase plane (B,M) for block 1. The largest change caused by SD was manifested by Owl AC1408 in terms of a 0.28 drop in B. All the other differences in B and M after SD were seen at the level of the second digit after the decimal point, and no statistical difference was found for both B (Wilcoxon Matched-Pairs Signed Rank Test, B: S_4 = 4.50, P = 0.31) and M (S_4 = - 3.50, P = 0.43). This is remarkable considering that the LS-mean number of head saccades estimated after SD (541.6, 95%CI: 304.9 - 961.8) had dropped by about a third of its value before SD (1723.4, 95%CI: 1248.9.9 - 2378.0). Comparing across blocks , the pre-/post-treatment differences in median values were not signifiant, neither for B (Kruskal-Wallis test, χ^2 = 0.63, P = 0.64), nor for M (χ^2 = 1.96, P = 0.58). For each experimental block, we also ran a Wilcoxon signed-rank to test whether these median differences were different from a zero median. In none of the blocks, this hypothesis was statistically supported. Thus, we conclude that the normal intermittent temporal dynamics of head saccades is maintained even under sleep pressure caused by SD.

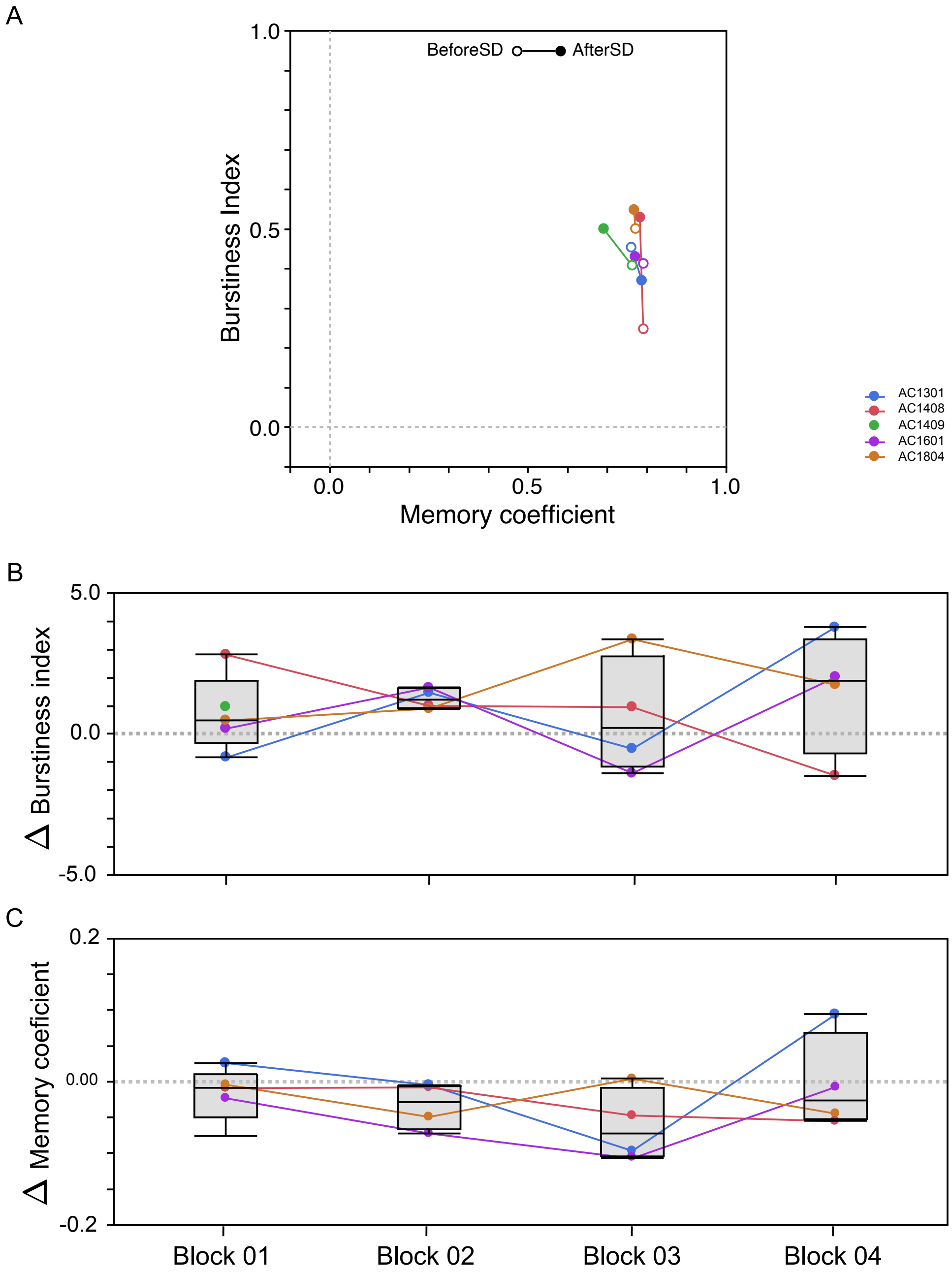


Figure 5.5. Effect of SD on the temporal organization of head saccades.
(A) Burstiness index and Memory coefficient space. Empty circles indicate the values for each owl before SD and filled circles index values after SD. **(B)** Boxplot Burstiness index difference (Δ) before and after SD across blocks and animals. The same animal is connected by lines. **(C)** Memory coefficient difference (Δ) before and after SD across blocks and animals. The same animal is connected by lines.

5.3 DISCUSSION

Cognizant of the complex scenario about the effect of sleep loss in birds, we were very surprised to find that burrowing owls exhibit a sleep rebound, even though our findings rest on behavioral indicators. Indeed, this is the first study that documents sleep recovery after a period of experimentally induced SD in owls.

5.3.1 A behavioral perspective on sleep homeostasis of a cathemeral bird

The behaviors showing a notable SD effect were the *rest pose*, binocular eye closure, isolated blinks and head saccades. Sleep compensation processes appeared to operate mostly during the first four hours following SD.

Hasselt et al. (2020) found that migratory geese had mixed sleep consolidation, but comparisons between their first hour of recovery sleep and their baseline sleep revealed longer sleep episodes. Furthermore, the authors reported that the increase in EEG delta power was limited. After 4 hours of SD, it increased slightly, but not after 8 hours. Thus, the geese compensated for sleep loss primarily by extending their sleep time rather than by increasing SWS intensity. On the other hand, pigeons and white-crowned sparrow exhibit marked sleep compensation associated with EEG-SWS intensity, but the time spent in SWS does not significantly differ from baseline sleep (Jones *et al.*, 2008; Martinez-Gonzalez *et al.*, 2008). Thus, a good indicator of sleep recovery in birds, is the time spent in sleep, more than the increase in EEG-SWS power.

Similarly, our study showed a considerable increase in sleep amount (*rest pose* + *overtly sleeping* behavior) during the first four hours after SD. Additionally, the features of sleep recovery presented here tend to decline over time, suggesting that a homeostasis process is at work. Our work supports the idea that beyond validating EEG-SWA intensity for the measurement of sleep compensation in different species, additional variables related to sleep should be analyzed, including detailed behavioral traits.

An important point to consider about the migratory geese study is that these birds did exhibit a sleep recovery during summer when their sleep is much more fragmented than during winter (Hasselt *et al.*, 2020). In our study, burrowing owls also exhibited a highly fragmented sleep and sleep compensation. This indicates the restorative value of the short sleep bouts for these species since, when prevented from engaging in short sleep cycles, they experience a compensatory sleep effect. Accordingly, Samson and colleagues (2019) demonstrated that sleep deprivation impaired task performance in two species of cathemeral lemurs and that sleep-disruptive effects were more potent in a diurnal lemur species.

Our analysis of transitions between wake and sleep states, P(Doze) and P(Wake), presented a profile consistent with what Wiggin *et al.,* (2020) reported in the fly, namely: after SD, P (Doze) increases as a function of sleep pressure, and P(Wake) decreases as a reflection of sleep consolidation. However, it was not possible to confirm the validity that those measures for our avian model due to a confounding factor introduced by our experimental design which reduced the statistical robustness of our data. The fly study is of relevance to explain this point. In this study, Wiggin *et al.,* (2020) demonstrated that P (Doze) is not only controlled by an endogenous process (sleep pressure) but that it is also influenced by the level of light, which is a robust circadian entrainment factor. In two owls, SD was ended at dusk, instead mid-afternoon, like the other three owls. Interestingly, these two owls did not seem to engage in a sleep recovery process. Environmental light at twilight hour probably must act as an entrainment factor resulting in peak activity in all owls. Therefore, for these two owls, the P(Doze) values might reflect an effect of the circadian system (process C) on sleep homeostasis (process S).

In line with this hypothesis, the homeostatic pressure in a crepuscular species, like the *Octodon degus*, is different depending on the ending time of SD. The amplitude level of slow-waves after ending SD at dawn was attenuated compared to that obtained after SD ended at the start of the subjective night (Kas & Edgar, 1999). The interaction between circadian regulation and sleep homeostasis has also been shown to occur in some species, such as cats and rats (Trachsel *et al.*,1986; Lancel *et al.*, 1991).

In contrast, sleep rebound in guinea pig, a species that lacks a distinct natural preference for sleeping in a determined circadian phase, indicates that sleep homeostasis is primarily independent of the circadian rhythm (Tobler & Franken, 1993). Also, the presence of sleep homeostasis was demonstrated in mice with deletions of circadian genes (Naylor *et al.*, 2000). It is evident that circadian regulation interferes with the homeostatic process, but this interaction is poorly understood.

In regards to REM sleep, birds species present different amounts of REM sleep, though the exact cause of this variation remains unclear (Blumberg *et al*., 2020; Hasselt *et al.*, 2020). Time spent in REM sleep has been shown to increase with sleep deprivation in pigeons (Tobler & Borbély, 1988; Martinez-Gonzalez *et al.*, 2008), but this does not appear to be the case in white-crowned sparrow and starlings (Jones *et al.*, 2008; Hasselt *et al.*, 2019). Chicks increase the duration of binocular eyes closure and percentage of total time sleep after sleep deprivation (Bobbo *et al.*, 2008). Similarly, the burrowing owls exhibited an increase in the duration of binocular eye closure, indicating that they are probably susceptible to REM sleep rebounds. According to Berger and Walker (1972), burrowing owls spend about 5% of their total sleep time in REM sleep. Thus, REM sleep recovery must be necessary to obtain the benefits associated with this state. Nevertheless, we do not discard the possibility that the longer duration of binocular eye closure was also partly associated with an increase in SWS.

5.3.2. 24 h of sleep deprivation is more than enough?

It has been demonstrated that 24 hours of sleep deprivation can disrupt sleep recovery effect due to a possible spill-over of SWS activity on wakefulness/ drowsiness states during the SD period (Tobler & Borbély, 1988; Martinez-Gonzalez *et al.*, 2008). Yet, we chose a 24 hour sleep deprivation protocol because burrowing owls exhibit a fragmented sleep. Thus, depending on the time window chosen for SD, we risked finding no effect of recovery. Also, shorter SD protocols do not guarantee that animals will need sleep compensation because the sleep loss effect is variable among species (Rattenborg *et al.*, 2019; Hasselt *et al*., 2019). EEG was not used to ascertain if our gentle manipulations were enough to maintain the owls awake most of the time during SD. Nonetheless, we are pretty confident that our approach was quite effective and at no apparent cost for the owl’s well being and health.

5.3.3 Final considerations

Although some similarities exist between mammals and birds, the primary markers of sleep recovery, such as EEG-SWS intensity, increase in time spent or duration of NREM and REM sleep, often do not respond as expected in avian species. Birds have to deal with ecological constraints as non-stop migration flights or constant foraging to feed their brood, a situation that is not so extreme in many terrestrial mammals. Thus, as well as aquatic mammals, birds seem to be biologically adapted to face long periods of almost complete sleep loss without full recovery (Rattenborg *et al.*, 2019). The burrowing owls have adapted to ecological demands over the course of their natural history by fragmenting the sleep-wake cycle across 24 hours. Having such a sleep architecture enables them to continuously monitor their surroundings and find food opportunistically. It is important to highlight that, although the need for sleep compensation is evident in burrowing owls, they still maintain the temporal organization of head saccades, a critical indicator of brief arousals. This leads us to question if, like other birds, burrowing owls show partial sleep recovery due to the constant calling of mother nature, which drives them to regularly survey their environment. Finally, we should pursue further studies in this regard by applying other protocols to investigate sleep homeostasis in owls, including EEG measures.

CHAPTER 6

CONCLUSION AND PERSPECTIVES

Altogether these findings support the need for more comprehensive behavioral studies of sleep-wake cycle in other species. Our study found that the distinction of immobile behavior in two different state-based postures (awake state vs. sleep state), namely the vigilant pose and the rest pose, proved to be robust and beneficial for gaining a deeper understanding of the sleep-wake behavior in burrowing owls.

In this study, we showed that burrowing owls are highly active during night and day, thus, confirming our hypothesis that this owl species is a cathemeral bird. Burrowing owls' sleep-wake cycle is highly fragmented throughout a 24h period, even in our more natural context. This trait is typical of cathemeral animals. Cathemerality has been largely neglected and scarcely investigated in birds. Recognizing a species as cathemeral has several implications for interpretations in the light of evolution. Also, having established that an cathemeral provided a rare opportunity to identify the environmental and biological factors that determine the distribution of activity across the 24-hour day.

Last but not least, in the first 4 hours after sleep deprivation, owls spent more time in sleep state, and the duration of binocular eye closures was longer. This is the first work to demonstrate that an owl species presents at least a partial sleep rebound after sleep deprivation.

Brain or behavior? What is the best offer?

We are quite aware that behavior is just one of many windows to access what is happening in the house of sleep. Sleep has many facets which go far beyond EEG brain data, especially in light of all the diversity in the animal kingdom. Behavior description is essential to understanding the life history of species, and can provide valuable insights into the unique features of animal sleep, particularly when firmly grounded into a comparative framework.

For example, platypuses exhibit rapid eye movements as well as head and bill twitching during sleep. Unexpectedly, their cortical EEG records show slow waves similar to non-REM sleep (Siegel *et al.*, 1999). This type of contradictions between behavior and cortical brain activity give us the notion of how far away we are to conceive sleep as a whole.

Furthermore, investigating animal behavior is a non-invasive way to probe awake and sleep states that can be associated with other physiological measures. For example, Coulombe (1970,1971) showed that the core temperature of burrowing owls decreases during the night and that locomotor activity patterns change between day and night depending on the ambient temperature. Had Coulombe's data been accompanied by detailed behavioral descriptions like ours, a number of potentially interesting and untapped correlations between behavioral traits and temperature would have been possible.

Now that we have detailed the behavioral repertoire of burrowing owls, less time-consuming strategies could be envisaged to measure their activity. For instance, the sampling methods described by Altman (1974) could be considered to compare owls' activity during the summer and winter seasons. Furthermore, the amount of knowledge we have accumulated scoring owl behavior from video records could be used to automate this process using, for example, deep learning machine methods. Considering the importance of head saccades to probe alert states and detect short awakenings in the burrowing, we believe that head tracking devices should also greatly benefit future investigations on both sleep and visual attention.

We believe that our work provides a sufficiently rich and quantified body of evidence to guide further investigations on the physiological bases of many behavioral traits described herein. Within this perspective, the use measurements such as heart rate, body temperature, breathing rate, molecular markers, and multi-scaled brain activity would be among the most needed types of correlates to obtain. For example, using extracellular unit or field recording techniques in the visual wulst, it would interesting to investigate the extent to which neural responses to a visual stimulus differ when burrowing owls sleep with one or two eyes opened, and how such responses differ from those recorded in different states of wakefulness.

Before the very eyes of burrowing owls, we could observe abrupt pupil contractions that seemed unrelated to the pupillary light reflex, and evidently related to sleep states. In line with our observations, recent studies in mice and pigeons have shown that pupil size is constricted during REM sleep and dilated during non-REM sleep (Weber, 2018; ; Ungurean *et al.,* 2021; but see Yuzgec *et al.*, 2018). Observing burrowing owls' behavior in detail has provided us with the same insight. However, a more thorough study is required to investigate this issue.

We think that our work stresses the importance of quantifying, with fine-grained temporal resolution, how animals deploy their behavioral repertoire around-the-clock. By doing so, one can access generative processes that mold the temporal organization of animal behavior. Physiological and behavioral processes may exhibit ultradian patterns that lack true periodicity. Such episodic patterns were particularly neglected in past studies (Blessing & Ootsuka, 2016; Guzman et al., 2017). The stochastic component of these ultradian events reflects complex interactions that arise among multiple physiological mechanisms (central and peripheral regulations), external environment, and volitive control (Guzman *et al.*, 2017). The foregoing considerations should motivate future analytical analyses on the database compiled in this study. For example, temporal analyses in the frequency domain of stationary and non-stationary behavioral responses (e.g., Fourier and wavelet analysis) could be used to investigate the presence of regular (true rhythms) and irregular (episodic) ultradian patterns for relevant behavioral traits, such as head movements, eye blinking, and REM-associated eye-closure.

Finally, we recognize the crucial importance of cortical and subcortical electrophysiological recordings to unfold the mysteries of sleep. In a near future, with the COVID-19 pandemic scenario behind us, our plan is to initiate this research program.

BIBLIOGRAPHY

Achermann, P., & Borbély, A. A. (2003). Mathematical models of sleep regulation. *Front Biosci*, *8*(Suppl.), S683-S693.

Akaike, H. (1974). A new look at the statistical model identification. *IEEE transactions on automatic control*, *19*(6), 716-723.

Altmann, J. (1974). Observational study of behavior: sampling methods. *Behaviour*, *49*(3-4), 227-266.

Amlaner, C.J. & McFarland, D.J. (1981) Sleep in the herring gull (Larus argentatus). *Anim Behav*, **29**, 551–556.

Amlaner, C.J. & Ball, N.J. (1983) A Synthesis of Sleep in Wild Birds. *Behaviour*, **87**, 85–119.

Andrade, A., Nabte, M.J., & Kun, M.E. (2010) Diet of the Burrowing Owl (Athene cunicularia) and its seasonal variation in Patagonian steppes: implications for biodiversity assessments in the Somuncurá Plateau Protected Area, Argentina. *Stud Neotrop Fauna E*, **45**, 101–110.

Andrew King, R., and James R. Belthoff (2001) "Post-fledging dispersal of Burrowing Owls in southwestern Idaho: characterization of movements and use of satellite burrows." *The Condor* 103.1: 118-126.

Aserinsky, E., & Kleitman, N. (1953). Regularly occurring periods of eye motility, and concomitant phenomena, during sleep. *Science*, *118*(3062), 273-274.

Ball, N.J., Amlaner, C.J., Shaffery, J.P., & Opp, M.R. (1988) Asynchronous eye-closure and unihemispheric quiet sleep of birds. *Sleep 86*, 151–153.

Ball, N.J. (1992). The phasing of sleep in animals. In *Why we nap* (pp. 31-49). Birkhäuser, Boston, MA.

Bartness, Timothy & Albers, H Elliott. (2000). Activity Patterns and the Biological Clock in Mammals. 10.1007/978-3-642-18264-8_3. In Activity Patterns in Small Mammals (pp.23-47)

Beauchamp, G. (2015). *Animal vigilance: monitoring predators and competitors.* Academic Press. (pp. 55).

Bennie, J.J., Duffy, J.P., Inger, R., & Gaston, K.J. (2014) Biogeography of time partitioning in mammals. *Proc National Acad Sci*, **111**, 13727–13732.

Blessing W, Ootsuka Y. Timing of activities of daily life is jaggy: How episodic ultradian changes in body and brain temperature are integrated into this process. Temperature (Austin). 2016 Apr 29;3(3):371-383.

Berger, R.J. & Walker, J.M. (1972) Sleep in the burrowing owl (Speotyto cunicularia hypugaea). *Behav Biol*, **7**, 183–194.

Berger, R. J., & Phillips, N. H. (1995). Energy conservation and sleep. *Behavioural brain research*, *69*(1), 65-73.

Blumberg, M.S., Dooley, J.C., & Sokoloff, G. (2020) The developing brain revealed during sleep. *Curr Opin Physiology*, **15**, 14–22.

Bobbo, D., Nelini, C., & Mascetti, G.G. (2008) Binocular and monocular/unihemispheric sleep in the domestic chick (Gallus gallus) after a moderate sleep deprivation. *Exp Brain Res*, **185**, 421–427.

Borbely, A. A., & Neuhaus, H. U. (1979). Sleep-deprivation: effects on sleep and EEG in the rat. *Journal of comparative physiology*, *133*(1), 71-87.

Borbély, A. A. (1980). Sleep: circadian rhythm versus recovery process. *Functional states of the brain: Their determinants*, 151-161.

Borbély, A. A. (1982a). Sleep regulation: circadian rhythm and homeostasis. In *Sleep* (pp. 83-103). Springer, Berlin, Heidelberg.

Borbély, A. A. (1982b). A two process model of sleep regulation. *Hum neurobiol*, *1*(3), 195-204.

Braga, A.C.R. & Motta-Junior, J.C. (2009) Weather Conditions and Moon Phase Influence on Tropical Screech Owl and Burrowing Owl Detection by Playback in Southeast Brazil. *Ardea*, **97**, 395–401.

Bravo, H., & Pettigrew, J. D. (1981). The distribution of neurons projecting from the retina and visual cortex to the thalamus and tectum opticum of the barn owl, Tyto alba, and the burrowing owl, Speotyto cunicularia. *Journal of Comparative Neurology*, *199*(3), 419-441.

Bryant, P. A., Trinder, J., & Curtis, N. (2004). Sick and tired: does sleep have a vital role in the immune system?. *Nature Reviews Immunology*, **4**(6), 457.

Burn, H., 1999. Burrowing Owl/Athene cunicularia. In: del Hoyo, J., Elliott, A., Sargatal, J. (Eds.), Handbook of the Birds of the World, Volume 5, Barn Owl to Hummingbirds, pp. 227–228. Lynx Editions, Barcelona, p. 760.

Burnham, K. P., & Anderson, D. R. (2002). A practical information-theoretic approach. *Model selection and multimodel inference*, *2*.

Butler, A. B., & Hodos, W. (2005). Comparative vertebrate neuroanatomy: evolution and adaptation. *John Wiley & Sons.*

Campbell, S.S. & Tobler, I. (1984) Animal sleep: A review of sleep duration across phylogeny. *Neurosci Biobehav Rev*, **8**, 269–300.

Campbell, S. S. (1992). The timing and structure of spontaneous naps. In *Why we nap* (pp. 71-81). Birkhäuser, Boston, MA.

Capellini, I., Nunn, C.L., McNamara, P., Preston, B.T., & Barton, R.A. (2008) Energetic constraints, not predation, influence the evolution of sleep patterning in mammals. *Funct Ecol*, **22**, 847–853.

Castro-Sa, M.J., Dias-Silva, R.H.P., & Barnett, A.A. (2021) Cathemeral activity by brown-throated three-toed sloths (Bradypus variegatus) in central Amazonian flooded igapó forests. *Can J Zool*, **99**, 832–838.

Catlin, D.H., Rosenberg, D.K., & Haley, K.L. (2005) The effects of nesting success and mate fidelity on breeding dispersal in burrowing owls. *Can J Zool*, **83**, 1574–1580.

Chandler, S.L., Tietz, J.R., Bradley, R.W., & Trulio, L. (2016) Burrowing Owl Diet at a Migratory Stopover Site and Wintering Ground on Southeast Farallon Island, California. *J Raptor Res*, **50**, 391–403.

Chipman, E.D., McIntyre, N.E., Strauss, R.E., Wallace, M.C., Ray, J.D., & Boal, C.W. (2008) Effects of Human Land Use on Western Burrowing Owl Foraging and Activity Budgets. *J Raptor Res*, **42**, 87–98.

Colquhoun, I.C. (2006) Predation and Cathemerality. *Folia Primatol*, **77**, 143–165.

Coulombe, H.N. (1970) Physiological and physical aspects of temperature regulation in the burrowing owl Speotyto cunicularia. *Comp Biochem Physiol*, **35**, 307–337.

Coulombe, H.N. (1971) Behavior and Population Ecology of the Burrowing Owl, Speotyto cunicularia, in the Imperial Valley of California. *Condor*, **73**, 162–176.

Cirelli, C., & Tononi, G. (2021). The why and how of sleep-dependent synaptic down-selection. In *Seminars in cell & developmental biology*. Academic Press.
Curtis, D.J. & Rasmussen, M.A. (2002) Cathemerality in lemurs. *Evol Anthropology Issues News Rev*, **11**, 83–86.

Curtis, D.J. & Rasmussen, M.A. (2006) The Evolution of Cathemerality in Primates and Other Mammals: A Comparative and Chronoecological Approach. *Folia Primatol*, **77**, 178–193.

Curtis, D.J., Zaramody, A., & Martin, R.D. (1999) Cathemerality in the mongoose lemur, Eulemur mongoz. *Am J Primatol*, **47**, 279–298.

de Souza Medeiros, S. L., de Paiva, M. M. M., Lopes, P. H., Blanco, W., de Lima, F. D., de Oliveira, J. B. C., ... & Ribeiro, S. (2021). Cyclic alternation of quiet and active sleep states in the octopus. *Iscience*, *24*(4), 102223.

Deboer, T. (2013). Behavioral and electrophysiological correlates of sleep and sleep homeostasis. In *Sleep, Neuronal Plasticity and Brain Function* (pp. 1-24). Springer Berlin Heidelberg.

Dement, W., & Kleitman, N. (1957). Cyclic variations in EEG during sleep and their relation to eye movements, body motility, and dreaming. *Electroencephalography and clinical neurophysiology*, *9*(4), 673-690.

Diekelmann, S., & Born, J. (2010). The memory function of sleep. *Nature Reviews Neuroscience*, **11**(2), 114-126.

Donati, G. & Borgognini-Tarli, S.M. (2006) From darkness to daylight: cathemeral activity in primates. *Journal of Anthropological Sciences*, **84**, 7–32.

Donati, G., Baldi, N., Morelli, V., Ganzhorn, J.U., & Borgognini-Tarli, S.M. (2009) Proximate and ultimate determinants of cathemeral activity in brown lemurs. *Anim Behav*, **77**, 317–325.

Donati, G. & Borgognini-Tarli, S.M. (2006) Influence of Abiotic Factors on Cathemeral Activity: The Case of Eulemur fulvus collaris in the Littoral Forest of Madagascar. *Folia Primatol*, **77**, 104–122.

Donati, G., Santini, L., Razafindramanana, J., Boitani, L., & Borgognini-Tarli, S. (2013) (Un-)expected nocturnal activity in “Diurnal” Lemur catta supports cathemerality as one of the key adaptations of the lemurid radiation. *Am J Phys Anthropol*, **150**, 99–106.

Edgar, D. M., Dement, W. C., & Fuller, C. A. (1993). Effect of SCN lesions on sleep in squirrel monkeys: evidence for opponent processes in sleep-wake regulation. *Journal of Neuroscience*, *13*(3), 1065-1079.

Ensing, E.P., Ciuti, S., Wijs, F.A.L.M. de, Lentferink, D.H., Hoedt, A. ten, Boyce, M.S., & Hut, R.A. (2014) GPS Based Daily Activity Patterns in European Red Deer and North American Elk (Cervus elaphus): Indication for a Weak Circadian Clock in Ungulates. *Plos One*, **9**, e106997.

Eppley, T.M., Ganzhorn, J.U., & Donati, G. (2015) Cathemerality in a small, folivorous primate: proximate control of diel activity in Hapalemur meridionalis. *Behav Ecol Sociobiol*, **69**, 991–1002.

Errington, P.L. (1930) The Pellet Analysis Method of Raptor Food Habits Study. *Condor*, **32**, 292–296.

Errington, P.L. (1932) Food Habits of Southern Wisconsin Raptors. Part I. Owls. *Condor*, **34**, 176–186.

Fernandez-Duque, E. (2003) Influences of moonlight, ambient temperature, and food availability on the diurnal and nocturnal activity of owl monkeys (Aotus azarai). *Behav Ecol Sociobiol*, **54**, 431–440.

Fernández-Juricic, E. & Tran, E. (2007) Changes in vigilance and foraging behaviour with light intensity and their effects on food intake and predator detection in house finches. *Anim Behav*, **74**, 1381–1390.

Fernández-Juricic, E., Beauchamp, G., Treminio, R., & Hoover, M. (2011) Making heads turn: association between head movements during vigilance and perceived predation risk in brown-headed cowbird flocks. *Anim Behav*, **82**, 573–577.

Fitak, R. R., & Johnsen, S. (2017). Bringing the analysis of animal orientation data full circle: model-based approaches with maximum likelihood. *Journal of experimental biology*, *220*(21), 3878-3882.

Friard, O. & Gamba, M. (2016) BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods Ecol Evol*, **7**, 1325–1330.

Gattermann, R., Johnston, R.E., Yigit, N., Fritzsche, P., Larimer, S., zkurt, S., Neumann, K., Song, Z., Colak, E., Johnston, J., & McPhee, M.E. (2008) Golden hamsters are nocturnal in captivity but diurnal in nature. *Biol Letters*, **4**, 253–255.

Gilestro, G.F. (2012) Video tracking and analysis of sleep in Drosophila melanogaster. *Nat Protoc*, **7**, 995–1007.

Giné, G.A.F., Cassano, C.R., Almeida, S.S. de, & Faria, D. (2015) Activity budget, pattern and rhythm of maned sloths (Bradypus torquatus): Responses to variations in ambient temperature. *Mammalian Biology - Zeitschrift Für Säugetierkunde*, **80**, 459–467.

Gleason, R. L., & Craig, T. H. (1979). Food habits of burrowing owls in southeastern Idaho. *The Great Basin Naturalist*, 274-276.

Goh, K.-I. & Barabási, A.-L. (2008) Burstiness and memory in complex systems. *Epl-europhys Lett*, **81**, 48002.

Goodman, I. (1974). The study of sleep in birds. *Birds: Brain and Behavior*, 133-152.
Green, G. A. (1983). Ecology of breeding burrowing owls in the Columbia Basin, Oregon.

Green, G. A., Fitzner, R. E., Anthony, R. G., & Rogers, L. E. (1993). Comparative diets of burrowing owls in Oregon and Washington. *Northwest Science*, *67*(2).

Guzmán DA, Flesia AG, Aon MA, Pellegrini S, Marin RH, Kembro JM. The fractal organization of ultradian rhythms in avian behavior. Sci Rep. 2017 Apr 6;7(1):684.

Hall, D.B., Greger, P.D., & Rosier, J.R. (2009) Regional and Seasonal Diet of the Western Burrowing Owl in South Central Nevada. *West N Am Naturalist*, **69**, 1–8.

Hall, M.I. & Ross, C.F. (2007) Eye shape and activity pattern in birds. *J Zool*, **271**, 437–444.

Halle, S. (1995) Diel Pattern of Locomotor Activity in Populations of Root Voles, Microtus oeconomus. *J Biol Rhythm*, **10**, 211–224.

Halle S, Weinert D (2000). Measuring and analysing activity of small mammals in the laboratory and in the field. In Activity Patterns in Small Mammals – an Ecological Approach (Halle S, Stenseth NC, eds.), pp 285–312. Berlin, Springer.

Halle, S. (2000). Voles—small graminivores with polyphasic patterns. In *Activity patterns in small mammals* (pp. 191-215). Springer, Berlin, Heidelberg.

Halle, S. (2006) Polyphasic Activity Patterns in Small Mammals. *Folia Primatol*, **77**, 15–26.

Hasselt, S.J.V., Rusche, M., Vyssotski, A.L., Verhulst, S., Rattenborg, N.C., & Meerlo, P. (2019) The European starling (Sturnus vulgaris) shows signs of NREM sleep homeostasis but has very little REM sleep and no REM sleep homeostasis. *Sleep*, **43**.

Hasselt, S.J. van, Mekenkamp, G.-J., Komdeur, J., Allocca, G., Vyssotski, A.L., Piersma, T., Rattenborg, N.C., & Meerlo, P. (2020) Seasonal variation in sleep homeostasis in migratory geese: a rebound of NREM sleep following sleep deprivation in summer but not in winter. *Sleep*, **44**, zsaa244-.

Haug, E. A. (1985). *Observations on the breeding ecology of Burrowing Owls in Saskatchewan* (Doctoral dissertation, University of Saskatchewan).

Haug, E.A. & Oliphant, L.W. (1990) Movements, Activity Patterns, and Habitat Use of Burrowing Owls in Saskatchewan. *J Wildl Management*, **54**, 27.

Hillar, C., Onnis, G., Rhea, D., & Tecott, L. (2018) Active State Organization of Spontaneous Behavioral Patterns. *Sci Rep-uk*, **8**, 1064.

Huber, R., Ghilardi, M. F., Massimini, M., & Tononi, G. (2004). Local sleep and learning. *Nature*, ***430***(6995), 78-81.

Ikeda, T., Uchida, K., Matsuura, Y., Takahashi, H., Yoshida, T., Kaji, K., & Koizumi, I. (2016) Seasonal and Diel Activity Patterns of Eight Sympatric Mammals in Northern Japan Revealed by an Intensive Camera-Trap Survey. *Plos One*, **11**, e0163602.

Jarvis, E.D., Güntürkün, O., Bruce, L., Csillag, A., Karten, H., Kuenzel, W., Medina, L., Paxinos, G., Perkel, D.J., Shimizu, T., Striedter, G., Wild, J.M., Ball, G.F., Dugas-Ford, J., Durand, S.E., Hough, G.E., Husband, S., Kubikova, L., Lee, D.W., Mello, C.V., Powers, A., Siang, C., Smulders, T.V., Wada, K., White, S.A., Yamamoto, K., Yu, J., Reiner, A., & Butler, A.B. (2005) Avian brains and a new understanding of vertebrate brain evolution. *Nat Rev Neurosci*, **6**, 151–159.

Jones, S.G., Vyazovskiy, V.V., Cirelli, C., Tononi, G., & Benca, R.M. (2008) Homeostatic regulation of sleep in the white-crowned sparrow (Zonotrichia leucophrys gambelii). *Bmc Neurosci*, **9**, 47.

Jouvet, M., (1965). Paradoxical sleep—a study of its nature and mechanisms. Progress in Brain Research **18**, 20–62.

Kappeler, P.M. & Erkert, H.G. (2003) On the move around the clock: correlates and determinants of cathemeral activity in wild redfronted lemurs (Eulemur fulvus rufus). *Behav Ecol Sociobiol*, **54**, 359–369.

Kas, M.J.H. & Edgar, D.M. (1999) Circadian Timed Wakefulness at Dawn Opposes Compensatory Sleep Responses After Sleep Deprivation in Octodon Degus. *Sleep*, **22**, 1045–1053.

Keene, A. C., & Duboue, E. R. (2018). The origins and evolution of sleep. *Journal of Experimental Biology*, *221*(11), jeb159533.

Kemna, C.J., Nagy-Reis, M.B., & Scrafford, M.A. (2020) Temporal segregation among sympatric boreal predators. *Mammal Res*, **65**, 565–572.

Kirk, E.C. (2006) Eye Morphology in Cathemeral Lemurids and Other Mammals. *Folia Primatol*, **77**, 27–49.

Kirsten, S.J. & Kirsten, E.B. (1983) Spontaneous Blink Rates of Birds. *The Condor*, Spontaneous Blink Rates of Birds, **85**, 92–93.

Kleitman, N., & Engelmann, T. G. (1953). Sleep characteristics of infants. *Journal of Applied Physiology*, *6*(5), 269-282.

König, C., & Weick, F. (2008). *Owls of the world*. A&C Black.

LaFever, D.H., LaFever, K.E., Catlin, D.H., & Rosenberg, D.K. (2008) Diurnal Time Budget of Burrowing Owls in a Resident Population During the Non-Breeding Season. *Southwest Nat*, **53**, 29–33.

Lancel, M., Riezen, H. van, & Glatt, A. (1991) Effects of circadian phase and duration of sleep deprivation on sleep and EEG power spectra in the cat. *Brain Res*, **548**, 206–214.

Lesku, J.A., Bark, R.J., Martinez-Gonzalez, D., Rattenborg, N.C., Amlaner, C.J., & Lima, S.L. (2008) Predator-induced plasticity in sleep architecture in wild-caught Norway rats (Rattus norvegicus). *Behav Brain Res*, **189**, 298–305.

Lesku, J.A., Martinez-Gonzalez, D., & Rattenborg, N.C. (2009) Sleep and Sleep States: Phylogeny and Ontogeny. *Subj Classif Neural Basis Behav Dreaming Arousal Neural Basis Behav Sleep Dreaming Arousal Article Titles S*, 953–961.

Lesku, J.A., Rattenborg, N.C., Valcu, M., Vyssotski, A.L., Kuhn, S., Kuemmeth, F., Heidrich, W., & Kempenaers, B. (2012) Adaptive Sleep Loss in Polygynous Pectoral Sandpipers. *Science*, **337**, 1654–1658.

Lesku, J. A., Martinez-Gonzalez, M. D., & Rattenborg, N. C. (2009a). Sleep and sleep states: phylogeny and ontogeny. In *Encyclopedia of Neuroscience* (pp. 963-971). Oxford Academic Press.

Lesku, J.A., Roth, T.C., Rattenborg, N.C., Amlaner, C.J., & Lima, S.L. (2009b) History and future of comparative analyses in sleep research. *Neurosci Biobehav Rev*, **33**, 1024–1036.

Lesku, J.A., Vyssotski, A.L., Martinez-Gonzalez, D., Wilzeck, C., & Rattenborg, N.C. (2011) Local sleep homeostasis in the avian brain: convergence of sleep function in mammals and birds? *Proc Royal Soc B Biological Sci*, **278**, 2419–2428.

Lesku, J.A., Meyer, L.C.R., Fuller, A., Maloney, S.K., Dell’Omo, G., Vyssotski, A.L., & Rattenborg, N.C. (2011) Ostriches sleep like platypuses. *Plos One*, **6**, e23203.

Lesku, J. A., Rattenborg, N. C., Valcu, M., Vyssotski, A. L., Kuhn, S., Kuemmeth, F., ... & Kempenaers, B. (2012). Adaptive sleep loss in polygynous pectoral sandpipers. *Science*, *337*(6102), 1654-1658.

Lewis, S.B., Fuller, M.R., & Titus, K. (2004) A comparison of 3 methods for assessing raptor diet during the breeding season. *Wildlife Soc B*, **32**, 373–385.

Libourel, P.-A., Barrillot, B., Arthaud, S., Massot, B., Morel, A.-L., Beuf, O., Herrel, A., & Luppi, P.-H. (2018) Partial homologies between sleep states in lizards, mammals, and birds suggest a complex evolution of sleep states in amniotes. *Plos Biol*, **16**, e2005982.

Lima, S.L., Rattenborg, N.C., Lesku, J.A., & Amlaner, C.J. (2005) Sleeping under the risk of predation. *Anim Behav*, **70**, 723–736.

Lisney, T.J., Iwaniuk, A.N., Bandet, M.V., & Wylie, D.R. (2012) Eye Shape and Retinal Topography in Owls (Aves: Strigiformes). *Brain Behav Evol*, **79**, 218–236.

Liu, X., Uchiyama, M., Shibui, K., Kim, K., Kudo, Y., Tagaya, H., ... & Okawa, M. (2000). Diurnal preference, sleep habits, circadian sleep propensity and melatonin rhythm in healthy human subjects. *Neuroscience letters*, *280*(3), 199-202.

Lutz, R.S. & Plumpton, D.L. (1999) Philopatry and nest site reuse by Burrowing Owls : Implications for productivity. *J RaptorRe*, **33**, 149–153.

Lyamin, O., Pryaslova, J., Lance, V., & Siegel, J. (2005). Animal behaviour: continuous activity in cetaceans after birth. *Nature*, *435*(7046), 1177.

Lyamin, O.I., Manger, P.R., Ridgway, S.H., Mukhametov, L.M., & Siegel, J.M. (2008) Cetacean sleep: An unusual form of mammalian sleep. *Neurosci Biobehav Rev*, **32**, 1451–1484.

McGinty, D., & Szymusiak, R. (1990). Keeping cool: a hypothesis about the mechanisms and functions of slow-wave sleep. *Trends in neurosciences*, *13*(12), 480-487.

Mahecha, G.A.B. & Oliveira, C.A. de (1998) An Additional Bone in the Sclera of the Eyes of Owls and the Common Potoo (Nictibius griseus) and Its Role in the Contraction of the Nictitating Membrane. *Cells Tissues Organs*, **163**, 201–211.

Mardia, K.V. & Jupp, P.E. (2018) Directional Statistics. *Wiley Ser Probab Statistics.*

Marti, C.D. (1974) Feeding Ecology of Four Sympatric Owls. *Condor*, **76**, 45–61.

Martin, D.J. (1973) Selected Aspects of Burrowing Owl Ecology and Behavior. *Condor*, **75**, 446.

Martinez-Gonzalez, D., Lesku, J.A., & Rattenborg, N.C. (2008) Increased EEG spectral power density during sleep following short-term sleep deprivation in pigeons (Columba livia): evidence for avian sleep homeostasis. *J Sleep Res*, **17**, 140–153.

Meij, J. van der, Martinez-Gonzalez, D., Beckers, G.J.L., & Rattenborg, N.C. (2018) Intra-“cortical” activity during avian non-REM and REM sleep: variant and invariant traits between birds and mammals. *Sleep*, **42**.

Merritt, J.F. & Vessey, S.H. (2000) Activity Patterns in Small Mammals, An Ecological Approach. *Ecol Stud*, 235–251.

Mikkola, H. (2014). Owls of the World-A Photographic Guide. A&C Black.

Motta-Junior & Bueno, & (2004) Trophic ecology of the Burrowing Owl in southeast Brazil. *Raptors worldwide: proceedings of the VI world conference on birds of prey and owls. World Working Group on Birds of Prey and Owls, MME BirdLife, Budapest, Hungary*, 763–775.

Mrykalo, R. (2005). The Florida Burrowing Owl in a Rural Environment: Breeding Habitat, Dispersal, PostBreeding Habitat, Behavior, and Diet.

Mukhametov, L. M., Supin, A. Y., & Polyakova, I. G. (1977). Interhemispheric asymmetry of the electroencephalographic sleep patterns in dolphins. *Brain research*, **134**(3), 581-584.

Naylor, E., Bergmann, B.M., Krauski, K., Zee, P.C., Takahashi, J.S., Vitaterna, M.H., Turek, F.W., 2, & 4 (2000) The Circadian Clock Mutation Alters Sleep Homeostasis in the Mouse.

Oleksenko, A.I., Mukhametov, L.M., Polyakova, I.G., Supin, A.Y., & Kovalzon, V.M. (1992) Unihemispheric sleep deprivation in bottlenose dolphins. *J Sleep Res*, **1**, 40–44.

Parmelee Jr, A. H., Wenner, W. H., & Schulz, H. R. (1964). Infant sleep patterns: from birth to 16 weeks of age. *The Journal of pediatrics*, 65(4), 576-582.

Pinto, L. & Baron, J. (2009) Spatiotemporal frequency and speed tuning in the owl visual wulst. *Eur J Neurosci*, **30**, 1251–1268.

Plumpton, D. L., & Lutz, R. S. (1993). Prey selection and food habits of Burrowing Owls in Colorado. *The Great Basin Naturalist*, 299-304.

Poulin, R.G. & Todd, L.D. (2006) Sex and nest stage differences in the circadian foraging of behaviors of nesting Burrowing Owls. *Condor*, **108**, 856–864.

Poulin, R. G., L. D. Todd, E. A. Haug, B. A. Millsap, and M. S. Martell (2020). Burrowing Owl (*Athene cunicularia*), version 1.0. In Birds of the World (A. F. Poole, Editor). Cornell Lab of Ornithology, Ithaca, NY, USA. https://doi.org/10.2173/bow.burowl.01

Rasphone, A., Kamler, J.F., & Macdonald, D.W. (2020) Temporal partitioning by felids, dholes and their potential prey in northern Laos. *Mammal Res*, **65**, 679–689.

Rands, S.A. (2021) Phylogenetically-controlled correlates of primate blinking behaviour. *Peerj*, **9**, e10950.

Rasmussen, M. A. (1999). Ecological influences on activity cycle in two cathemeral primates, the mongoose lemur (Eulemur mongoz) and the common brown lemur (Eulemur fulvus fulvus). Duke University.

Rattenborg, N.C., Lima, S.L., & Amlaner, C.J. (1999) Half-awake to the risk of predation. *NATURE*, **397**, 397–398.

Rattenborg, N.C., Amlaner, C.J., & Lima, S.L. (2000) Behavioral, neurophysiological and evolutionary perspectives on unihemispheric sleep. *Neuroscience and Biobehavioral Reviews*, **24**, 817–842.

Rattenborg, N.C. (2006) Evolution of slow-wave sleep and palliopallial connectivity in mammals and birds: A hypothesis. *Brain Res Bull*, **69**, 20–29.

Rattenborg, N.C., Martinez-Gonzalez, D., & Lesku, J.A. (2009) Avian sleep homeostasis: Convergent evolution of complex brains, cognition and sleep functions in mammals and birds. *Neurosci Biobehav Rev*, **33**, 253–270.

Rattenborg, N.C., Lima, S.L., & Lesku, J.A. (2012) Sleep Locally, Act Globally. *Neurosci*, **18**, 533–546.

Rattenborg, N.C., Voirin, B., Cruz, S.M., Tisdale, R., Dell'Omo, G., Lipp, H.-P., Wikelski, M., & Vyssotski, A.L. (2016) Evidence that birds sleep in mid-flight. *Nat Commun*, **7**, 12468.

Rattenborg, N.C. (2017) Sleeping on the wing. *Interface Focus*, **7**, 20160082.

Rattenborg, N.C., Iglesia, H.O. de la, Kempenaers, B., Lesku, J.A., Meerlo, P., & Scriba, M.F. (2017) Sleep research goes wild: new methods and approaches to investigate the ecology, evolution and functions of sleep. *Philosophical Transactions Royal Soc B Biological Sci*, **372**, 20160251.

Rattenborg, N.C., Meij, J. van der, Beckers, G.J.L., & Lesku, J.A. (2019) Local Aspects of Avian Non-REM and REM Sleep. *Front Neurosci-switz*, **13**, 567.

Rea, M.S., Figueiro, M.G., Jones, G.E., & Glander, K.E. (2014) Daily activity and light exposure levels for five species of lemurs at the duke lemur center. *Am J Phys Anthropol*, **153**, 68–77.

Refinetti, R. (2006) Variability of diurnality in laboratory rodents. *J Comp Physiology*, **192**, 701–714.

Refinetti, R. (2008) The diversity of temporal niches in mammals. *Biol Rhythm Res*, **39**, 173–192.

Rial, R. V., Nicolau, M. C., Gamundí, A., Akaârir, M., Aparicio, S., Garau, C., ... & Esteban, S. (2007). The trivial function of sleep. *Sleep medicine reviews*, *11*(4), 311-325.

Rodriguez-Martínez, S., Carrete, M., Roques, S., Rebolo-Ifrán, N., & Tella, J.L. (2014) High Urban Breeding Densities Do Not Disrupt Genetic Monogamy in a Bird Species. *Plos One*, **9**, e91314.

Roth, T. C., Rattenborg, N. C., & Pravosudov, V. V. (2010). The ecological relevance of sleep: the trade-off between sleep, memory and energy conservation. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *365*(1542), 945-959.

Samson, D.R., Vining, A., & Nunn, C.L. (2019) Sleep influences cognitive performance in lemurs. *Anim Cogn*, **22**, 697–706.

Savage, V.M. & West, G.B. (2007) A quantitative, theoretical framework for understanding mammalian sleep. *Proc National Acad Sci*, **104**, 1051–1056.

Schmidt, M.H. (2014) The energy allocation function of sleep: A unifying theory of sleep, torpor, and continuous wakefulness. *Neurosci Biobehav Rev*, **47**, 122–153.

Schnute, J.T. & Groot, K. (1992) Statistical analysis of animal orientation data. *Anim Behav*, **43**, 15–33

Scobie, C., Bayne, E., & Wellicome, T. (2014) Influence of anthropogenic features and traffic disturbance on burrowing owl diurnal roosting behavior. *Endanger Species Res*, **24**, 73–83.

Scriba, M.F., Rattenborg, N.C., Dreiss, A.N., Vyssotski, A.L., & Roulin, A. (2014) Sleep and vigilance linked to melanism in wild barn owls. *J Evolution Biol*, **27**, 2057–2068.

Shein-Idelson, M., Ondracek, J.M., Liaw, H.-P., Reiter, S., & Laurent, G. (2016) Slow waves, sharp waves, ripples, and REM in sleeping dragons. *Science*, **352**, 590–595

Siegel, J.M., Manger, P.R., Nienhuis, R., Fahringer, H.M., Shalita, T., & Pettigrew, J.D. (1999) Sleep in the platypus. *Neuroscience*, **91**, 391–400.

Siegel, J.M. (2008) Do all animals sleep? *Trends Neurosci*, **31**, 208–213.

Siegel, J. M. (2009). Sleep viewed as a state of adaptive inactivity. *Nature Reviews Neuroscience*, *10*(10), 747.

Sibley, C. G., & Monroe, B. L. (1990). Distribution and taxonomy of birds of the world. Yale University Press.

Sigrist, T. (2009).The Avis Brasilis Field Guide to the Birds of Brazil. In Field Guide AvisBrasilis - Species Accounts. Ed. AvisBrasilis. *105p.*

Silva-Porto, F., & Cerqueira, R. (1990). Seasonal variation in the diet of the Burrowing Owl Athene cunicularia in a restinga of Rio de Janeiro State. *Ciência & Cultura*, *42*, 1182-1186.

Silva, S. (1995). Numerical and functional response of burrowing owls to long-term mammal fluctuations in Chile.

Smarr, B.L., Schwartz, M.D., Wotus, C., & Iglesia, H.O. de la (2013) Re-examining "Temporal Niche." *Integr Comp Biol*, **53**, 165–174.

Stampi, C. (1992a). Evolution, chronobiology, and functions of polyphasic and ultrashort sleep: main issues. In *Why we nap* (pp. 1-20). Birkhäuser, Boston, MA.

Stampi, C. (1992b). The effects of polyphasic and ultrashort sleep schedules. In *Why we nap* (pp. 137-179). Birkhäuser, Boston, MA.

Steinbach, M. J., & Money, K. E. (1973). Eye movements of the owl. *Vision research*, *13*(4), 889-891.

Stickgold, R. (2013). Parsing the role of sleep in memory processing. *Current opinion in neurobiology*, *23*(5), 847-853.

Šušić, V.T. & Kovačević, R.M. (1973) Sleep patterns in the owl Strix aluco. *Physiol Behav*, **11**, 313–317.

Szymczak, J.T., Kaiser, W., Helb, H.W., & Beszczyńska, B. (1996) A study of sleep in the European blackbird. *Physiol Behav*, **60**, 1115–1120.

Tattersall, I. (1977) Ecology and behavior of Lemur fulvus mayottensis (Primates, Lemuriformes). *Anthropological papers of the AMNH*, **54**, 425–481.

Tattersall, I. (1979) Patterns of Activity in the Mayotte Lemur, Lemur fulvus mayottensis. *J Mammal*, **60**, 314–323.

Tattersall, I. (1987) Cathemeral Activity in Primates: A Definition. *Folia Primatol*, **49**, 200–202.

Tattersall, I. (2006) The Concept of Cathemerality: History and Definition. *Folia Primatol*, **77**, 7–14.

Tattersall, I. (2008) Avoiding commitment: cathemerality among primates. *Biol Rhythm Res*, **39**, 213–228.

Teixeira, L.A.V. and R.L. (2008) Diet of Athene cunicularia (Molina, 1782) from a sandy coastal plain in southeast Brazil. *BOL. MUS. BIOL. MELLO LEITÃO*,.

Thomsen, L. (1971) Behavior and Ecology of Burrowing Owls on the Oakland Municipal Airport. *Condor*, **73**, 177–192.

Tobler, I. & Borbély, A.A. (1988) Sleep and EEG spectra in the pigeon (Columba livia) under baseline conditions and after sleep deprivation. *J Comp Physiology*, **163**, 729–738.

Tobler, I., Franken, P., & Jaggi, K. (1993). Vigilance states, EEG spectra, and cortical temperature in the guinea pig. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *264*(6), R1125-R1132.

Tobler, I. & Franken, P. (1993) Sleep homeostasis in the guinea pig: similar response to sleep deprivation in the light and dark period. *Neurosci Lett*, 164, 105–108.

Tobler, I. (1995). Is sleep fundamentally different between mammalian species?. *Behavioural brain research*, *69*(1), 35-41.

Tononi, G., & Cirelli, C. (2003). Sleep and synaptic homeostasis: a hypothesis. *Brain research bulletin*, *62*(2), 143-150.

Tononi, G., & Cirelli, C. (2006). Sleep function and synaptic homeostasis. *Sleep medicine reviews*, *10*(1), 49-62.

Trachsel, L., Tobler, I., & Borbely, A.A. (1986) Sleep regulation in rats: effects of sleep deprivation, light, and circadian phase. *Am J Physiol Regul Integr Comp Physiol*,.

Traber, S.Y. & Müller, A.E. (2006) A Note on the Activity Cycle of Captive White-Fronted Lemurs (Eulemur fulvus albifrons). *Folia Primatol*, **77**, 139–142.

Ungurean, G., Martinez-Gonzalez, D., Massot, B., Libourel, P.-A., & Rattenborg, N.C. (2021) Pupillary behavior during wakefulness, non-REM sleep, and REM sleep in birds is opposite that of mammals. *Curr Biol*, **31**, 5370-5376.e4.

Valdez-Gómez, H.E., Holroyd, G.L., Trefry, H.E., & Contreras-Balderas, A.J. (2018) Home Ranges, Habitats, and Roosts of Wintering Burrowing Owls In Agricultural Landscapes In Central Mexico. *J Raptor Res*, **52**, 178–190.

Vieira, P.G., Sousa, J.P.M. de, & Baron, J. (2016) Contrast response functions in the visual wulst of the alert burrowing owl: a single-unit study. *J Neurophysiol*, **116**, 1765–1784.

Vyazovskiy, V. V., & Tobler, I. (2008). Handedness leads to interhemispheric EEG asymmetry during sleep in the rat. *Journal of neurophysiology*, *99*(2), 969-975.

Webb, W. B., & Agnew, H. W. (1977). Analysis of the Sleep Stages in Sleep-Wakefulness Regimens of Varied Length. *Psychophysiology*, *14*(5), 445-450.

Weber, F.D. (2018) Sleep: Eye-Opener Highlights Sleep's Organization. *Curr Biol*, **28**, R217–R220.

Weitzman E.D, Nogeire C., Perlow, M., Fukushima, D., Sassin, J.F., McGregor, P., Hellman, L. (1974). Effects of a prolonged 3-hour sleep-wake cycle on sleep stages, plasma cortisol, growth hormone and body temperature in man.*The Journal of Clinical Endocrinology & Metabolism*, *38*(6), 1018-1030.

Wiggin, T.D., Goodwin, P.R., Donelson, N.C., Liu, C., Trinh, K., Sanyal, S., & Griffith, L.C. (2020) Covert sleep-related biological processes are revealed by probabilistic analysis in Drosophila. *Proc National Acad Sci*, **117**, 10024–10034.

Wu, Y. & Wang, H. (2019) Convergent evolution of bird-mammal shared characteristics for adapting to nocturnality. *Proc Royal Soc B*, **286**, 20182185.

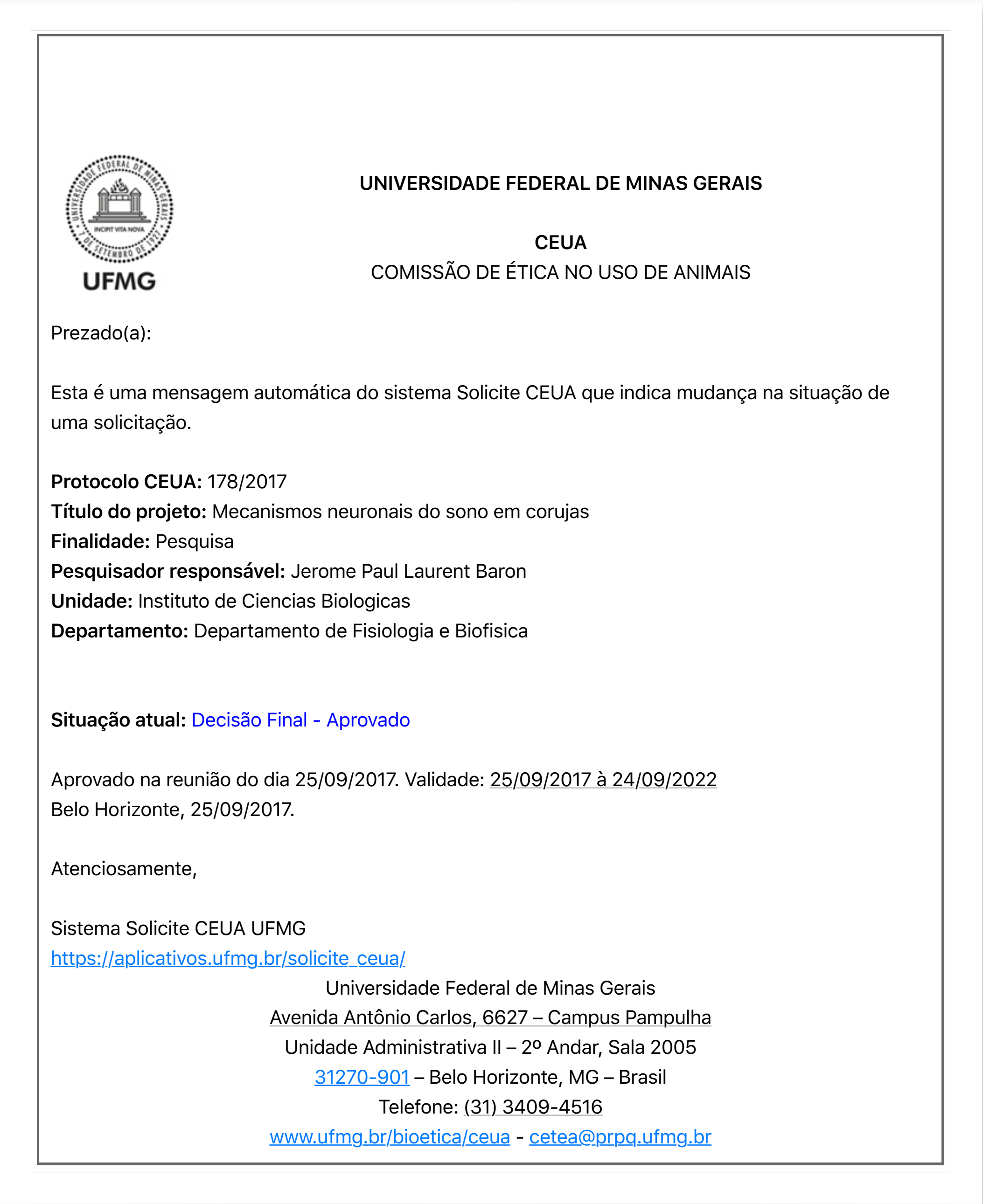
York, M. M., Rosenberg, D. K., & Sturm, K. K. (2002). Diet and food-niche breadth of Burrowing Owls (Athene cunicularia) in the Imperial Valley, California. *Western North American Naturalist*, 280-287.

Yorzinski, J.L. (2016) Eye blinking in an avian species is associated with gaze shifts. *Sci Rep-uk*, **6**, 32471.

Yorzinski, J.L. (2020) A songbird inhibits blinking behaviour in flight. *Biol Letters*, **16**, 20200786.

Yüzgeç Ö, Prsa M, Zimmermann R, Huber D. Pupil Size Coupling to Cortical States Protects the Stability of Deep Sleep via Parasympathetic Modulation. Curr Biol. 2018

APPENDIX A



UFMG

UNIVERSIDADE FEDERAL DE MINAS GERAIS

CEUA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

Prezado(a):

Esta é uma mensagem automática do sistema Solicite CEUA que indica mudança na situação de uma solicitação.

Protocolo CEUA: 178/2017
Título do projeto: Mecanismos neuronais do sono em corujas
Finalidade: Pesquisa
Pesquisador responsável: Jerome Paul Laurent Baron
Unidade: Instituto de Ciencias Biologicas
Departamento: Departamento de Fisiologia e Biofisica

Situação atual: Decisão Final - Aprovado

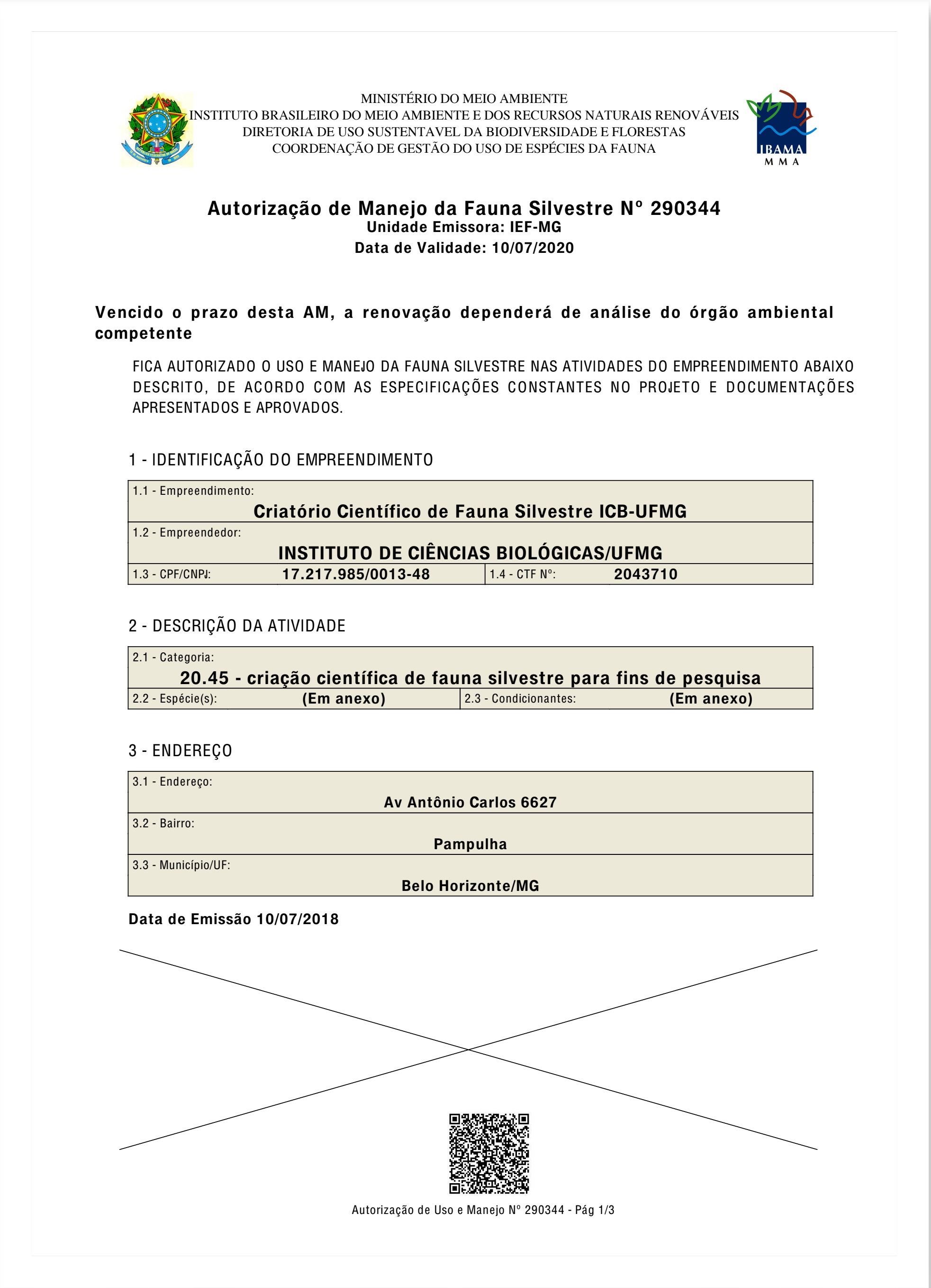
Aprovado na reunião do dia 25/09/2017. Validade: 25/09/2017 à 24/09/2022
Belo Horizonte, 25/09/2017.

Atenciosamente,

Sistema Solicite CEUA UFMG
https://aplicativos.ufmg.br/solicite_ceua/

Universidade Federal de Minas Gerais
Avenida Antônio Carlos, 6627 – Campus Pampulha
Unidade Administrativa II – 2º Andar, Sala 2005
31270-901 – Belo Horizonte, MG – Brasil
Telefone: (31) 3409-4516
www.ufmg.br/bioetica/ceua - cetea@prpq.ufmg.br

APPENDIX B



MINISTÉRIO DO MEIO AMBIENTE
INSTITUTO BRASILEIRO DO MEIO AMBIENTE E DOS RECURSOS NATURAIS RENOVÁVEIS
DIRETORIA DE USO SUSTENTAVEL DA BIODIVERSIDADE E FLORESTAS
COORDENAÇÃO DE GESTÃO DO USO DE ESPÉCIES DA FAUNA

Autorização de Manejo da Fauna Silvestre Nº 290344
Unidade Emissora: IEF-MG
Data de Validade: 10/07/2020

Vencido o prazo desta AM, a renovação dependerá de análise do órgão ambiental competente

FICA AUTORIZADO O USO E MANEJO DA FAUNA SILVESTRE NAS ATIVIDADES DO EMPREENDIMENTO ABAIXO DESCRITO, DE ACORDO COM AS ESPECIFICAÇÕES CONSTANTES NO PROJETO E DOCUMENTAÇÕES APRESENTADOS E APROVADOS.

1 - IDENTIFICAÇÃO DO EMPREENDIMENTO

1.1 - Empreendimento:	**Criatório Científico de Fauna Silvestre ICB-UFMG**		
1.2 - Empreendedor:	**INSTITUTO DE CIÊNCIAS BIOLÓGICAS/UFMG**		
1.3 - CPF/CNPJ:	**17.217.985/0013-48**	1.4 - CTF Nº:	**2043710**

2 - DESCRIÇÃO DA ATIVIDADE

2.1 - Categoria:	**20.45 - criação científica de fauna silvestre para fins de pesquisa**		
2.2 - Espécie(s):	**(Em anexo)**	2.3 - Condicionantes:	**(Em anexo)**

3 - ENDEREÇO

3.1 - Endereço:	**Av Antônio Carlos 6627**
3.2 - Bairro:	**Pampulha**
3.3 - Município/UF:	**Belo Horizonte/MG**

Data de Emissão 10/07/2018

Autorização de Uso e Manejo Nº 290344 - Pág 1/3

APPENDIX C

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO
SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Certidão
Cadastro nº A0792B6

Declaramos, nos termos do art. 41 do Decreto nº 8.772/2016, que o cadastro de acesso ao patrimônio genético ou conhecimento tradicional associado, abaixo identificado e resumido, no Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado foi submetido ao procedimento administrativo de verificação e não foi objeto de requerimentos admitidos de verificação de indícios de irregularidades ou, caso tenha sido, o requerimento de verificação não foi acatado pelo CGen.

Número do cadastro:	**A0792B6**
Usuário:	**Universidade Federal de Minas Gerais**
CPF/CNPJ:	**17.217.985/0001-04**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa**

Espécie

Athene cunicularia

Título da Atividade:	**Estudo sobre os padrões do ciclo sono vigilia nas corujas buraqueiras**

Equipe

Jerome Paul Armand Laurent Baron	**Universidade Federal de Minas Gerais**
Cíntia Aparecida de Souza Garcia	**Universidade Federal de Minas Gerais**

Data do Cadastro:	**07/10/2020 19:22:10**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **16:50** de **16/09/2021**.

SISTEMA NACIONAL DE GESTÃO
DO PATRIMÔNIO GENÉTICO
E DO CONHECIMENTO TRADICIONAL
ASSOCIADO - SISGEN