UNIVERSIDADE DE SÃO PAULO
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA

ISIS ZANINI DAS CANDEIAS

Relação temporal de parâmetros fisiológicos, comportamentais e ecológicos na raposa-do-campo (*Lycalopex vetulus*)

São Paulo
2021

ISIS ZANINI DAS CANDEIAS

Relação temporal de parâmetros fisiológicos, comportamentais e ecológicos na raposa-do-campo (*Lycalopex vetulus*)

Tese apresentada ao Programa de Pós-Graduação em Reprodução Animal da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo para a obtenção do título de Doutor em Ciências.

Departamento:
Reprodução Animal

Área de concentração:
Reprodução Animal

Orientador:
Prof. Dr. Claudio Alvarenga de Oliveira

São Paulo

2021

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO

(Biblioteca Virginie Buff D'Ápice da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo)

T. 4122
FMVZ

Candeias, Isis Zanini das
Relação temporal de parâmetros fisiológicos, comportamentais e ecológicos na raposa-do-campo (*Lycalopex vetulus*) / Isis Zanini das Candeias. – 2021.
83 f. : il.

Tese (Doutorado) – Universidade de São Paulo. Faculdade de Medicina Veterinária e Zootecnia. Departamento de Reprodução Animal, São Paulo, 2021.

Programa de Pós-Graduação: Reprodução Animal.

Área de concentração: Reprodução Animal.

Orientador: Prof. Dr. Claudio Alvarenga de Oliveira.

1. Ecologia comportamental. 2. Ritmo comportamental. 3. Canídeo selvagem. 4. Área de vida. 5. Ciclo reprodutivo. I. Título.

Ficha catalográfica elaborada pela bibliotecária Camila Molgara Gamba, CRB7070-8, da FMVZ/USP.

UNIVERSIDADE DE SÃO PAULO
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA
Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que a proposta intitulada "Avaliação dos hormônios reprodutivos como moduladores da ecologia e comportamento da raposa-do-campo (Lycalopex vetulus)", protocolada sob o CEUA nº 1778211118 (ID 005952), sob a responsabilidade de **Cláudio Alvarenga de Oliveira** *e equipe; Ísis Zanini das Candeias* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (CEUA/FMVZ) na reunião de 17/12/2018.

We certify that the proposal "Evaluation of reproductive hormones as ecological and behavioral modulators in hoary fox (Lycalopex vetulus).", utilizing 10 Brazilian wild species (males and females), protocol number CEUA 1778211118 (ID 005952), under the responsibility of **Cláudio Alvarenga de Oliveira** *and team; Ísis Zanini das Candeias* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/FMVZ) in the meeting of 12/17/2018.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 01/2019 a 12/2020 Área: Reprodução Animal

Origem:	Não aplicável biotério						
Espécie:	Espécies silvestres brasileiras	sexo:	Machos e Fêmeas	idade:	1 a 10 anos	N:	10
Linhagem:	Lycalopex vetulus			Peso:	2500 a 3500 g		

Registro IBAMA/Sisbio/Etc: SiSBio 65816-1

Método de Captura: Os animais serão capturados em armadilhas tipo caixa, com um total de 20 armadilhas espalhadas pela área de estudo. As armadilhas serão posicionadas na sombra, com abrigo da luz. Os animais serão anestesiados com uma associação de quetamina (15mg/kg)/ midazolam (0,5 mg/kg)/ butorfanol (10mg/kg). Após a anestesia serão realizados os seguintes procedimentos: avaliação clínica, pesagem, biometria, coleta de amostras, colocação de brinco, microchip e instalação do colar GPS. Após o procedimento os animais serão colocados novamente na caixa de captura até sua total recuperação e soltos no mesmo local da captura.

Local do experimento: A área de estudo está localizada na porção sudeste de Goiás, no município de Cumari. Esse local foi escolhido devido a parceria do presente projeto com o Programa de Conservação Mamíferos do cerrado que possui estudos na região desde 2009 e uma grande experiência na captura e monitoramento de carnívoros na área.

São Paulo, 17 de dezembro de 2018

Anneliese Traldi

Profa. Dra. Anneliese de Souza Traldi
Presidente da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Roseli da Costa Gomes
Secretária
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia
Universidade de São Paulo

São Paulo, 01 de dezembro de 2020
CEUA N 1778211118
(ID 007156)

Ilmo(a). Sr(a).
Responsável: Cláudio Alvarenga De Oliveira
Área: Reprodução Animal

Título da proposta: "Relação temporal de parâmetros fisiológicos, comportamentais e ecológicos na raposa-do-campo (Lycalopex vetulus)".

CERTIFICADO (Alteração do cadastro versão de 24/setembro/2020)

A Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, no cumprimento das suas atribuições, analisou e **APROVOU** a Alteração do cadastro (versão de 24/setembro/2020) da proposta acima referenciada.

Resumo apresentado pelo pesquisador: "Gostaria de pedir a alteração do título do projeto em virtude de adequações no delineamento do projeto. Não ocorreram alterações em relação ao uso dos animais propostos.".

Comentário da CEUA: Título da proposta alterado atendendo solicitação do pesquisador.

Camilla Mota Mendes

Prof. Dr. Marcelo Bahia Labruna
Coordenador da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Camilla Mota Mendes
Vice-Coordenadora da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

FOLHA DE AVALIAÇÃO

Autor: CANDEIAS, Isis Zanini Das

Título: **Relação temporal de parâmetros fisiológicos, comportamentais e ecológicos na raposa-do-campo (*Lycalopex vetulus*)**

Tese apresentada ao Programa de Pós-Graduação em Reprodução Animal da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo para obtenção do título de Doutor em Ciências.

Data: _____/_____/_____

Banca Examinadora

Prof. Dr.____

Instituição:___________________________ Julgamento:___________________

Prof. Dr.____

Instituição:___________________________ Julgamento:___________________

Prof. Dr.____

Instituição:___________________________ Julgamento:___________________

Prof. Dr.____

Instituição:___________________________ Julgamento:___________________

Prof. Dr.____

Instituição:___________________________ Julgamento:___________________

À pessoa que tem minha tecla SAP...
Meu melhor amigo...
Parceiro de votos pela proteção da
Natureza e de todas as formas de vida...
Dedico essa tese a você...
Caio Motta

AGRADECIMENTOS

Aos meus pais Elias e Lúcia, por estarem do meu lado a cada momento e a cada conquista. Obrigada pelos momentos inesquecíveis nas nossas viagens, por todo o carinho em cada visita, pelos bons momentos em cada almoço em família, pelo cuidado em chegar em casa depois de uma viagem cansativa e encontrar uma refeição de presente na geladeira. Sou hoje um reflexo de todo o amor, cuidado, apoio e ensinamento que vocês me deram, muito obrigada!

Ao Caio Motta, pela parceria em todas as etapas desse projeto, da concepção à escrita. Obrigada por que trazer para a realidade em todas as vezes que eu quis abraçar o mundo na tese... por ser aquela voz analítica sempre me perguntando: “mas que variável você vai usar para medir isso? Qual a reposta que você espera?”. Com certeza o resultado desse trabalho passa pela sua contribuição.

Ao meu professor e orientador Professor Claudio Alvarenga de Oliveira pela confiança no projeto, toda a orientação e ensinamentos. Obrigada por dar uma segunda casa a mim e ao meu trabalho.

Ao Programa de Conservação Mamíferos do Cerrado – PCMC: Fernanda Cavalcanti, Frederico Lemos, Mozart Caetano, Fabiana Rocha e Caio Motta. Obrigada por me tornarem uma pessoa melhor a cada reunião, campanha de campo e reflexão em grupo. Cresço a cada dia com vocês, e espero podermos continuar crescendo juntos, com os mesmos objetivos e trabalhando para proporcionar um futuro melhor para nossas espécies.

Ao Frederico Lemos por me apresentar essa espécie cativante que é a raposa-do-campo. Obrigada por compartilhar seu amor por essa espécie, por toda sua orientação e ensinamentos, que vão muito além do campo científico.

À Fernanda Cavalcanti, por abrir as portas da sua casa, por toda sua amizade, carinho, atenção e paciência.

Ao professor Gustavo Oliveira, que comprou minhas ideias malucas. Obrigada pelos ensinamentos, inspiração, dedicação, por abrir as portas da sua

universidade e da sua casa. Sua contribuição foi fundamental para realização dessa pesquisa e em ampliar meus horizontes no mundo da ecologia do movimento e comportamento dos animais.

Ao professor James Forester, que me recebeu na Universidade de Minnesota de abraços e ideias abertas, me apresentando um campo novo de estudos o qual vou incorporar e levar para minha carreira de pesquisadora.

Ao Lucas Resende, por sua determinação e dedicação no trabalho. Sem sua ajuda esse trabalho não seria possível, muito obrigada!

À todos os pesquisadores, colaboradores e estagiários que participaram das atividades desse projeto ao longo dos anos. Cada pessoa trouxe diferentes visões e reflexões que contribuíram para o amadurecimento do trabalho.

À Fundação Parque Zoológico de São Paulo (FPZSP), nas pessoas de Paulo Bressan, Patricia Locosque e Caio Motta, pela iniciativa em realizar pesquisas *in situ* e pela parceria firmada com PCMC, fundamental para realização desse trabalho.

À CAPES. O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001. Programa de Doutorado Sanduíche, processo: CAPES-PRINT - 88887.368643/2019-00.

Ao povo brasileiro que através do pagamento de seus impostos, permitiram a realização desse trabalho, em diferentes níveis.

“Uma rã se achava importante
Porque o rio passava nas suas margens.
O rio não teria grande importância para a rã
Porque era o rio que estava ao pé dela.
Pois Pois.

Para um artista aquele ramo de luz sobre uma
lata desterrada no canto de uma rua, talvez para
um fotógrafo, aquele pingo de sol na lata seja
mais importante do que o esplendor do sol nos
oceanos.

Pois Pois.

Em Roma, o que mais me chamou atenção foi um
prédio que ficava em frente das pombas.
O prédio era de estilo bizantino do século IX.
Colosso!
Mas eu achei as pombas mais importantes do que
o prédio.

Agora, hoje, eu vi um sabiá pousado na
Cordilheira dos Andes
Achei o sabiá mais importante do que a
Cordilheira dos Andes.

O pessoal falou: seu olhar é distorcido.
Eu, por certo, não saberei medir a importância
das coisas: alguém sabe?
Eu só queria construir nadeiras para botar nas
minhas palavras.”

Manoel de Barros

RESUMO

CANDEIAS, I. Z. **Relação temporal de parâmetros fisiológicos, comportamentais e ecológicos na raposa-do-campo (*Lycalopex vetulus*)**. 2021. 83 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2021.

Desde seu surgimento, os organismos convivem com processos rítmicos no ambiente como o ciclo dia e noite, estações do ano, e fases da lua. Neste ambiente essencialmente variável, os animais evoluem restringindo muitas de suas atividades comportamentais a nichos temporais específicos, maximizando o fitness e a sobrevivência. Esse ajuste se dá através dos relógios biológicos, que integram o *timing* endógeno com as informações ambientais. Embora esses processos temporais sejam determinantes na relação entre os animais e seu meio, a interface entre a cronobiologia e a ecologia ainda é uma fronteira pouco explorada da ciência. É neste território que buscamos trabalhar nesta tese. Monitoramos dez raposas-do-campo (*Lycalopex vetulus*) no Cerrado do Brasil Central. Trata-se da única espécie de carnívoro endêmica do Cerrado e um dos sete canídeos menos estudados do mundo. Estudamos o comportamento da espécie em diferentes escalas, conciliando dados detalhados de observação de campo com monitoramento espacial por transmissores GPS. Propusemos métodos analíticos que pudessem elucidar como os diferentes parâmetros ecológicos variam ao longo do tempo. Nossos resultados demostraram que a raposa-do-campo é noturna e que concentram comportamentos específicos em determinados horários dentro deste período de atividade. Mostramos também que esta rotina diária de comportamentos sofre alterações ao longo do ano, a depender da fase reprodutiva. Também comprovamos a existência de importantes processos rítmicos em relação ao uso do espaço e às interações sociais da raposa-do-campo. Houve variações significativas ao longo do ano no tamanho da área utilizada, deslocamento dos abrigos, fidelidade a regiões específicas e compartilhamento da área entre o casal. Estas variações foram consistentes, cíclicas, e relacionadas com o ciclo reprodutivo da espécie. Nossos resultados trouxeram *insights* sobre a importância dos processos rítmicos para estudos de ecologia comportamental, e os avanços metodológicos propostos aqui servirão de base para que estudos futuros sigam explorando esta fronteira do conhecimento.

Palavras-chave: Ecologia comportamental. Ritmo comportamental. Canídeo selvagem. Área de vida. Ciclo reprodutivo.

ABSTRACT

CANDEIAS, I. Z. **Temporal relationship of physiological, behavioral, and ecological parameters in the hoary fox (*Lycalopex vetulus*)**. 2021. 83 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2021.

Since their emergence, organisms have lived with rhythmic processes in the environment such as the day and night cycle, seasons of the year, and moon phases. In this essentially variable environment, animals evolve by restricting many of their behavioral activities to specific temporal niches, maximizing fitness and survival. This adjustment takes place through biological clocks, which integrate endogenous timing with environmental information. Although these temporal processes are decisive in the relationship between animals and their environment, the interface between chronobiology and ecology is still a little-explored frontier in science. It is in this territory that we worked on this thesis. We monitored ten hoary foxes (*Lycalopex vetulus*) in the Cerrado of Central Brazil. It is the only carnivore species endemic to the Cerrado and one of the seven least studied canids in the world. We studied the species' behavior at different scales, combining detailed field observation data with spatial monitoring by GPS transmitters. We proposed analytical methods that could elucidate how different ecological parameters vary over time. Our results showed that the hoary fox is nocturnal and concentrates specific behaviors at certain times within this period of activity. We also showed that this daily behavior routine changes throughout the year, depending on the reproductive stage. We also proved the existence of important rhythmic processes concerning the hoary fox’s space use and social interactions. Throughout the year, there were significant variations in the size of the used area, shelter displacement, fidelity to specific regions, and couple home range overlap. These variations were consistent, cyclic, and related to the reproductive cycle of the species. Our results brought insights into the importance of rhythmic processes for behavioral ecology studies, and the methodological advances proposed here will serve as a basis for future studies to continue exploring this frontier of knowledge.

Keywords: Behavioral ecology. Behavioral rhythm. Wild canid. Home range. Reproductive cycle.

SUMÁRIO

1. INTRODUÇÃO

O que chamamos de comportamento nada mais é do que a categorização das inúmeras formas com que os corpos se movem no espaço e no tempo. À movimentação sutil dos focinhos de uma raposa-do-campo (*Lycalopex vetulus*) nos solos do Cerrado, chamamos "forrageio". Aos milhares de quilômetros percorridos pelos Gnus (*Connochaetes taurinus*) nas planícies do Serengeti, chamamos "migração". Podemos observar e classificar os comportamentos dos animais em diferentes escalas de tempo e espaço, e é sobre isso que se trata esta tese. Aqui apreciaremos os comportamentos da raposa-do-campo sob diferentes escalas, buscando elucidar sobre como este pequeno canídeo brasileiro expressa-se em seu meio. Mas sobretudo, exploraremos como tal expressão pode variar ao longo do tempo.

Os animais precisam ajustar seus comportamentos a um ambiente essencialmente instável, com processos rítmicos que possuem ciclos de comprimento muito distintos, como ciclo claro-escuro, fases da lua, e estações climáticas (KRONFELD-SCHOR et al., 2017). Esse ajuste se dá através dos relógios biológicos, que integram o *timing* endógeno com as informações ambientais. Assim, são geradas representações internas do tempo, para que, a qualquer momento, o organismo esteja em um estado temporal específico que chamamos de 'tempo do relógio interno'(HELM et al., 2017)

Todos os mamíferos possuem relógio biológico, mas por quê? Por que é tão necessário para os animais dispenderem energia em um mecanismo complexo de relação temporal interna e externa? A resposta passa pelos mecanismos de antecipação e previsibilidade advindos desta marcação temporal, os ritmos endógenos. Um animal diurno não pode ser meramente responsivo aos primeiros raios solares para despertar. Horas antes os níveis de melatonina já começam a cair, assim como o cortisol começa a subir ainda na fase de escuro, preparando o corpo para o despertar (GÜLDÜR; OTLU, 2017). Os ritmos endógenos não só possuem uma relação de fase estável com os ciclos ambientais, mas também se encadeiam temporalmente formando a ordem temporal interna (MORENO, 2018).

Além de prever o tempo correto para determinada atividade, o relógio biológico possui mecanismos para realizar ajustes neste *timing*, em resposta às variações nos componentes bióticos e abióticos que podem ocorrer entre os anos em uma dada localidade (KRONFELD-SCHOR et al., 2017). A cronobiologia é a ciência que promove o estudo sistemático destas características temporais dos seres vivos, em todos os níveis de organização (ROTENBERG; MARQUES; MENNA-BARRETO, 2003).

Enquanto cronobiólogos vêm estudando esta maquinaria do relógio endógeno, os ecólogos já demonstraram a importância que o *timing* anual pode ter no fitness individual dos animais. Desvios no *timing* ideal podem resultar em reduções no sucesso de forrageio, nas taxas de sobrevivência e no sucesso reprodutivo (HELM et al., 2017). No entanto, embora haja um potencial inato de conexão entre estas duas áreas da ciência, ainda são escassos os estudos que abordem esta fronteira do conhecimento. Estudos de cronobiologia são comumente focados no ambiente de laboratório, dada a dificuldade em se realizar monitoramentos precisos e contínuos com animais na natureza. Por outro lado, estudos ecológicos normalmente desconsideram os processos rítmicos finos que regulam e permeiam os diferentes aspectos da ecologia comportamental de uma espécie.

Na presente tese de doutorado buscamos aliar elementos do arcabouço teórico e do ferramental analítico destas duas áreas da ciência, buscando demonstrar o rico potencial que surge do estreitamento entre elas. Utilizamos como espécie modelo a raposa-do-campo, *Lycalopex vetulus*, único carnívoro endêmico do Cerrado (JUAREZ; MARINHO-FILHO, 2002) e um dos sete canídeos menos estudados do mundo (*IUCN canid specialist group*).

A raposa-do-campo é um canídeo de pequeno porte (2.5 – 4kg) e possui um sistema monogâmico de acasalamento, com um ciclo reprodutivo anual e uma fase reprodutiva marcadamente sazonal, com filhotes nascendo entre agosto e setembro e ficando na mesma área dos pais até os 7 meses de idade, aproximadamente (CANDEIAS et al., 2020; DALPONTE, 2003, 2009; LEMOS, 2016). Os filhotes nascem em tocas abandonadas de tatu-peba (*Euphractus sexcinctus*), em ninhadas que podem variar de um a cinco filhotes (três em média) (COURTENAY et al., 2006; DALPONTE, 2009; LEMOS; FACURE; DE

AZEVEDO, 2011). Atualmente a raposa-do-campo é classificada pela União Internacional para a Conservação da Natureza (IUCN) como quase ameaçada (*NT*). Nas avaliações nacionais é classificada como vulnerável na Lista Vermelha de Espécies Ameaçadas de Extinção (LEMOS et al., 2013); em perigo no estado de Minas Gerais (CHIARELLO et al., 2008); e ameaçada de extinção no estado de São Paulo (DECRETO 63.853, 2018).

Apesar de ser um dos 25 ecossistemas mais ricos em biodiversidade, o Cerrado tem sido intensamente impactado pela expansão da fronteira agrícola e urbanização (COLLI; VIEIRA; DIANESE, 2020). Com esta perda crescente de habitats naturais, as raposas-do-campo têm, cada dia mais, utilizado paisagens modificadas, como áreas de pastagem exótica e agricultura. Ao viverem próximas de casas e estradas enfrentam ameaças crescentes relacionadas ao conflito com pessoas, predação por cães domésticos, atropelamentos e transmissão de doenças oriundas de animais domésticos (BICKLEY et al., 2020; DALPONTE, 2009; LEMOS et al., 2013). Além disso, é estimado que as mudanças climáticas representem uma grande ameaça à sobrevivência da espécie, com uma estimativa de drástica redução na disponibilidade de habitat em menos de cinquenta anos (GUTIÉRREZ et al., 2019).

Assim, vivemos em um mundo que passa por um acelerado processo de mudanças ambientais causadas por nós, seres humanos. Estas alterações podem afetar negativamente a complexa sincronia entre os ritmos biológicos. A progressiva mudança climática, os crescentes níveis de luz à noite, a intensificação da agricultura e as consequentes alterações na cadeia alimentar podem gerar efeitos perturbadores no *timing* biológico (GIENAPP; REED; VISSER, 2014; HOFFMANN; SGRÒ, 2011; VISSER, 2008). Portanto, é essencial que compreendamos os processos mecânicos e ecológicos que determinam as respostas dos organismos selvagens a este mundo em rápida transformação (HELM et al., 2017).

Diante deste cenário, buscamos desenvolver uma abordagem integrativa entre a cronobiologia, a ecologia comportamental, e a ecologia espacial. Monitoramos dez raposas-do-campo em um agroecossistema do sudeste de Goiás. Conciliamos dados detalhados de observação de campo, através de etogramas, com dados precisos de uso do espaço, através de transmissores GPS.

Estes foram os primeiros animais desta espécie a serem monitorados com esta tecnologia. Propusemos métodos analíticos que pudessem elucidar como os diferentes parâmetros ecológicos variam ao longo do tempo, e qual sua relação com processos rítmicos e aspectos reprodutivos da espécie. Exploramos variações comportamentais em diferentes escalas, desde descrições das rotinas comportamentais diárias, até variações sazonais no uso do espaço e na interação intraespecífica. E em nossas discussões, não nos limitamos à compreensão da espécie alvo, procuramos explorar como nossos resultados e métodos podem contribuir para expandir esta fronteira do conhecimento relacionada ao significado funcional do tempo na natureza.

2. THE TIME ON HOARY FOXES CLOCK: UNCOVERING DAILY AND SEASONAL FINE-SCALE BEHAVIOR RHYTHMS OF A WILD CANID

THE TIME ON HOARY FOXES CLOCK: UNCOVERING DAILY AND SEASONAL FINE-SCALE BEHAVIOR RHYTHMS OF A WILD CANID

Isis Zanini das Candeias[1,2], Caio Filipe da Motta Lima[3,2], Fernanda Cavalcanti de Azevedo[4,2], Gustavo Oliveira Santos[5], James Forester[6], Frederico Gemesio Lemos[4,2], Claudio Alvarenga de Oliveira[1]

[1] Departamento de Reprodução Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, SP 05508-270, Brazil

[2] Programa de Conservação Mamíferos do Cerrado, Catalão, GO 75704-020, Brazil

[3] Fundação Parque Zoológico de São Paulo, São Paulo, SP 041301-905, Brazil

[4] Departamento de Ciências Biológicas, Universidade Federal de Catalão, Catalão, GO 75704-020, Brazil

[5] Universidade Federal do Mato Grosso do Sul, Instituto de Biociências, Campo Grande, MS, 79070-900, Brazil

[6] Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota, St. Paul, Minnesota, 55108 USA

ABSTRACT

Animals evolved by restricting their behavioral activities to specific temporal niches to maximize their fitness according to rhythmic environmental processes such as the day/night cycle and seasons. This coordination between the unstable environment and the internal state of animals was only possible through the development of anticipatory mechanisms, the endogenous rhythms coordinated by biological clocks. However, rapid global anthropogenic changes can interfere with the perfect timing of intra- and enter-specific interactions. This scenario is crucial for species threatened with extinction, such as the hoary fox (*Lycalopex vetulus*), an endemic species of the Brazilian Savanna (Cerrado) that has been suffering from loss of natural areas, intensification of agriculture, and urbanization. We monitored six individuals of hoary fox for eleven months through GPS collars and direct observations with ethograms to determine the species' daily and seasonal behavioral activity rhythm. Our results demonstrated that the hoary foxes are nocturnal and consistently select activity periods during the night, presenting a bimodal activity pattern within the night, which can be part of a mechanism to allow the coexistence of sympatric species. The hoary foxes had an annual and markedly seasonal reproductive cycle with offspring born in mid-September and staying with their parents for 7-8 months. During this phase, significant changes occur in the activity pattern due to the emergence of parental care, changing mainly the feeding and sleeping dynamics, which became spread through the night and presented an overall reduction. The increased energetic demand with decreased foraging and resting could lead to a critical energetic imbalance in this phase, critical for parents and offspring. However, this phase coincides with greater food availability, with the termites swarming with the beginning of the rain season. Nevertheless, climatic changes can affect this fine synchrony, impacting the offspring survival and the conservation of the species.

Keywords: Behavior ecology. Chronobiology. Behavior rhythms. Wild canid. Conservation

2.1. INTRODUCTION

Along its evolution, all the species must deal with rhythmic processes of the environment, such as day and night cycle, seasons, moon phases, and tidal oscillations. Due to this essentially unstable environment, animals have evolved by restricting their behavior activities, such as feeding, mating, and locomotion, to specific temporal niches (NELSON, 2005). These adaptations were only possible by developing the species' anticipatory mechanisms, the endogenous rhythms (MORENO, 2018). The temporal coordination of biological processes is critical to maximizing survival and consequently fitness, as they optimize the balance between foraging, reproduction, and predation risk (VAN DER VEEN et al., 2017).

Animals' circadian system provides a fascinating example of a system that paradoxically demonstrates rigidity and plasticity (HELM et al., 2017). The rigidity of the system is essential to organize animal physiology and behavior precisely. They are critical for measuring the day's length (photoperiod) that permeates many annual and circa-annual rhythms (GWINNER, 1986). On the other hand, recent studies highlighted the plasticity contained in this system (PEZUK et al., 2010; VAN DER VEEN et al., 2017), revealed, for example, by the discrepancy between rhythms described for the same species on in situ and laboratory. While laboratory studies, under stable conditions, essentially neutralize the need or triggers for plasticity, plasticity is probably the rule in a constantly changing natural world (HELM et al., 2017).

The physiological and behavioral patterns derive from a complex network formed between the central clock in the suprachiasmatic nucleus (SCN) and peripheral clocks present throughout the tissues (ALBRECHT, 2012). These biological clocks can be entrained by periodic external signals known as zeitgebers (SCHWARTZ; HELM; GERKEMA, 2017). In the circadian context, light is the predominant zeitgeber. Zeitgebers do not induce a rhythm but determine its period length and set its phase angle. In addition, the peripheral clock system can be synchronized with many non-photic zeitgebers, such as food availability, temperature, and glucocorticoid levels (BALSALOBRE et al., 2000; BUHR; YOO; TAKAHASHI, 2010; DAMIOLA et al., 2000). Thus, virtually, every cell in the body can express a circadian clock, forming an extensive, complex system that expresses many rhythms with different phases.

Although endogenous rhythms result from thousands of years of co-evolution between biotic and abiotic factors, currently, the species have been subjected to significant environmental changes in a short period. Global anthropogenic changes such as climate change, agricultural intensification and urbanization can influence the finely tuned and orchestrated timing of intra and interspecific interactions (KRONFELD-SCHOR et al., 2017). This scenario is crucial for species threatened to extinction (DI MARCO et al., 2018; HOFFMANN; SGRÒ; KRISTENSEN, 2017), as the hoary fox (*Lycalopex vetulus*), the only Brazilian endemic carnivore. The hoary fox is a small canid (2.5 - 4kg) more common in open areas of the Brazilian Savanna (Cerrado) (DALPONTE, 2009; LEMOS et al. 2020). Although, globally the Cerrado figures as one of the 25 wealthiest ecosystems in biodiversity, it has been intensely impacted by the expansion of the agricultural frontier (COLLI; VIEIRA; DIANESE, 2020). Furthermore, GUTIÉRREZ et al., 2019 described that climate change is likely to drastically reduce habitat availability for L. vetulus in no longer than five decades. Finding solutions to such major challenges will be facilitated by a fundamental understanding of the mechanistic and ecological processes that determine the responses of wildlife to a changing world (SMARR et al., 2013).

Hoary fox is a monogamic canid, and it has a markedly seasonal reproductive period, with births occurring once a year, between August and September (DALPONTE, 2009; LEMOS, 2016; CANDEIAS et al., 2020). Puppies are born in abandoned armadillo burrows and remain exclusively inside the burrows for the first 30 days of life, approximately (DALPONTE, 2003). Among carnivores, the social system ranges from solitary to highly social, and for the Canidae family, monogamy is the most common system (KLEIMAN; EISENBERG, 1973; MACDONALD et al., 2019). Monogamous canids typically have both male and female parental care (BRUIN; GANSWINDT; ROUX, 2016). The degree of male care may vary from poor care, as in *Vulpes cana*, to essential care, as in *Otocyon megalotis* (BRUIN; GANSWINDT; ROUX, 2016; WRIGHT et al., 2010). In the latter, the male care is directly related to the offspring's survival until dispersion, maximizing reproductive fitness. Unfortunately, to this date, few studies on parental care and social behavior of hoary fox have been carried (COURTENAY et al., 2006).

Despite the highlighted importance of integrating behavior ecology and chronobiology (SCHWARTZ; HELM; GERKEMA, 2017), this still remains a poorly explored frontier of science. It is understandable, given that assessing detailed animal behavior in the wild on a fine temporal scale is a challenge hard to overcome. It is an even more defiant task on cryptic species such as carnivores. Hence, field ethological studies in this group are rare, and there are no studies regarding behavior rhythms on the hoary fox, one of the seven less studied canids of the world (IUCN/SSC Canid Specialist Group).

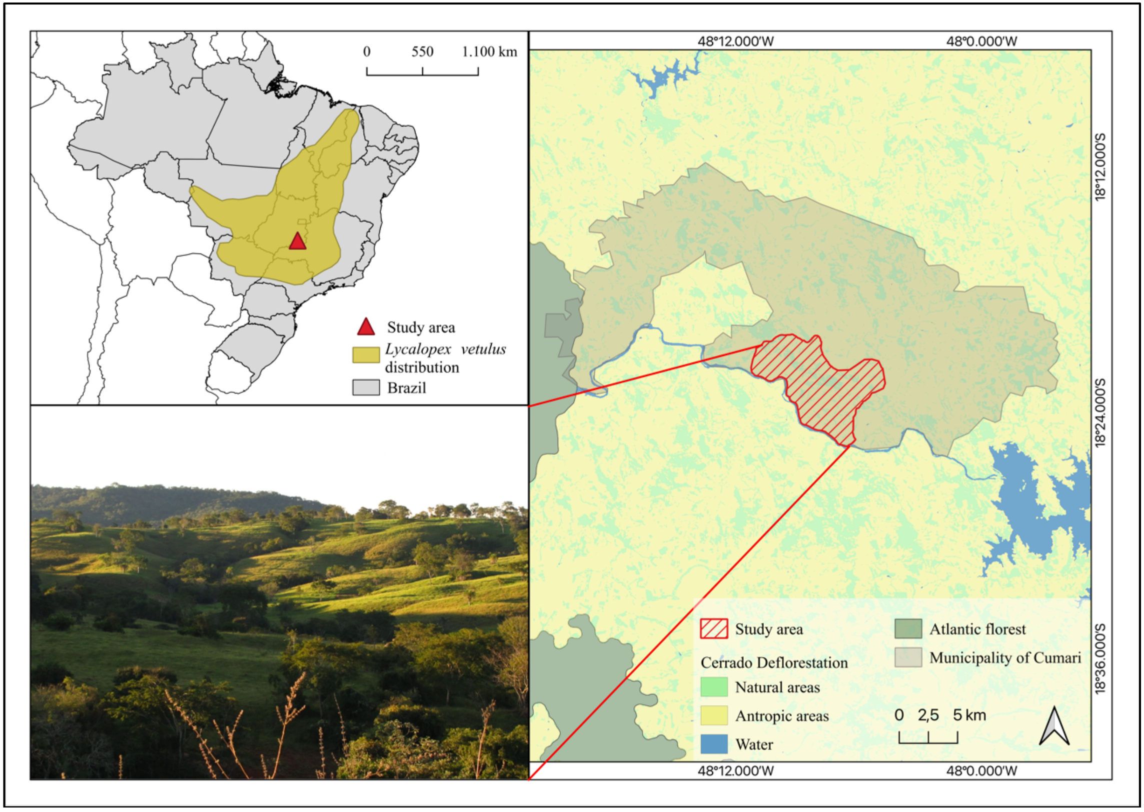
Here, we present a detailed ethological monitoring of this wild canid from Central Brazil, and fine-scale analysis to elucidate the species daily and seasonal behavior rhythms. We hypothesized that: 1. hoary foxes are nocturnal and have a behavioral routine, consistently concentrating specific activities at certain periods of the night; 2. hoary foxes' behavioral routine changes at different reproductive phases. Finally, we explored the connections between the described chronobiological rhythms and the hoary fox ecology and conservation aspects.

2.2. METHODS

2.2.1 Study Area

The study took place in a tropical region known as Limoeiro, a rural zone in the municipality of Cumari, state of Goiás, Brazil (Lat -18.382039°/Long -48.117714°) (Figure 1). The Limoeiro region holds small and medium-sized private cattle farms and was declared a municipal Environmental Protection Area (EPA) in 2017. Despite its creation, there is still no specific management in the EPA toward environmental protection. The region is characterized by an ecotone of intrusions of Atlantic Forest vegetation into the Cerrado domain (Savanna biome). More than 70% of the landscape 9300ha is composed of exotic pasture (*Brachiaria sp.*) used for cattle grazing, followed by small patches of crops and forestry (~ 2%) (LEMOS; FACURE, 2011). These human-modified areas forms a mosaic with patches of natural vegetation constituted by semidecidual seasonal and gallery forests (~21%) and cerrado *sensu stricto* (woodland savanna; ~ 4%) (LEMOS, 2016). There were no areas of native open vegetation. The climate is tropical savanna (Koppen's AW), constituted by dry fall and winter, with respective mean temperatures of 22.35oC and 22.71oC, and season precipitation of 210mm and 0.4mm; and humid spring and summer, with respective mean temperatures of 24.19 and 24.16, and season precipitation of 650mm and 397mm. The annual temperature ranged from 6.60C to 360C. These are the specific climate metrics for the studied region during the studied period (INMET, 2019). Sunset time varied from 17:44 to 18:55 (71 min difference), and sunrise from 5:28 to 6:46 (78 min difference) (BRT – Brasília Time, UTC-03:00). Night period (from sunset to sunrise) ranged from 10.8 hours to 13 hours (2.2 hours difference).

Figure 1 – Study area in Central Brazil, municipality of Cumari, Goias state. The red line delimits the study area, composed of exotic pasture areas and forest remnants.



2.2.2 Captures and Handling

Six hoary foxes (three females and three males) were captured using Tomahawk box traps during March 2018 and June 2019. We anesthetized foxes with an association of 15 mg/kg of ketamine (Cetamin 10 mg/ml, Syntec, Santana de Parnaiba, SP, Brazil), 0,5 mg/kg of midazolam (Dormire 5mg/ml, Cristália Chemical and Pharmaceutical products, São Paulo, SP, Brazil) and 0,2 mg/kg butorphanol (Torbugesic 10 mg/ml, Fort Dodge Laboratories, Fort Dodge, Iowa, U.S.A.), administered intramuscularly in a single injection into the gluteal muscle. Each fox was weighed, measured, tagged with numbered plastic ear tags, and adults equipped with a GPS collar (Tigrinus®, Timbó, SC, Brazil). After full anesthetic recovery, foxes were released in the same spot of capture. All captures and handlings were approved by the Chico Mendes Institute for Biodiversity Conservation – ICMBIO under permit no 65816-1. The study followed the rules issued by the National Council for Control of Animal Experimentation (CONCEA) and was approved by the Ethics Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/FMVZ; protocol number 1778211118).

2.2.3 Activity Patterns

A species activity pattern is traditionally determined by measuring locomotor activity (VALENTINUZZI et al., 1997). Locomotor activity concentrated during the day or/and night typifies the species as diurnal, mostly diurnal, nocturnal, mostly nocturnal, or cathemeral (GÓMEZ et al., 2005; VAN SCHAIK; GRIFFITHS, 1996). Whereas activity during dawn and dusk makes the species crepuscular (SMARR et al., 2013). We established the hoary fox main activity pattern using movement data from GPS collars of four adult hoary foxes (M1, F1, F2, F3). We obtained 40 fox-days of data with a fix interval of 30 min. Location errors of GPS units were <20m. To infer the foxes' activity, we estimated their movement by calculating the step length (SL) (*amt* package, program R®, version 4.1.0, 2021). The SL is not the size of a natural step of the animal but a virtual measure where a straight line is made between two consecutive fixes, and then we calculate the distance traveled between these fixes (IRONSIDE et al., 2017). Here, SL refers to the fox displacement (meters) in 30 minutes. We classified each step as being at dawn (between 30min after and before sunrise), day (between 30min after sunrise and 30 min before sunset), dusk (between 30 min before and after sunset), and night (between 30min after sunset and 30 min before sunrise). We considered the first fix of each step for this classification, and we crossed it with the specific sunrise/sunset time of the respective day (*suncalc* package, program R®, version 4.1.0, 2021). Variations on sunrise (6:13 – 6:22) and sunset (18:04 – 18:07) times were minimal during these sampling days. This preliminary analysis was used to highlight the general activity pattern of the hoary fox, so that we could refine their behaviors within the activity period in the following analysis.

2.2.4 Ethogram

We collected behavior data through direct observations using a focal scan ethogram. The ethogram sampling occurred during 14 consecutive days of each month from 15:00h to 8:00h, between November 2018 and September 2019. We registered 27 behaviors, including intraspecific and interspecific interactions. We previously described each behavior to standardize the data collection (Supplementary 1). We localized the foxes in the area through radiotelemetry, using a directional antenna (Telonics, Inc., Mesa, AZ) and a VHF receiver (Advanced Telemetry Systems, Isanti, MN). GPS data was downloaded remotely with a UHF receiver (Tigrinus®, Timbó, SC, Brazil). All the observations were carried by the same observer, which was always alone when approaching the animals. Once the observer encountered the individuals, the observation was carried from approximately 30 meters, on foot, using a flashlight of 960 lumens (Fenix, LoneTree, CO). Focal scan ethogram sessions were performed every 30 seconds. The ethogram session began when the observer had visual contact with the focal animal and finished when the observer loosed visual contact with the individual for more than 10 minutes or after a maximum period of eight hours. If the same animal were tracked again in the same night, a new ethogram session was initiated.

2.2.5 Statistical Analysis

To refine the activity pattern analysis, we grouped the behaviors on inactive (sleeping) or active (all the other behaviors). Then we calculated the activity frequency within each hour of the ethogram for each individual. For the following statistical analysis, we grouped the 27 recorded behaviors into seven categories: Sleeping, Low activity, Feeding, Locomotion, Surveillance, Parental care, and Others, that group behaviors registered in low quantities. (Supplementary 1). Then we calculated the frequency of each behavior per hour for each individual. For the descriptive statistics, we averaged the behavior frequency of the different individuals on each hour.

To infer the daily behavior rhythm of the hoary fox and possible changes along the year, we used statistical modeling. Observations were classified into parental phase (October to May), when couples are denning, and non-parental phase (June to September), the period after puppies' dispersion and before the next birth (DALPONTE, 2009; LEMOS et al., 2020). We created four harmonic vectors for each observation, applying the following formulas to the observation hour: $s1=\sin(hour.2.\pi/24)$; $s2=\sin(hour.4.\pi/24)$; $c1=\cos(hour.2.\pi/24)$; $c2=\cos(hour.4.\pi/24)$. We used Multinomial Logit Models (*mclogit* package, program R®, version 4.1.0, 2021) with the seven categorical behaviors as dependent variables, considering "sleeping" as the reference variable. We considered the interaction between the four harmonics (s1, s2, c1,c2) and the two parental phases as independent variables. By modeling the combination of these four harmonic curves, we allowed the model to adjust the best from a vast possibility of curves, including a flat line if there was no behavior variation through time. We also included the individual ID as a random intercept to consider individual variations on size effect. We investigated the assumptions of the fitted model by checking for normal distribution of the residuals using histogram and normal Q-Q plot. We also checked the autocorrelation of the data regarding the time and the individuals. We extracted the fitted model's prediction values and highlighted the periods of behavior concentration by calculating the 50% isopleth for each behavior in each parental phase. The 50% isopleth represents the periods that accumulate 50% of the behavior probability throughout determined time. Finally, we calculated the integral of both parental phases' curves to compare the total probability volume of each behavior between the phases.

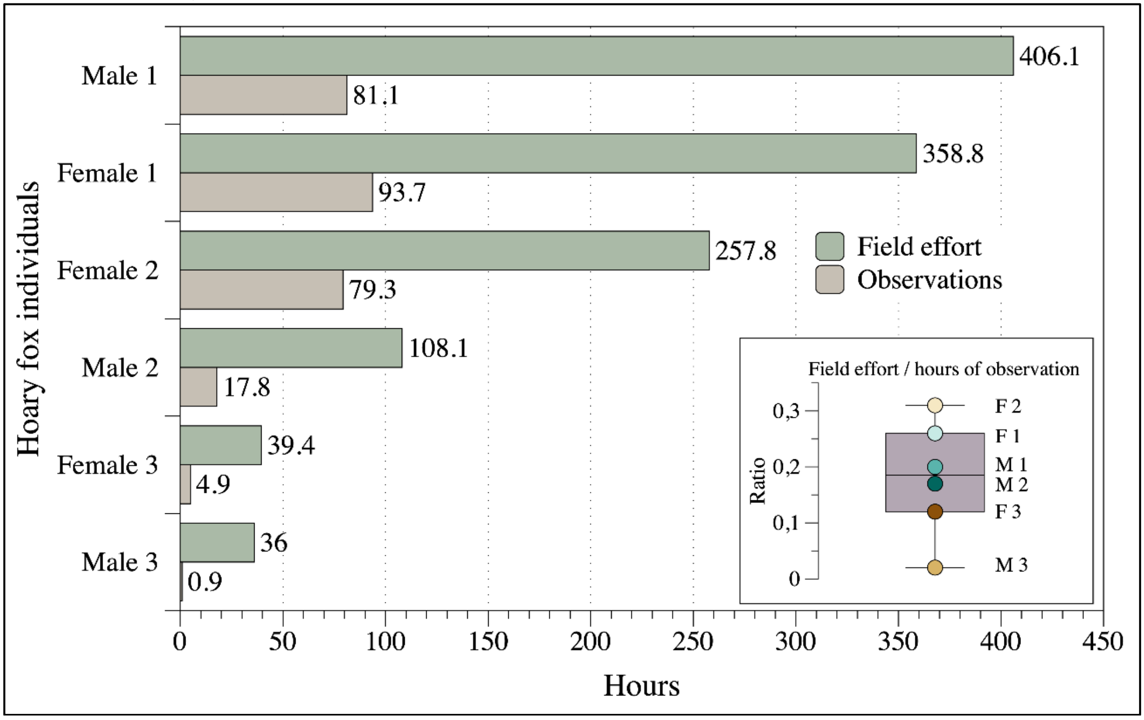
2.3 RESULTS

Between November 2018 and September 2019, six adult hoary foxes were accompanied and observed, three males and three females (Table 1). One pair (F1, M1) was observed together and shared the same home range though considered a couple. The other four individuals occupied four different ranges, and at least two of them (M2, F3) were paired with non-monitored individuals. Observations occurred between 15:00h and 8:00h, totalizing 278 hours of observation after 1,318 hours of field effort. Average observation success rate (observation time/effort time) was 21%, but there was a wide variation between individuals (range: 2% - 31%), suggesting an individual heterogeneity in tolerating the observer (Figure 2). On 21.4% of the observation time during the same observation session, individuals were close to their pair. However, was no observation of intraspecific interactions with other individuals beyond the pair unit. We observed interspecific interactions with gray brocket deer (*Mazama gouazoubira*), giant anteater (*Mymercophaga tridactyla*), lesser anteater (*Tamandua tetradactyla*), striped hog-nosed skunk (*Conepatus amazonicus*), and crab-eating fox (*Cerdocyon thous*). Yet, these events were rare, less than one event per month, and none of them lead to agonistic interactions.

Table 1 – General description of hoary foxes (*Lycalopex vetulus*) monitored in the Limoeiro region, between 2018 and 2019.

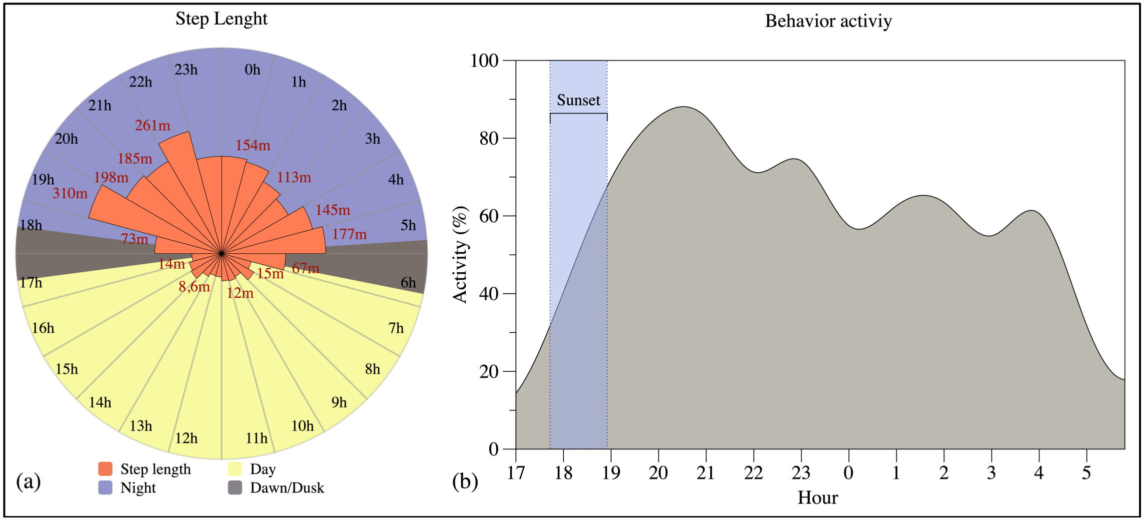
ID	Sex	Weight (kg)	Reproduction During study	Monitoring period (months)
F1	Female	4,0	Yes	11
M1	Male	3,9	Yes	11
F2	Female	3,42	No	7
M2	Male	3,4	Yes	2
F3	Female	3,85	Yes	11
M3	Male	3,3	No	5

Figure 2 – Field effort time and hours of ethogram obtained from hoary foxes (*Lycalopex vetulus*) observation. Boxplots represent the observation success rate for each hoary fox.



Hoary foxes were nocturnal, with activity concentrated mainly during the night (mean SL = 171.2m, SE = 22.2), starting to increase during the dusk (mean SL = 71.42m, SE = 13.97), and with a decreased activity during the dawn (mean SL = 24.6, SE = 9.45) (Figure 3-a). During the day, foxes rested in dense bushes and yellow armadillo burrows (*Euphractus sexcinctus*) (LEMOS, 2016), and the SL observed (mean SL = 14.18m, SE = 2.23) results from GPS errors between fixes. Through direct observation of foxes behavior, it was possible to notice an activity peak on the first four hours of the night (> than 80% of activity frequency), a decrease through the following six hours ~(60% of activity frequency), and finally a sharp drop close to the sunrise (< 20% of activity frequency) (Figure 3-b).

Figure 3 – (a) Hoary fox average step length (fox displacement in 30 min) within each hour of the day, obtained by GPS movement data. Dawn and dusk periods were considered 30min before and 30 min after the time of sunrise and sunset, respectively. (b) Mean activity frequency of hoary foxes in each hour of the night obtained by direct observation. The blue range represents the year variation in the sunset time (photoperiod variation).



The multinomial model generated two different outputs for each behavior (Table 2), one for each parental phase (Figures 4 and 5). Prediction values from models represent the odds ratio between the specific behavior and "sleeping", the reference variable. At the non-parental phase, there were high peaks of all behaviors (feeding, locomotion, surveillance, low activity) in the first hours of the night, between 18:00 and 22:00, except parental care since this behavior is not present in this phase. A decrease follows it between 23:00 and 2:00, representing the increase of sleeping behavior. There was a second short peak from 2:00 to 4:00, followed by a second decrease before 6:00. Although it´s possible to notice a bimodal activity pattern during the night, the first peak was higher than the second, concentrating all the isopleth 50% within it. Feeding and locomotion achieved the highest prediction values during the first peak, followed by low activity, and the least frequent behavior, the surveillance. Although there were differences in the prediction values among these behaviors, their fluctuation patterns along the activity period were similar.

During the parental phase, hoary foxes live with and take care of offspring (DALPONTE, 2009; LEMOS et al., 2020), and it seems to have influenced their behavior. Parental care was concentrated between 18:00 and 2:00, with 50% isopleth between 20:00 and 23:00. Regarding the other behaviors, although we can still observe a peak at the beginning of the night, it greatly decreased when compared to the non-parental phase, especially to feeding, locomotion, and surveillance behaviors. Both peaks concentrated similar prediction values at the parental phase, intensifying the bimodal pattern within the night. This effect is clear when we analyze the isopleth 50%, which in this phase was divided between the first (19:00 - 21:00 hours) and second peaks (1:00 - 3:00 hours) for feeding, locomotion, and surveillance. Low activity behavior was also bimodal, but it was spreader through the night, with isopleth 50% between 19:00-2:00 and 4:00–6:00. We can also notice a decrease in inactivity in the middle of the night; the prediction values of surveillance and low activity between the peaks were higher in the parental phase. It represents a decrease in sleeping during this period, which was replaced mainly by parental care, surveillance, and low activity behaviors.

Besides these differences in the behavior's fluctuation through the night, total probability volume also differed between phases. Compared to non-parental phase, at the parental phase there was a reduction of feeding (-31%) and locomotion (-41%) behaviors, and an increase of low activity category (+18%). Effects detected by the models can be observed checking the descriptive behavior data of couple F1 and M1 (Figure 6). At the non-parental phase, the couple presented two prominent feeding peaks (18:00-22:00; 2:00-4:00), separated by a marked sleeping period in the middle of the night (23:00 – 2:00). The inclusion of parental care changed feeding and sleeping dynamics, which are spread through the night and present an overall reduction.

Figure 4 – Model predict of hoary fox behaviors (feeding, locomotion, low activity, surveillance, and parental care) along hours of the day in the non-parental and parental phases.

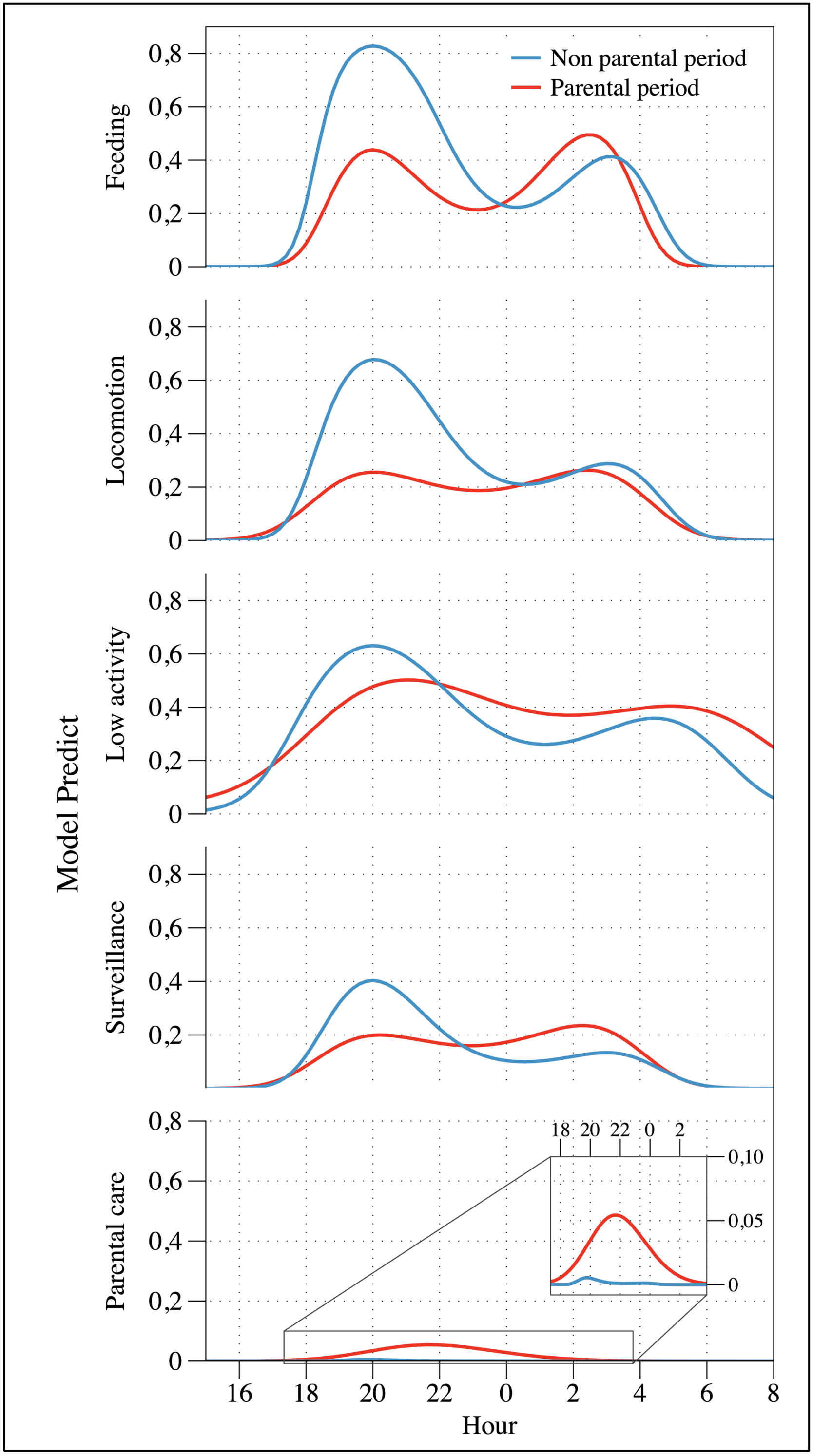


Figure 5 – 50% Isopleth of the model predict for hoary fox behaviors (feeding, locomotion, low activity, surveillance, and parental care) along hours of the day in the parental and non-parental phases.

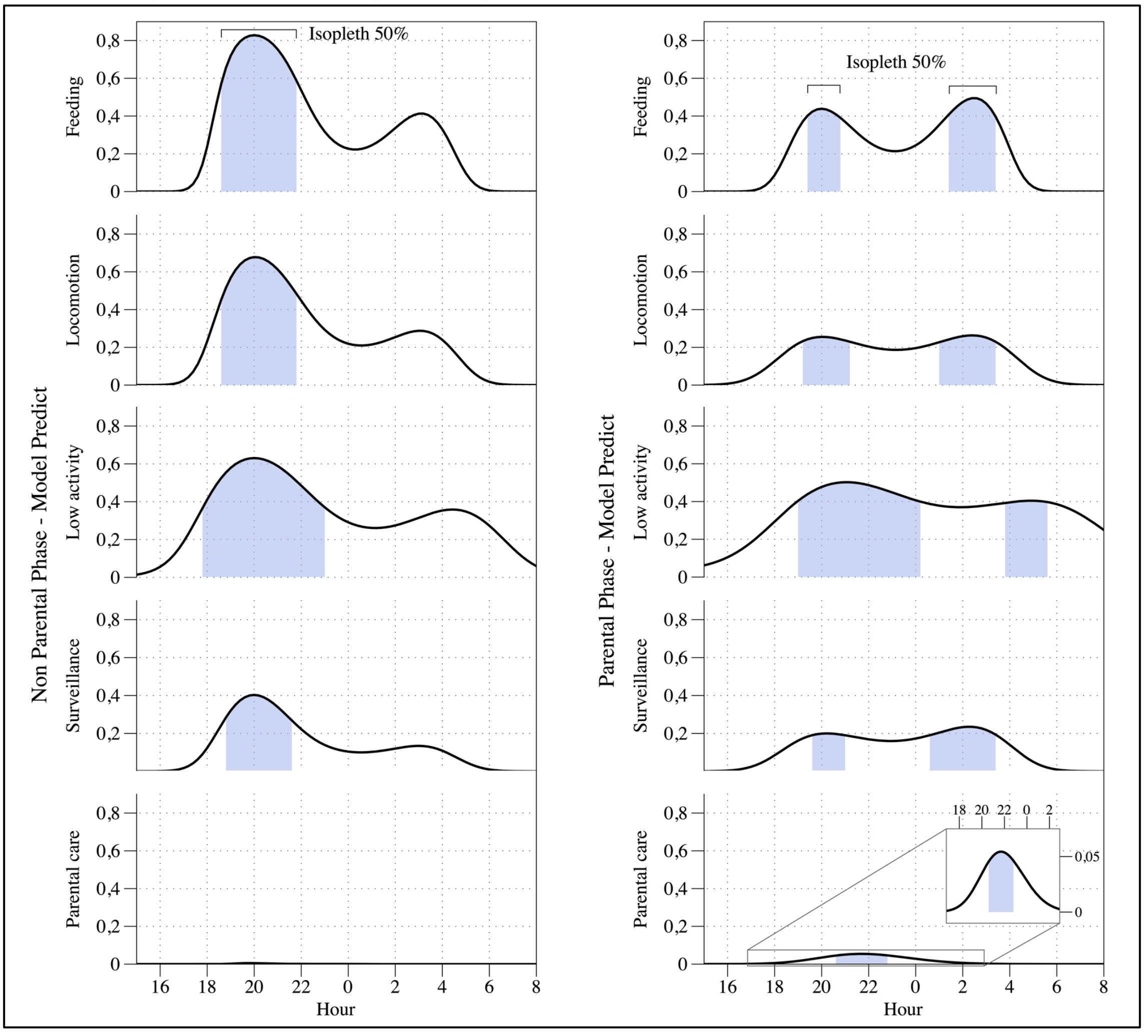
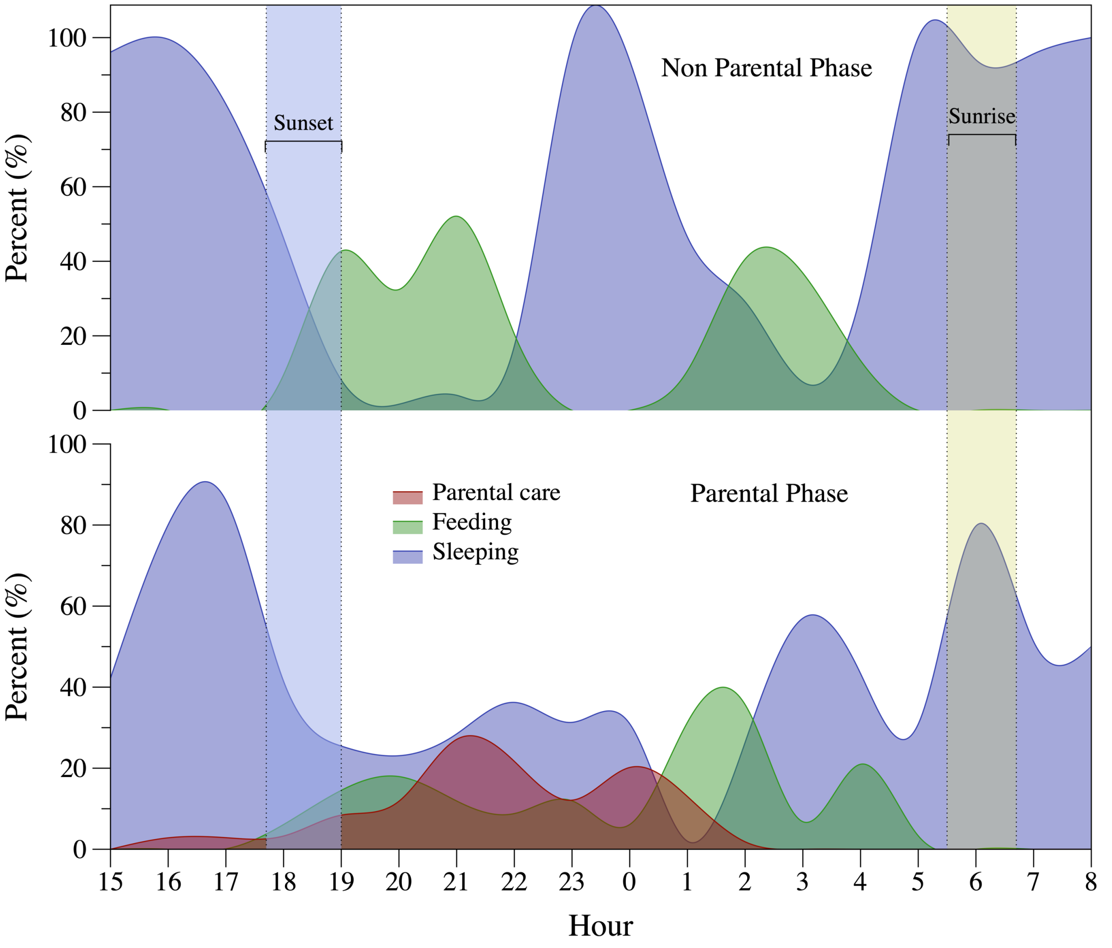


Table 2 – Model coefficients of behaviors in hoary foxes (*Lycalopex vetulus*) with the intercept and the interaction with each of the four harmonic vectors in the parental and non-parental phases.

		Value	**Std. Error**	**z-value**	**p-value**
Low activity vs Sleeping	Intercept	-2.16	0.19	-11315	<2e-16
	S1	0.31	0.04	8.27	<2e-16
	C1	-2.76	0.14	-19.60	<2e-16
	S2	-0.35	0.05	-6.79	1.10e-11
	C2	-1.49	0.09	-16.97	<2e-16
	Parental	0.95	0.11	9.00	<2e-16
	S1:Parental	-0.52	0.05	-10.21	<2e-16
	C1:Parental	1.39	0.18	7.67	1.66e-14
	S2:Parental	-0.05	0.07	-0.71	0.48
	C2:Parental	0.95	0.11	8.42	<2e-16
Feeding vs Sleeping	Intercept	-8.40	0.54	-15.60	<2e-16
	S1	1.99	0.13	15.25	<2e-16
	C1	-12.42	0.45	-27.11	<2e-16
	S2	0.67	0.11	6.01	1.82e-09
	C2	-5.24	0.18	-27.98	<2e-16
	Parental	-1.17	0.53	-2.23	0.03
	S1:Parental	0.61	0.25	2.44	0.01
	C1:Parental	-0.64	0.77	-0.83	0.41
	S2:Parental	1.36	0.20	6.87	6.57e-12
	C2:Parental	0.63	0.29	2.16	0.03
Locomotion vs Sleeping	Intercept	-6.09	0.42	-14.46	<2e-16
	S1	1.41	0.11	12.69	<2e-16
	C1	-8.29	0.38	-21.78	<2e-16
	S2	0.32	0.10	3.05	0.00
	C2	-3.47	0.17	-20.23	<2e-16
	Parental	1.28	0.35	3.68	0.00
	S1:Parental	-0.34	0.18	-1.92	0.05
	C1:Parental	3.10	0.54	5.78	7.52e-09
	S2:Parental	0.48	0.16	2.95	0.00
	C2:Parental	1.67	0.24	7.04	1.86e-12
Surveillance vs Sleeping	Intercept	-6.22	0.56	-11.09	<2e-16
	S1	1.33	0.16	8.27	<2e-16
	C1	-7.02	0.53	-13.25	<2e-16
	S2	0.34	0.16	2.18	0.03
	C2	-2.94	0.25	-11.87	<2e-16
	Parental	0.84	0.47	1.79	0.07
	S1:Parental	-0.19	0.25	-0.79	0.43
	C1:Parental	1.33	0.72	1.85	0.06
	S2:Parental	0.55	0.22	2.48	0.01
	C2:Parental	1.07	0.32	3.35	0.00
Parental Care vs Sleeping	Intercept	-82.22	35.27	-2.33	0.02
	S1	57.46	27.28	2.10	0.03
	C1	-88.37	41.35	-2.14	0.03
	S2	27.11	13.12	2.07	0.04
	C2	-13.00	6.69	-1.94	0.05
	Parental	74.11	35.26	2.10	0.04
	S1:Parental	-55.10	27.29	-2.02	0.04
	C1:Parental	83.25	41.36	2.01	0.04
	S2:Parental	-27.01	13.12	-2.06	0.04
	C2:Parental	12.44	6.69	1.86	0.06
Others vs Sleeping	Intercept	-8.36	0.89	-9.39	<2e-16
	S1	1.58	0.32	5.00	5.64e-07
	C1	-9.20	1.06	-8.72	<2e-16

	S2	0.74	0.28	2.64	0.01
	C2	-4.22	0.45	-9.48	<2e-16
	Parental	2.78	0.83	3.37	0.00
	S1:Parental	-1.67	0.37	-4.52	6.11e-06
	C1:Parental	5.69	1.28	4.46	8.20e-06
	S2:Parental	-1.55	0.37	-4.22	2.42e-05
	C2:Parental	2.64	0.58	4.51	6.38e-06

Figure 6 – Average frequency of three behaviors (sleeping, feeding, and parental care) for couple F1 – M1 in the parental and non-parental phases.



2.4 DISCUSSION

Chronobiology is an emergent science branch, and recent studies have emphasized the importance of investigating rhythm processes to understand not only animals but nature as a whole (VAN DER VEEN et al., 2017). However, literature regarding this theme comes from laboratory studies, which are limited to capture the environmental complexity within these processes (SCHWARTZ; HELM; GERKEMA, 2017). That is a consequence of the difficulty to assess detailed wild animal behaviors, an even more challenging task for elusive species as carnivores. The hoary fox is a great model for carrying behavioral observations of wild specimens. Originally common to open landscapes of Cerrado, the species is also observed in pastureland agroecosystems, sometimes living close to human houses and domestic animals (BICKLEY et al., 2020). Hoary foxes live in small home-ranges (0.36 – 7.4Km2) (LEMOS, 2016), facilitating animal tracking with no need for large displacements. Despite these, at Limoeiro each hour of ethogram required on average 4.8 hours of field effort. Besides, there was a marked individual variation in tolerating the observer, and the shyest individual (M3) would require 50 hours of field effort to achieve one hour of ethogram. For these reasons, wild carnivore behavior studies are rare, and when carried, usually are limited to few individuals.

Although the hoary fox has been cited as a nocturnal species (COURTENAY et al., 2006; DALPONTE, 2003; JUAREZ; MARINHO-FILHO, 2002), any study has consistently quantified and described its activity pattern. We collected data on foxes movement 24 hours a day and corroborated that the species is nocturnal, with little crepuscular activity and no activity during the day. Temporal niche specialization represents a crucial ecological strategy to respond to variations in biotic, abiotic, and sometimes anthropogenic factors (HUT et al., 2012). Species with similar ecology and morphologies, using the same habitat and resources, may reduce the adverse effects of interspecific competition through partial temporal segregation (RAMESH et al., 2012). However, the temporal niche segregation can be more specific than the usual classification of a species in diurnal, nocturnal, crepuscular, or cathemeral. Our results highlight how a nocturnal species can consistently select particular activity periods during the night. This fine-scale differentiation of activity peaks may be a more efficient mechanism to allow the coexistence of sympatric species (DI BITETTI et al., 2009; MARINHO et al., 2020; ULLAS KARANTH et al., 2017).

The hoary fox is sympatric to three other canids, the bush dog (*Speothos venaticus*, average weight = 6Kg), the crab-eating fox (*Cerdocyon thous*, average weight = 6.5kg), and the maned wolf (*Chrysocyon brachyurus*, average weight = 25 kg) (LEMOS; FACURE, 2011; SILLERO-ZUBIRI, 2009). When occurring sintopically, hoary foxes may overlap the home-range with one or more of these species at a certain degree (LEMOS, 2016). Although it presents a more insectivorous diet, food niche overlap with the not so rare canids (e.g. crab-eating foxes and maned wolves) have been described for several regions (JÁCOMO; SILVEIRA; DINIZ-FILHO, 2004; KOTVISKI et al., 2019; LEMOS; FACURE, 2011). As the smallest canid of the Cerrado (average weight = 3.5kg), the hoary fox faces the highest risk on agonistic interactions, making coexistence mechanisms essential for its survival. Although all three canids are classified as nocturnal (CERUTTI et al., 2021; COURTENAY et al., 2006; MARINHO et al., 2020), maned wolves have activity peaks at sunset (SPANÒ et al., 2021) and can be cathemeral (EMMONS; JOHNSON, 2012) depending on the environment. The crab-eating fox is active throughout the night, with an activity peak at dawn (DI BITETTI et al., 2009). Our study describes that hoary fox activity peak occurs in the early evening hours, between the other larger canids. It could represent a fine-scale temporal niche segregation mechanism and explain the rarity of interspecific interactions observed in our results and the literature (LEMOS, 2016; LEMOS; AZEVEDO; BEISIEGEL, 2004) Therefore, further studies on fine-scale activity patterns may add to our knowledge on temporal niche and species coexistence.

Nevertheless, behavior rhythms are far beyond the understanding of activity patterns. Although many studies classify animal behaviors into active or inactive, more recent studies have shown that periods of activity can be more detailed (DI BITETTI et al., 2009; MARINHO et al., 2020; SPANÒ et al., 2021) with concentrations at particular intervals within the general phase of activity; this allows us to understand the temporal segregation strategies. Here we collected detailed behavior data of a wild carnivore in the field and built statistical models to identify each behavior's daily rhythm and explore its variations between the reproductive phases. Although we recognize the study's limitations due to the number of individuals and encourage repetitions with more individuals, the results obtained have relevance and biological correspondence for the species. The hoary fox is a monogamic canid with an annual reproductive cycle (CANDEIAS et al., 2020; DALPONTE et al., 2018). The couple share the same range through the role year and shelter their offspring from birth to dispersion (parental phase) (COURTENAY et al., 2006). At the non-parental phase, all the active behaviors were concentrated mainly in one activity peak in the first hours of the night (18:00 – 22:00); feeding was the most frequent behavior. It is followed by a rest period (23:00-2:00) and a small increase in activity at the end of the night.

However, at parental phase this marked behavior rhythm suffers important variations. Both male and female play essential roles in parental care (COURTENAY et al., 2006), as do most monogamous canids (MACDONALD et al., 2019). After birth, puppies remain exclusively inside the den for 30 days (DALPONTE, 2003), and parents rest in different shelters (Lemos, F.G. personal communication). Female parental care consists mainly on nursing puppies, while males are responsible for guarding, feeding with prey, and grooming the puppies (COURTENAY et al., 2006; DALPONTE, 2003). In this study, all these parental care behaviors were concentrated between 18:00 and 2:00, impacting the other behaviors rhythms initially concentrated within this period. Hence, the first peak of feeding and locomotion decays to similar probability frequencies as the second smaller peak, and surveillance and low activity behaviors are spread through the night. Besides these behavior rhythm changes, which intensify the bimodal pattern within the night, at the parental phase there were a substantial reduction in the overall frequency of essential behaviors such as feeding and sleeping. Though, when the couple is caring for their offspring, parents rearrange their activity routine and reduce total foraging and resting time.

The combination of increased energetic demand with a decrease in foraging and resting could lead to a critical energetic imbalance that could be life-threatening for both parents and puppies. However, feeding on items with a higher energetic intake during this period could partially compensate for it.

Insects constitute hoary foxes primary diet, and termites are the species main food source, complemented by fruits and small vertebrates (KOTVISKI et al., 2019). Foraging on ground termites is common throughout the year, and it requires extended periods to achieve the necessary energy. However, the period of more intensive parental care coincides with the season of most food abundance for hoary foxes. At the Cerrado, after an extensive dry season, the first rains (from end September to November) mark the transition to the wet season, and termites swarming (PRESTES, 2012). Thus, foxes could increase their energy intake in shorter foraging periods. However, it does not fully compensate for the energy imbalance. Direct observation of hoary foxes during this and a long-term study (Lemos, F.G. personal communication) suggests individuals visually lose weight and fur quality during parental care, probably result from intensive parental care between September and December. As puppies develop, the need to provide food diminishes, puppies forage with their parents, and parental care turns into protection rather than providing food.

Therefore, this reproductive timing is crucial for species fitness and survival (DOMINONI et al., 2017). However, the zeitgebers that regulate this physiological rhythm on hoary foxes are still unknown. Photoperiod influences reproduction rhythms in several mammal species through the melatonin mechanism in the pineal gland, influencing the Hypothalamic-Pituitary-Gonadal (HPG) axis (HAU et al., 2017). Reproduction cycle of African wild dogs (*Lycaon pictus*), for example, varies from seasonal at high latitudes to non-seasonal at low latitudes (MCNUTT; GROOM; WOODROFFE, 2019). Nevertheless, recent studies described the effect of peripheral clock systems in reproduction (MODEL et al., 2015; MONG et al., 2011). They can be entrained with non-photo environmental oscillators such as temperature and food availability. The gray wolf (*Canis lupus*) is a canid species with the reproduction rhythm regulated by factors other than photoperiod since removing their pineal gland was insufficient to interrupt seasonal reproduction (ASA et al., 1987). Hence, food availability could be a potential non-photo environmental oscillator for the reproductive cycle in hoary foxes. However, it has not been studied yet if its reproductive activity is determined by photoperiod or also influenced by other factors.

Therefore, the synchronicity of the hoary fox reproductive phases with environmental conditions may result from a dynamic adaptation process involving a myriad of interactions with biotic and abiotic factors on an evolutionary time scale. However, rapid anthropogenic changes we have been carrying can lead to disruptive effects on this fine adjustment between the species' biological clock and its environment (STEVENSON et al., 2015). Anthropogenic impacts can affect biological rhythms negatively at different levels. They could directly affect external cues by increasing artificial light at night, interfering with important reproductive zeitgebers as photoperiod and lunar cycles (HELM et al., 2017). Additionally, agricultural intensification can affect the daily and seasonal timing of critical parts of food chain. It decreases biodiversity, produces intense soil and water management changes, and generates artificial biomass cycles through manipulated crops. Furthermore, the increasing use of agrochemicals could indirectly act on animals' nervous systems, potentially affecting their clock systems (HORAK; HORN; PIETERS, 2021). Finally, climate changes can affect the biological timekeeping of several species (GIENAPP; REED; VISSER, 2014; HOFFMANN; SGRÒ, 2011; VISSER, 2008), including the hoary fox (GUTIÉRREZ et al., 2019). These alterations are taxonomic substantially variated, leading to changes in the relationships between species and food chain (STEVENSON et al., 2015). Therefore, several anthropogenic mechanisms could lead to a mismatch between the hoary fox parental phase and food availability.

We concluded that the hoary fox is a nocturnal species and described the daily and seasonal fine-scale behavior rhythms. We described how the behaviors can be consistently concentrated at specific times within the species activity period and how these behavior rhythms can substantially vary between the reproductive phases. We suggest meaningful synchronicity between the hoary fox reproductive cycle and environmental conditions such as food availability. However, anthropogenic factors could disrupt this complex and sensible system negatively impacting the species conservation. Therefore, we highlight the importance of further studies unraveling the hoary fox endogenous clocks' machinery and its relation with a dynamic environment. Integrating ecology, chronobiology, and conservation is urgent given the accelerated advance of habitat loss, agricultural intensification, urbanization, and climate change. Hoary foxes and several other species may be running out of time.

BIBLIOGRAPHY

ALBRECHT, U. Timing to Perfection: The Biology of Central and Peripheral Circadian Clocks. **Neuron**, v. 74, n. 2, p. 246–260, 2012.

ASA, C. S. et al. Pinealectomy or superior cervical ganglionectomy do not alter reproduction in the wolf (Canis lupus). **Biology of reproduction**, v. 37, n. 1, p. 14–21, 1987.

BALSALOBRE, A. et al. Resetting of circadian time in peripheral tissues by glucocorticoid signaling. **Science**, v. 289, n. 5488, p. 2344–2347, 2000.

BICKLEY, S. M. et al. Human perceptions of and interactions with wild canids on cattle ranches in central Brazil. **Oryx**, v. 54, n. 4, p. 546–553, 17 jul. 2020.

BRUIN, R. DE; GANSWINDT, A.; ROUX, A. LE. From killer to carer: Steroid hormones and paternal behaviour. **African Zoology**, v. 51, n. 4, p. 173–182, 2016.

BUHR, E. D.; YOO, S.-H.; TAKAHASHI, J. Temperature as a universal resetting cue for mammalian circadian oscillators. **Science**, v. 330, n. 6002, p. 379–385, 2010.

CANDEIAS, Í. Z. DAS et al. First assessment of hoary fox (Lycalopex vetulus) seasonal ovarian cyclicity by non-invasive hormonal monitoring technique. **Conservation Physiology**, v. 8, n. 1, p. 1–12, 2020.

CERUTTI, R. D. et al. Interspecies comparison of daily total locomotor activity between maned wolves (Chrysocyon brachyurus) and domestic dogs (Canis familiaris) maintained in captivity. **Journal of Veterinary Behavior**, v. 43, p. 24–27, 2021.

COLLI, G. R.; VIEIRA, C. R.; DIANESE, J. C. Biodiversity and conservation of the Cerrado: recent advances and old challenges. **Biodiversity and Conservation**, v. 29, n. 5, p. 1465–1475, 2020.

COURTENAY, O. et al. First observations on South America's largely insectivorous canid: The hoary fox (Pseudalopex vetulus). **Journal of Zoology**, v. 268, n. 1, p. 45–54, 2006.

DALPONTE, J. C. **História natural, comportamento e conservação da raposa-do-campo, Pseudalopex vetulu (Canidae)**. [s.l.] Universidade de Brasilia, 2003.

DALPONTE, J. C. Lycalopex vetulus (Carnivora: Canidae). **Mammalian Species**, n. 847, p. 1–7, 2009.

DALPONTE, J. C. et al. Home range and spatial organization by the Hoary Fox Lycalopex vetulus (Mammalia: Carnivora: Canidae): Response to social disruption of two neighboring pairs. **Journal of Threatened Taxa**, v. 10, n. 6, p. 11703–11709, 2018.

DAMIOLA, F. et al. Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. **Genes and Development**, v. 14, n. 23, p. 2950–2961, 2000.

DI BITETTI, M. S. et al. Time partitioning favors the coexistence of sympatric Crab-eating foxes (Cerdocyon Thous) and pampas foxes (Lycalopex Gymnocercus). **Journal of Mammalogy**, v. 90, n. 2, p. 479–490, 2009.

DI MARCO, M. et al. Changes in human footprint drive changes in species extinction risk. **Nature Communications**, v. 9, n. 1, 2018.

DOMINONI, D. M. et al. Methods in field chronobiology. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 372, n. 1734, 2017.

EMMONS, L. H.; JOHNSON, B. C. M. The Maned Wolves of A Chronology of Noel Kempff Mercado Middle Missouri Plains National Park Village Sites Edited by. **Smithson. Contrib. Zool.**, v. 639, n. 639, 2012.

GIENAPP, P.; REED, T. E.; VISSER, M. E. Why climate change will invariably alter selection pressures on phenology. **Proceedings of the Royal Society B: Biological Sciences**, v. 281, n. 1793, p. 20141611, 22 out. 2014.

GÓMEZ, H. et al. Dry season activity periods of some Amazonian mammals. **Studies on Neotropical Fauna and Environment**, v. 40, n. 2, p. 91–95, 2005.

GUTIÉRREZ, E. E. et al. Climate change and its potential impact on the conservation of the Hoary Fox, Lycalopex vetulus (Mammalia: Canidae). **Mammalian Biology**, v. 98, n. August, p. 91–101, 2019.

GWINNER, E. **Circannual Rhythms**. Berlin, Heidelberg: Springer Berlin Heidelberg, 1986. v. 18

HAU, M. et al. Timing as a sexually selected trait: The right mate at the right moment. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 372, n. 1734, 2017.

HELM, B. et al. Two sides of a coin: Ecological and chronobiological perspectives of timing in the wild. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 372, n. 1734, 2017.

HOFFMANN, A. A.; SGRÒ, C. M. Climate change and evolutionary adaptation. **Nature**, v. 470, n. 7335, p. 479–485, 23 fev. 2011.

HOFFMANN, A. A.; SGRÒ, C. M.; KRISTENSEN, T. N. Revisiting Adaptive Potential, Population Size, and Conservation. **Trends in Ecology and Evolution**, v. 32, n. 7, p. 506–517, 2017.

HORAK, I.; HORN, S.; PIETERS, R. Agrochemicals in freshwater systems and their potential as endocrine disrupting chemicals: A South African context. **Environmental Pollution**, v. 268, n. January, p. 115718, jan. 2021.

HUT, R. A. et al. **In search of a temporal niche: Environmental factors**. 1. ed. [s.l.] Elsevier B.V., 2012. v. 199

IRONSIDE, K. E. et al. Quantifying animal movement for caching foragers: the path identification index (PII) and cougars, Puma concolor. **Movement Ecology**, v. 5, n. 1, p. 24, 23 dez. 2017.

JÁCOMO, A. T.; SILVEIRA, L.; DINIZ-FILHO, J. A. F. Niche separation between the maned wolf (Chrysocyon brachyurus), the crab-eating fox (Dusicyon thous) and the hoary fox (Dusicyon vetulus) in central Brazil. **Journal of Zoology**, v. 262, n. 1, p. 99–106, 2004.

JUAREZ, K. M.; MARINHO-FILHO, J. Diet, Habitat Use, and Home Ranges of Sympatric Canids in Central Brazil. **Journal of Mammalogy**, v. 83, n. 4, p. 925–933, 2002.

KLEIMAN, D. G.; EISENBERG, J. F. Comparisons of canid and felid social systems from an evolutionary perspective. **Animal behaviour**, v. 21, n. 4, p. 637–59, nov. 1973.

KOTVISKI, B. M. et al. Trophic niche overlap and resource partitioning among wild canids in an anthropized neotropical ecotone. **Mastozoologia Neotropical**, v. 26, n. 2, p. 368–376, 2019.

KRONFELD-SCHOR, N. et al. Chronobiology of interspecific interactions in a changing world. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 372, n. 1734, 2017.

LEMOS, F. G. **Ecologia e conservação da raposa-do-campo (Lycalopex vetulus) e interações com caídeos simpátricos em áreas antropizadas do Brasil Central**. [s.l.] Universidade Federal de Uberlândia, 2016.

LEMOS, F. G. et al. Lycalopex vetulus. **The IUCN Red List of Threatened Species 2020**, 2020.

LEMOS, F. G.; AZEVEDO, F. C. DE; BEISIEGEL, B. D. M. Lycalopex vetulus. 2004.

LEMOS, F. G.; FACURE, K. G. Seasonal variation in foraging group size of crab-eating foxes and hoary foxes in the Cerrado Biome, Central Brazil. **Mastozoología Neotropical**, v. 18, n. 2, p. 239–245, 2011.

MACDONALD, D. W. et al. Monogamy: Cause, Consequence, or Corollary of Success in Wild Canids? **Frontiers in Ecology and Evolution**, v. 7, n. September, p. 341, 2019.

MARINHO, P. H. et al. Temporal niche overlap among mesocarnivores in a Caatinga dry forest. **European Journal of Wildlife Research**, v. 66, n. 2, 2020.

MCNUTT, J. W.; GROOM, R.; WOODROFFE, R. Ambient temperature provides an adaptive explanation for seasonal reproduction in a tropical mammal. **Journal of Zoology**, v. 309, p. 153–160, 2019.

MODEL, Z. et al. Suprachiasmatic nucleus as the site of androgen action on circadian rhythms. **Hormones and Behavior**, v. 73, p. 1–7, 2015.

MONG, J. A. et al. Sleep, rhythms, and the endocrine brain: Influence of sex and gonadal hormones. **Journal of Neuroscience**, v. 31, n. 45, p. 16107–16116, 2011.

MORENO, C. R. C. The recognition of chronobiology in science. **Sleep Science**, v. 11, n. 1, p. 1, 2018.

NELSON, R. J. Biological Rhythms. In: NELSON, R. J. (Ed.). . **Behavioral Endocrinology**. third ed. [s.l.] Sinauer Associates, 2005. p. 587–667.

PEZUK, P. et al. Circadian organization is governed by extra-SCN pacemakers. **Journal of Biological Rhythms**, v. 25, n. 6, p. 432–441, 2010.

PRESTES, A. C. Padrão de revoadas de cupins (Isoptera) em duas áreas de Cerrado no Brasil Central. p. 48, 2012.

RAMESH, T. et al. Spatio-temporal partitioning among large carnivores in relation to major prey species in Western Ghats. **Journal of Zoology**, v. 287, n. 4, p. 269–275, 2012.

SCHWARTZ, W. J.; HELM, B.; GERKEMA, M. P. Wild clocks: Preface and glossary. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 372, n. 1734, 2017.

SILLERO-ZUBIRI, C. Family CANIDAE (dogs). In: **Handbook of the Mammals of the World**. [s.l: s.n.]. p. 352–446.

SMARR, B. L. et al. Re-examining “temporal Niche”. **Integrative and Comparative Biology**, v. 53, n. 1, p. 165–174, 2013.

SPANÒ, N. et al. Evaluation of the patterns of daily total locomotor activity in maned wolf (Chryosocyon brachyurus) maintained in captivity. **Biological Rhythm Research**, v. 52, n. 3, p. 454–461, 2021.

STEVENSON, T. J. et al. Disrupted seasonal biology impacts health, food security and ecosystems. **Proceedings of the Royal Society B: Biological Sciences**, v. 282, n. 1817, 2015.

ULLAS KARANTH, K. et al. Spatio-temporal interactions facilitate large carnivore sympatry across a resource gradient. **Proceedings of the Royal Society B: Biological Sciences**, v. 284, n. 1848, 2017.

VALENTINUZZI, V. S. et al. Effects of aging on the circadian rhythm of wheel-running activity in C57BL/6 mice. **American Journal of Physiology-Regulatory, Integrative and Comparative Physiology**, v. 273, n. 6, p. R1957–R1964, 1 dez. 1997.

VAN DER VEEN, D. R. et al. Flexible clock systems: Adjusting the temporal programme. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 372, n. 1734, 2017.

VAN SCHAIK, C. P.; GRIFFITHS, M. Activity Periods of Indonesian Rain Forest Mammals. **Biotropica**, v. 28, n. 1, p. 105, mar. 1996.

VISSER, M. E. Keeping up with a warming world; assessing the rate of adaptation to climate change. **Proceedings of the Royal Society B: Biological Sciences**, v. 275, n. 1635, p. 649–659, 22 mar. 2008.

WRIGHT, H. W. Y. et al. Mating tactics and paternity in a socially monogamous canid, the bat-eared fox (Otocyon megalotis). **Journal of Mammalogy**, v. 91, n. 2, p. 437–447, 2010.

SUPPLEMENTARY 1

Table A1- Ethogram of behaviors exhibited by adults hoary foxes. Each category represents a grouped behavior used for the activity patterns analysis.

Category	Behavior	Description
Sleeping	Sleeping	The animal is lying down, the head is touching the ground, or its hind limbs and eyes are closed for more than 15 seconds.
Low activity	Lying	The animal is lying down, the head is touching the ground or its paws back, and the eyes are open.
	Seated	The animal has its hind limbs on the ground and its forelimbs erect.
	Yawning	The animal moves to open and close its mouth; it can be stationary, sitting, or lying down.
	Stretching	The animal has its hind limbs standing and its forelimbs beyond the snout line, stretched out, with its head lowered towards the forelimbs.
	Smelling the air	Animal with its head up sniffing the air.
Feeding	Feeding	The animal is standing still, putting the food in its mouth and chewing the food.
	Foraging	The animal is actively looking for food, with its snout close to the ground.
Locomotion	Walking	The animal is erecting, looking ahead, moving with a walk or trot’s rhythm.
	Running	The animal is in an erect posture, looking ahead, moving with the rhythm of a run.
Surveillance	Patrolling	Withstanding posture, moving with walking rhythm, animals remain with their snout upwards, looking sideways, as if observing the territory while walking.

	Alert	Animal standing still, looking around, ears curved forward.
	Interspecific tolerance	Individual observes another wild animal from a distance, remains stationary and vigilant.
	Interspecific escape	The animal flees the stimulus, typically in the opposite direction, reflex response.
	Interspecific fight	Individuals address the other individual, chases or vocalize, curved body, piloerection, physical contact between individuals, with bites.
	Interspecific vocalization	Stationary individual, erect, vocalizes towards another individual.
Parental care	Feeding	The act of breastfeeding (female) or providing food to the young (male or female bring fruits and small vertebrates to the young).
	Grooming	Parents lick the puppies on different parts of the body.
	Lair with puppies	Parents interact and play with the puppies.
	Chaperoning	Parents remain close to the pups (standing or lying down) and watch the puppies.
Others	Intraspecific greeting	Individuals approach, smell, touch their snouts, lick, or rub against each other. The tails are typically upright.
	Female urinating for male	Standing female urinates when the male approaches, male smells the female’s urine.
	Olfactory exchange	Male and female sniff each other, especially near the genitals.
	Grooming	Self-care, scratching, licking, or shaking the body. The individual can also rollover or rub the body on the floor.
	Defecating	Fox with lower hind limbs and upright tail to expel fecal material.

	Urinating	Female squats and males raise a hind limb to expel urine for more than 3 seconds.
	Marking	Female squats and male raise a hind limb to expel urine for less than 3 seconds.

3. MOVING AROUND TIME: EXPLORING UNDERLYING RHYTHMS ON HOARY FOXES’ (*LYCALOPEX VETULUS*) SPACE USE BEHAVIOR

MOVING AROUND TIME: EXPLORING UNDERLYING RHYTHMS ON HOARY FOXES' (*LYCALOPEX VETULUS*) SPACE USE BEHAVIOR

Isis Zanini das Candeias[1,2], Caio Filipe da Motta Lima[3,2], Fernanda Cavalcanti de Azevedo[4,2], Gustavo Oliveira Santos[5], James Forester[6], Frederico Gemesio Lemos[4,2], Claudio Alvarenga de Oliveira[1]

[1] Departamento de Reprodução Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, SP 05508-270, Brazil

[2] Programa de Conservação Mamíferos do Cerrado, Catalão, GO 75704-020, Brazil

[3] Fundação Parque Zoológico de São Paulo, São Paulo, SP 041301-905, Brazil

[4] Departamento de Ciências Biológicas, Universidade Federal de Catalão, Catalão, GO 75704-020, Brazil

[5] Universidade Federal do Mato Grosso do Sul, Instituto de Biociências, Campo Grande, MS, 79070-900, Brazil

[6] Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota, St. Paul, Minnesota, 55108 USA

ABSTRACT

The home range (HR) is the area used by an individual to develop its normal activities such as feed, mate, care for the offspring, and shelter. The HR behavior results from a dynamic relationship between the individual internal state and the environment. However, the relationship between movement ecology and biological rhythms is still little studied. Here we explored the rhythms related to the hoary fox (*Lycalopex vetulus)* space use behavior. The hoary fox is a small canid endemic to the Brazilian Savanna (Cerrado) open areas, with a small HR, monogamic mating system, and an annual reproductive cycle. We monitored the space use of ten individuals in Central Brazil using GPS collars between 2018 and 2019. We built statistical models to detect rhythmic variations in HR size, fidelity to specific HR regions; shelter displacement; and intra-couple HR overlap. We also estimated the parturition dates of monitored females. Our results showed consistent variations in space use throughout the year. Foxes increased their movement during mating phase, with more extensive HRs and longer shelter displacements. During birth and first care of puppies, HRs and shelter displacement decreased. As the offspring developed, couple overlap decreased, and HRs and individual overlap increased. After offspring dispersal, HRs increased, and the couple overlap increased again, starting a new cycle. Therefore, we have shown that under apparently stable HRs, there are dynamic and rhythmic processes regarding space use and social interactions of hoary foxes, which are related to their annual reproductive cycle. Knowing the HR of a species is essential to understand its biological and ecological needs. In addition, understanding how animals use the space and which rhythmic processes regulate this use can help predict the animals' responses to environmental changes and facilitate the identification of critical points for species conservation.

Keywords: Behavior ecology. Chronobiology. Spatial ecology. Reproductive cycle. Wild canid

3.1. INTRODUCTION

Darwin observed that many animals restricted their movement in areas much smaller than would be expected according to their motor capacity (DARWIN, 1859). This area was later defined as Home Range (HR) “That area traversed by an individual in its normal activities of food gathering, mating and caring for young” (BURT, 1943). He also added that occasional departures from this area should not be considered part of the HR. Space use behavior has significant consequences for many ecological processes such as species distribution and abundance, reproductive system, habitat selection, predator-prey dynamics, and disease dispersion (GRASSEL; RACHLOW; WILLIAMS, 2015; JOHNSON et al., 2010; MCLOUGHLIN; FERGUSON; MESSIER, 2000). Home range studies may also guide species conservation strategies and support the selection of areas for habitat conservation or restoration (BÖRGER et al., 2019; BÖRGER; DALZIEL; FRYXELL, 2008).

Home ranges vary interspecifically, intraspecifically, and even within the same individual over time (POWELL; MITCHELL, 2012). Hence, it is crucial to define the time scale when analyzing home range behavior (BÖRGER et al., 2006; BÖRGER; DALZIEL; FRYXELL, 2008). Thus, HR is established after an individual demonstrates *site fidelity*, defined as an animal's tendency to remain in the same area for an extended period (WHITE; GARROTT, 1990). However, the specific period necessary to establish a stable HR varies among species, and the length of time that location data is collected can have a marked effect on identifying temporal patterns (BÖRGER et al., 2006).

Once the individual is defined as a resident animal, its use of space is often presented as a utilization distribution (UD), which shows the probabilities of where an animal might have been found at any randomly chosen time (POWELL; MITCHELL, 2012). This approach enables to explore different intensities of space use through the HR, emphasizing that animals have not homogeneous use of space within their HR. Therefore, the UD allows to estimate important attributes as apparent preferences over the HR. However, it is usually disregarded that these preferences could vary over time in resident animals. Although fluctuations in HR sizes have been described for many species

(MORELLET et al., 2013; YAN et al., 2017; ZOELLICK; SMITH, 1992), these studies were usually related to migratory animals or animals with extensive HR. Should resident animals that live in small HR have the same space use over time? Are there any rhythmic processes regarding movement behavior on apparently stable HR?

Animals evolve in an essentially unstable environment, dealing with several rhythmic processes such as day and night cycles, seasons, moon phases, and tidal oscillations (KRONFELD-SCHOR et al., 2017). Hence, the behavior entrainment with daily and seasonal environmental cycles is crucial for species survival (DOMINONI et al., 2017), and it is only possible by developing anticipatory mechanisms, the endogenous rhythms (MORENO, 2018). Biological clocks integrate endogenous timekeeping with environmental information to generate internal representations of time so that the organism will be in a particular temporal state at a given moment (HELM et al., 2017). This temporal coordination of biological processes maximizes fitness by optimizing the balance between reproduction, foraging, and predation risk (VAN DER VEEN et al., 2017). Therefore, considering that HR behavior results from the dynamic relationship between the individual internal state and the environment (FORESTER et al., 2007), we should expect a strong association between biological rhythms studied by chronobiology and behaviors described on spatial ecology.

Aiming to expand this knowledge frontier, we explored the relationships between biological rhythms and space use fluctuations in the hoary fox (*Lycalopex vetulus*). The hoary fox is a small canid (2.5 - 4kg), with small HR (average 2.68 km^2), monogamic mating system, and annual reproductive cycle with a markedly seasonal reproductive period (CANDEIAS et al., 2020; DALPONTE, 2009; LEMOS, 2016). Thus, we chose it as a model species to investigate possible underlying rhythmic fluctuations in movement behavior of animals living in apparently stable HR. The hoary fox is endemic to open areas of the Brazilian Savanna (*Cerrado*) (COURTENAY et al., 2006; DALPONTE, 2009; DALPONTE et al., 2018). The Cerrado is one of the 25 wealthiest ecosystems in biodiversity (COLLI; VIEIRA; DIANESE, 2020), but this biome has lost about 46% of its native vegetation area and the remaining areas have been intensely impacted by the expansion of the agricultural frontier (TRIGUEIRO; NABOUT; TESSAROLO, 2020). In this scenario, it is estimated that habitat availability for *L. vetulus* will drastically reduce in no longer than five decades (GUTIÉRREZ et al., 2019). Therefore, understanding the dynamics regarding the species space use will be crucial to estimate its possible responses in a constantly changing world.

We monitored the space use of hoary foxes living in an agroecosystem of Central Brazil using GPS tracking technology. First, we present general HR metrics: 1. Total HR size; 2. HR Core size; 3. Total couple overlap. Then, we estimated different metrics to elucidate how HR use varies over time and built statistical models to detect consistent rhythmic variations among individuals. We hypothesize that: 1. There are significant variations on hoary foxes′ space use along the year. 2. These variations occur cyclically and are related to the reproductive cycle of the species. To test these hypotheses, we estimated fluctuations in: HR size; fidelity to specific HR regions; shelter displacements; and couple HR overlap. Finally, we estimated parturition dates of monitored females, and explored the relationships between space use rhythmic variations and hoary foxes′ reproductive phases.

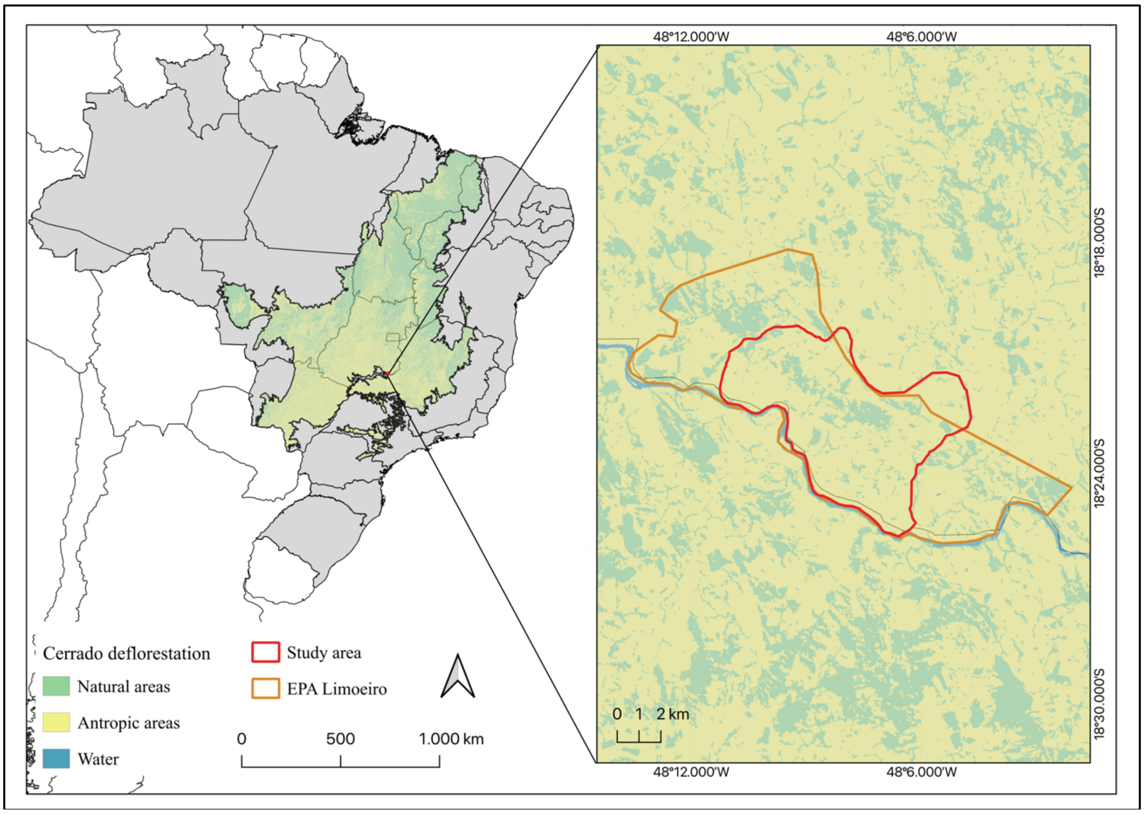
3.2. METHODS

3.2.1. **Study Area**

The study took place in a tropical region known by Limoeiro, municipality of Cumari, state of Goiás, Brazil (Lat -18.382039°/Long -48.117714°) (Figure 1). The Limoeiro region held small and medium-sized private cattle farms. Most part of the study area (90%) was declared a municipal Environmental Protection Area (EPA) in 2017. Despite its creation, there is still no specific management in the EPA toward environmental protection. The area is an ecotone with intrusions of Atlantic Forest in the Cerrado, the predominant biome. More than 70% of the 58km^2 studied was composed of exotic pasture (*Brachiaria* sp.) used for cattle grazing, and there were small patches of crops and forestry (LEMOS, 2016). These human-modified areas forms a mosaic with patches of natural forested vegetation constituted by seasonal and gallery forests

(~21%) and Cerrado *sensu stricto* (woodland savanna; 4%) (LEMOS, 2016). There were no areas of open native vegetation. The climate is tropical savanna (Koppen's AW), constituted by dry fall and winter, with respective mean temperatures of 22.35°C and 22.71°C, and season precipitation of 210mm and 0.4mm; and humid spring and summer, with respective mean temperatures of 24.19 and 24.16, and season precipitation of 650mm and 397mm. The annual temperature ranged from 6.6^{0}C to 36^{0}C. These are the specific climate metrics for the studied region during the studied period (INMET, 2019).

Figure 1 – Study area in Central Brazil, municipality of Cumari, Goias state. The red line delimits the study area, composed of exotic pasture areas and forest remnants.



3.2.2. Data Collection

The study area is home to the Cerrado Mammals Conservation Program (PCMC), a research group that has carried a long-term research and conservation program on the Hoary fox ecology and conservation. The species has been monitored in the region since 2006. Initially, animals were monitored by direct observation and collection of non-invasive samples (LEMOS, 2007; LEMOS;

FACURE; COSTA, 2007). Then, from 2009 to 2016, foxes and other wild canids were monitored by telemetry using VHF collars (LEMOS, 2016; LEMOS; FACURE, 2011; LEMOS; FACURE; DE AZEVEDO, 2011). Finally, for the development of the present study, we monitored the first hoary foxes with GPS technology. To the best of our knowledge, there are no previous studies with GPS tracking on this species.

Animals were captured using box-type traps baited with sardines. We anesthetized hoary foxes with an association of 15 mg/kg of ketamine (Cetamin 10 mg/ml, Syntec, Santana de Parnaiba, SP, Brazil), 0,5 mg/kg of midazolam (Dormire 5mg/ml, Cristália Chemical and Pharmaceutical products, São Paulo, SP, Brazil) and 0,2 mg/kg butorphanol (Torbugesic 10 mg/ml, Fort Dodge Laboratories, Fort Dodge, Iowa, U.S.A.) administered intramuscularly in a single injection into the gluteal muscle. We realized clinical evaluation, weighing, biometrics, and sample collection. Each animal received a numbered ear tag, a subcutaneous microchip, and a GPS collar (Tigrinus®, Timbó, SC, Brazil). After the full anesthetic recovery, we released the foxes in the same spot of capture.

The first capture campaign was carried between March and April 2018, totalizing 60 days of trapping. We installed 20 traps in the area and captured ten hoary foxes (five males and five females). Collar's batteries were planned to last for 12 months; therefore, in March 2019, we performed a second capture campaign with 30 days of trapping. During this period, we exchanged the collars of four individuals.

All the procedures of the wild carnivores followed the American Society of Mastozoology procedures (SIKES; GANNON, 2011). The Chico Mendes Biodiversity Conservation Institute – ICMBIO provided permission for capture and handling (nº 65816-1). The study followed the rules issued by the National Council for Control of Animal Experimentation (CONCEA) and was approved by the Ethics Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/FMVZ) (protocol number CEUA 1778211118).

The foxes' monitoring was carried out over 19 months (March 2018 to September 2019). The GPS radio collars were programmed to collect one location every seven hours, averaging 3.5 fixes per day. We selected this specific interval of time to assure equal data collection from different day hours in the long term. Fixes were registered at the collars' GPS transmitter, though we tracked foxes using VHF telemetry, and every 60 days, we remotely downloaded collected fixes using a UHF receiver (Tigrinus®, Timbó, SC, Brazil).

3.2.3. Data Analysis

There were some differences in the monitoring time of males and females within each couple. Therefore, to calculate total home range (HR) size and HR overlap within couples, we used a subset of locations containing only data with temporal overlap within each couple. We estimated hoary foxes' home range (HR) size calculating the Autocorrelated Kernel Density Estimates (AKDE) (FLEMING et al., 2015). The AKDE estimates the probability density of an animal occurrence based on the individual's own movement parameters, considering the time sequence of the set of locations used. This approach results in more precise home range estimates and eliminates the bias of autocorrelation between fixes, generally associated with other traditional methods (FLEMING et al., 2015) such as the conventional Kernel Density Estimator (WORTON, 1989). We used the R package "ctmm" for HRs analyses (FLEMING; CALABRESE, 2017). The HR and its core were derived from the 95% and 50% isopleth levels of utilization distribution (UD), respectively. We explored sex effect on HRs size with a generalized linear model (GLM), considering each couple ID as a random variable. We used the adehabitatHR R package (CALENGE, 2006) with Bhattacharyya's affinity method (FIEBERG; KOCHANNY, 2005) to estimate the kernel HR overlap between individuals of each couple. This method calculates the general similarity between the utilization distribution (UD), providing a symmetric index with one value for each comparison pair. This index ranges from 0 to 1, where zero indicates non-overlap, and 1 represents a high overlap of areas intensively used by the individuals (FIEBERG; KOCHANNY, 2005).

We selected four metrics to investigate possible variations on how hoary foxes use their home ranges throughout the year: 1. Biweekly home range (BiwHR), it represents the estimated size of areas used in fourteen-day periods and how it varies over periods. We divided the whole year into fortnights, starting from the first day of the year. Then we classified locations within each year's fortnight and calculated the estimated foxes' space use within each fortnight, using the AKDE 95%. 2. Shelter displacement, it represents the variation in shelter fidelity through time. Hoary foxes are mainly nocturnal, with reduced crepuscular activity and no activity during the day (CANDEIAS, 2021; DALPONTE, 2009; LEMOS, 2016) and have been described to use different types of shelters, mainly armadillo holes, besides clump of grass with different sizes and clump of grass with bush (DALPONTE, 2009; LEMOS, 2016). Therefore, we considered that animals were inactive between 9:00h and 15:59h, and locations within this period represented the shelter spot. Once we classified shelter locations, we calculated the Euclidian distances between shelter locations of two consecutive days. We only considered shelter distances between two straight days. 3. Individual overlap, used to quantify the degree of fidelity to the area being used (KERNOHAN; GITZEN; SPAUGH, 2001). For each fox, we calculated the kernel overlap between the UD of two consecutive year's fortnights. We used it to explore the periods when animals concentrated their space use in specific regions within their total HR. 4. Biweekly couple overlap: it represents the variations on how individuals in a couple share the space through the year. We calculated the couple overlap within each years' fortnight. We calculated both overlap metrics using Bhattacharyya's affinity method, described above (FIEBERG; KOCHANNY, 2005). For metrics 1 to 3, we used the entire fixes' database. For metric 4, we used the couple's simultaneous subset.

We used statistical modeling to identify the existence of rhythmic patterns on these four metrics throughout the year. We created four harmonic vectors applying the following formulas to the month of each observation: $s1=\sin(\text{month}.2.\pi/12)$; $s2=\sin(\text{month}.4.\pi/12)$; $c1=\cos(\text{month}.2.\pi/12)$; $c2=\cos(\text{month}.4.\pi/12)$. We used Linear mixed-effects models ('nlme' R package), one for each of the following dependent variables: BiwHR, shelter displacement, individual overlap, and biweekly couple overlap. We considered the four harmonics (s1, s2, c1, c2) as independent variables. By modeling the combination of these four harmonic curves, we allowed the model to adjust the best from a vast possibility of curves, including a flat line if there was no behavior variation through time. We included the individual ID as a random intercept to consider individual variations on size effect. We included time and individual ID in the within-group correlation structure of the model and checked the autocorrelation regarding these variables. In the BiwHR model, we also included the number of fixes of each fortnight as a dependent variable to control its possible influence on the results. We investigated the assumptions of the fitted model by checking for the normal distribution of the residuals using histograms and normal Q-Q plots. Finally, we extracted the fitted model's prediction values. We used the software R (R Development Core Team, 2021) for all the statistical analyses. To interpret models' outputs, we used as reference a general estimate of the hoary fox' reproductive phases: Mating (May to mid-July); Gestation (end of June to mid-September), First care (mid-August to late-October); Parental care (late-October to end of March); and Offspring dispersal (April to early May). First care represents a period of intensive parental care, when offspring's demands are higher; that is why we highlighted this period separated from the following parental care.

We calculated the fluctuations on failure events of GPS data collection to estimate parturition dates of monitored females. Missing GPS positions and subsequent data loss are expected for GPS radiotracking. It can occur randomly or with consistent patterns related to specific animal behaviors (WALTON; MATTISSON, 2021). Denning behavior, for example, may lead to failure to acquire GPS fixes. Hence, since hoary foxes give birth inside armadillo burrows (COURTENAY et al., 2006; DALPONTE, 2009), we used gaps originated from unsuccessful GPS fixes to identify parturition events. The same approach was previously used in another species with similar underground parturition behavior, the red fox (*Vulpes vulpes*) (WALTON; MATTISSON, 2021). We calculated the number of successful GPS fixes in five-day clusters to reduce the effects of single days with random low fix success rates. After calculating the fixes' success in the first cluster, we moved it in one day until the end of the sampling period (e.g., days 1 to 5; 2 to 6; 3 to 7, etc.). Thus, we could identify the five-day cluster with the highest gap in GPS collection, suggesting the parturition event. We confirmed parturition events by finding active den sites or through subsequent observation of pups in the field.

3.3. RESULTS

We monitored ten hoary fox individuals (five males and five females) for 19 months with GPS collars totalizing 8,486 locations between March 2018 and September 2019, with an average of 848 locations per individual (range: 235 - 1357) (Table 1). Eight of these monitored individuals were couples (C1, C2, C3, and C4), and three of these couples had litters during monitoring (C1, C2, C3). Although we could observe that M5 had a female and also had offspring during the study, we could not capture his female to install the GPS collar. Mean HR size was 1.59 km^2 (range: 0.52 km^2 – 3.13 km^2), and mean core area was 0.31km^2 (range: 0.08 km^2 - 0.62 km^2) (Figure 2). Males had slightly larger HRs than females in all couples (Figure 3). However, we did not detect significant effect of sex on HR sizes ($p > 0.05$). Home range overlap between individuals of the same couple ranged from 0.87 to 0.93 (mean: 0.89). Two individuals lost their respective pairs during monitoring (M4 and F2). F4 was preyed by a puma (*Puma concolor*) in August 2018, and M2 was no longer found after October 2018.

Biweekly space use ranged between 0.08 km^2 – 4.95 km^2 (mean: 1.17 km^2; SD 1.0). Shelter displacement ranged from 0.34 m to 2,472.48 m (mean: 375.18m; SD 411.2m). Individual overlap ranged from 0.57 to 0.93 (mean: 0.82; SD 0.07). Biweekly intra-couple overlap ranged from 0.64 to 0.9, with a mean of 0.81 (SD 0.06) (Figure 4). Linear mixed-effects models identified non-linear variations of the four metrics, evidencing consistent rhythm patterns among individuals (Table 2). The size of BiwHR was maximum at the pre-mating phase (April-May), decreased during mating, and was minimum through offspring birth and first care (August-September). After this period, the BiwHR increased as the puppies became more independent, and reached the maximum size again after puppies' dispersal (April-May). Shelter displacement presented a peak in the middle of the mating phase (June), followed by a sharp decrease and reaching the lowest values at puppies' birth (September). After first care, it increased, reaching a second peak in December, followed by a slighter decrease at the end of the parental care phase (March-April), until it increases again, ending the cycle at the mating phase peak. Individual overlap was maximum in the middle of the mating phase (June), decreased during birth (September), and wavered up in December but with lower values than the mating phase peak. It decreased again at the end of the parental care (March) and progressively increased at the pre-mating phase. Biweekly couple overlap remained in a plateau of high coefficients from offspring dispersal to first care (April-September). At the end of first care phase, it started to decrease until it reached the lowest values in December and January and progressively increased again from February to March (Figure 5).

The GPS collars were programmed to collect one location every seven hours. Therefore, each five-day cluster should have 17 or 18 fixes. In general, there was a high success in acquiring GPS fixes. Previous to the parturition the success per five-day cluster were constantly high (F1 median: 99.9%, min: 64%; F2 median: 99.1%, min: 64%; F3 median: 0.99.1%, min: 70%). All monitored females presented an abrupt decrease in fix collection at the parturition phase, collecting about a third of the expected locations for the period (F1: 23.3%; F2: 35%; F3: 27.6%). This gap in data collection may be due to the increased time inside burrows during parturition and postpartum periods. Therefore, we were able to infer parturition periods of the three monitored females that had offspring by detecting the five-day cluster with the lowest data collection success. Parturition periods were: F1, September 13-17; F2: September 10-14; F3: August 19-23. After presumed parturition, data collection slowly increased its success, but smaller gaps still occurred during the postpartum phase (Figure 6). We corroborated this result with the field encounter of 2 litters from these females and another litter from male 5 (with a non-monitored female) in the following months.

Table 1 –Spatial parameters of Hoary foxes (*Lycalopex vetulus*) monitored at the Limoeiro region, municipality of Cumari, Goiás, between 2018 and 2019. Home range area (HR) and its core. ML represents the model predicted area, and the Low and High values are the area with and without the confidence interval.

Couple	Id	Sex	Weight (kg)	Sampling period (days)	N Locations	HR (km^2) AKDE 95%			Core (km^2) AKDE 50%		
						Low	ML	High	Low	ML	High
1	F1	Female	4.06	433	1357	0.48	0.52	0.55	0.08	0.09	0.10
	M1	Male	3.9	261	827	0.52	0.56	0.60	0.07	0.08	0.08
2	F2	Female	3.85	537	1828	0.83	0.93	1.04	0.16	0.18	0.20
	M2	Male	3.76	125	394	1.39	1.55	1.71	0.22	0.25	0.27
3	F3	Female	3.48	242	676	2.84	3.11	3.40	0.55	0.60	0.66
	M3	Male	4.19	235	782	2.88	3.13	3.39	0.57	0.62	0.68
4	F4	Female	3.4	72	235	0.65	0.75	0.86	0.16	0.19	0.22
	M4	Male	3.3	303	798	0.82	0.95	1.09	0.21	0.24	0.28
-	F5	Female	3.42	171	771	1.16	1.31	1.47	0.31	0.35	0.39
-	M5	Male	3.4	240	818	2.75	3.10	3.47	0.49	0.55	0.62

Figure 2 – *Lycalopex vetulus* home ranges areas estimated by AKDE with 95% isopleth level (light gray) and Core area using 50% isopleth level (dark gray); red lines are the confidence range. Each map represents an individual; Legend: F indicates females, M males, and C couple; individuals F5 and M5 were not a pair and both had respective pairs not monitored.

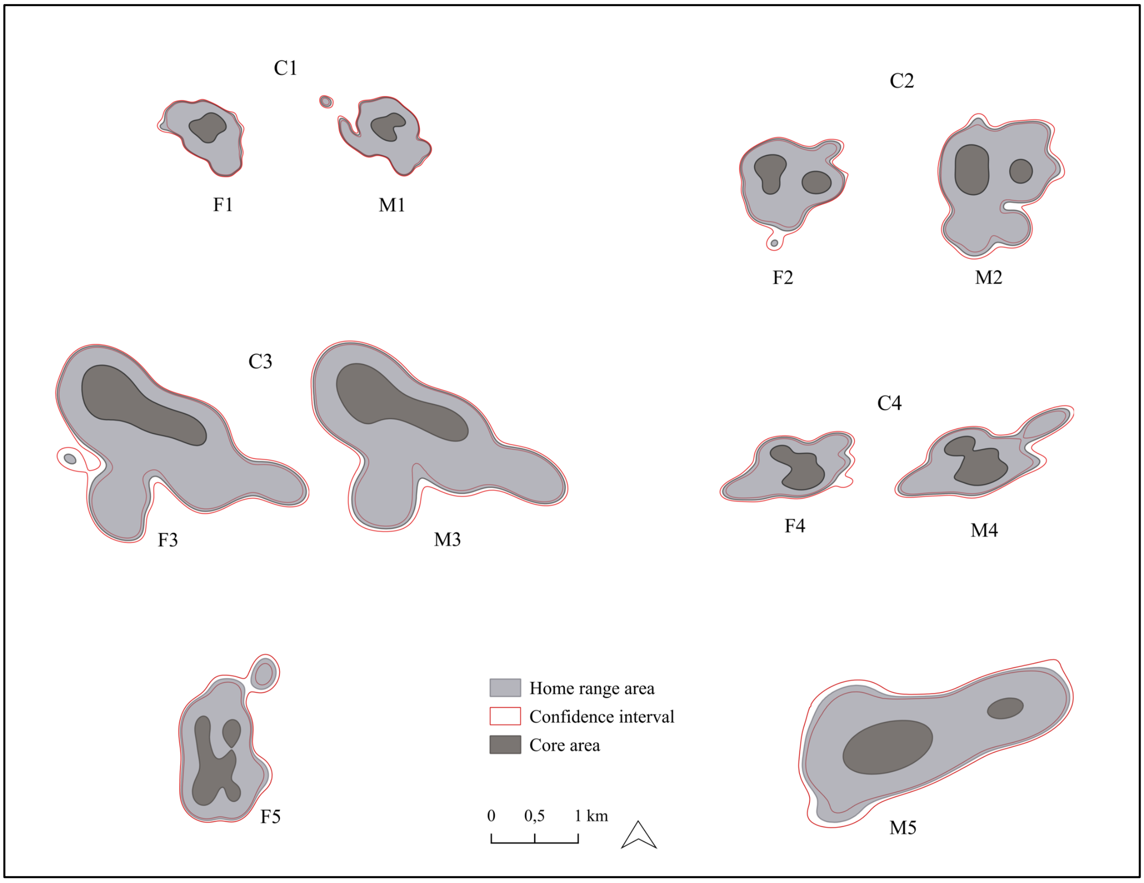


Figure 3 - *Lycalopex vetulus* home ranges (km2) of simultaneous data of each couple. Each dot represents an individual; red dots represent females and blue dots males; Cs identifies couples; vertical bars are confidence intervals.

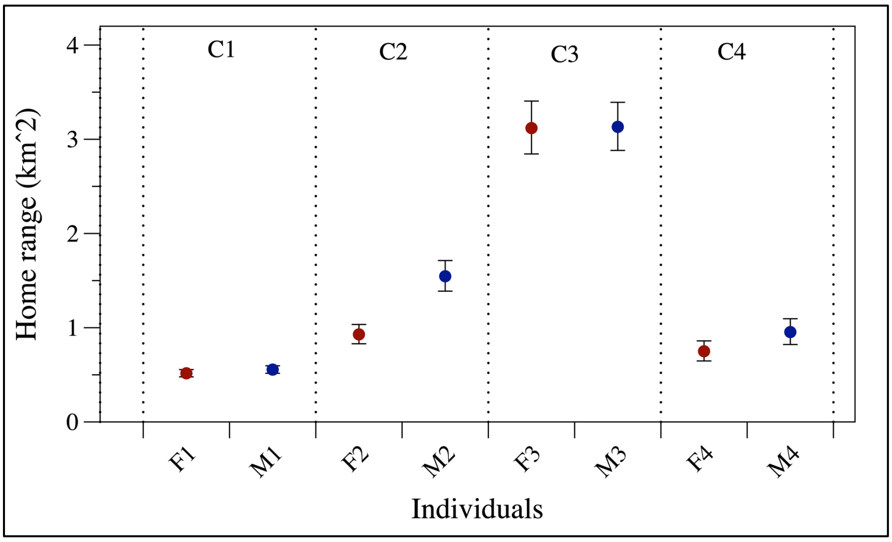


Figure 4 – Boxplot panel with *Lycalopex vetulus* metrics: (A) - Biweekly home ranges (HR) (km2) of each individual; (B)- Overlap coefficient from biweekly HR for each individual; (C)- Shelter displacement (m); (D)- Couple overlap coefficient, which uses individual biweekly HR of simultaneous data for each couple. Red color indicates females and blue males; vertical dots lines separate couples, and C identifies those; F5 and M5 were not a pair.

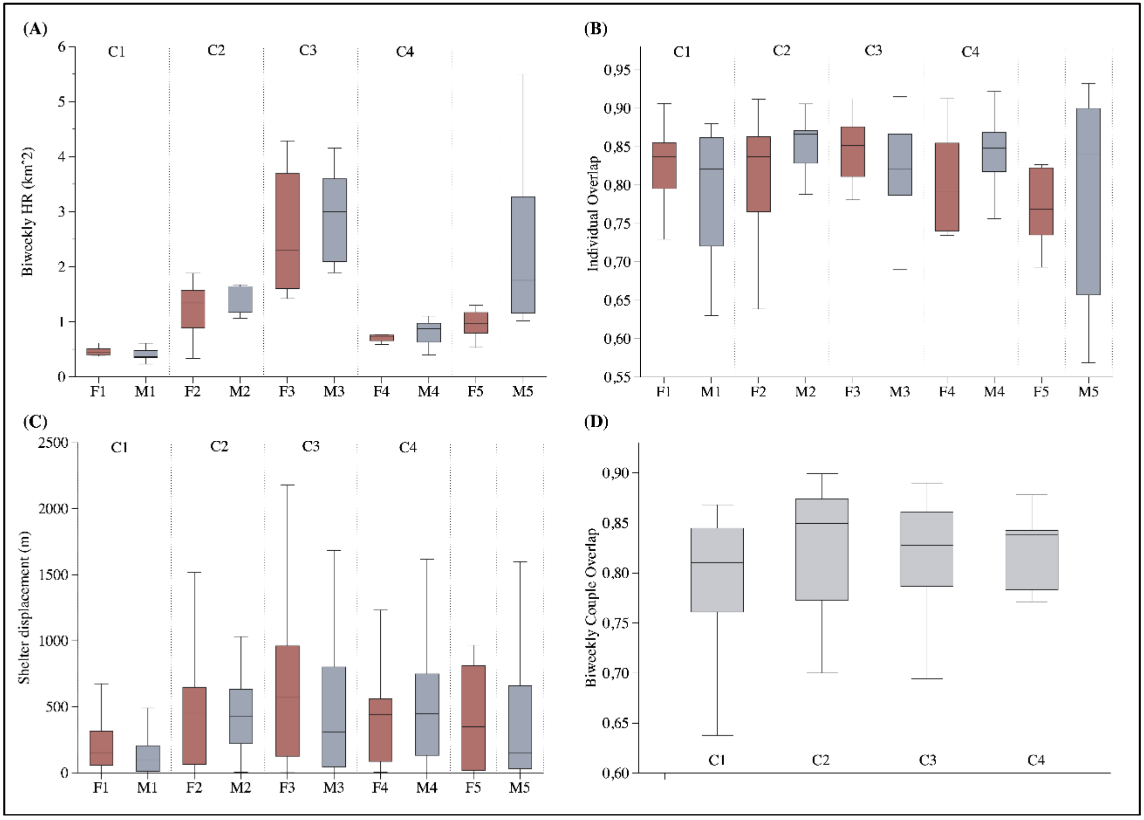


Figure 5 - Model annual prediction for the variables: home range, shelter displacement, intra-individual overlap; and couple overlap. Dashed lines represent the model's confidence interval; vertical range colors and dotted lines mark the different phases of an annual hoary fox reproductive cycle.

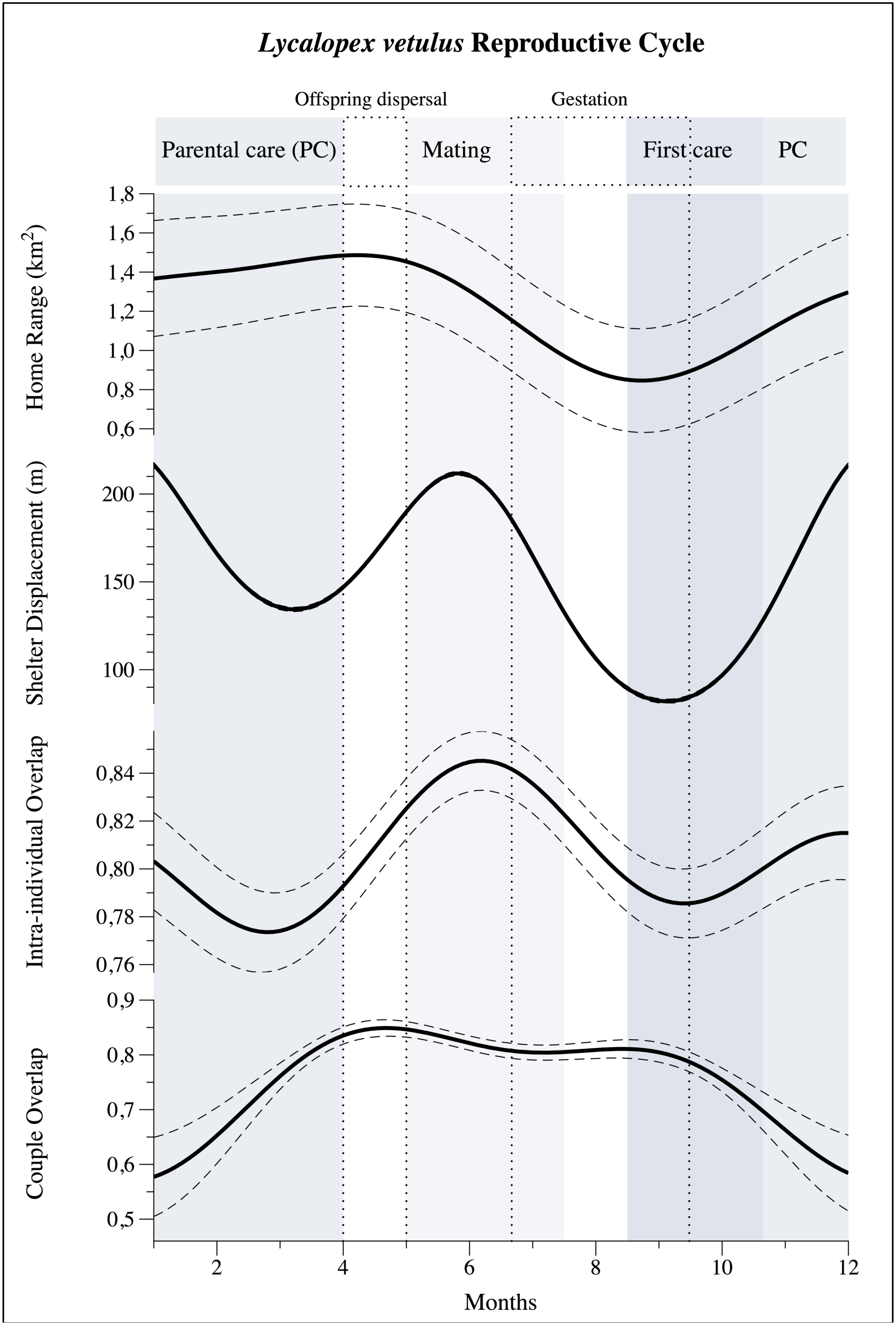
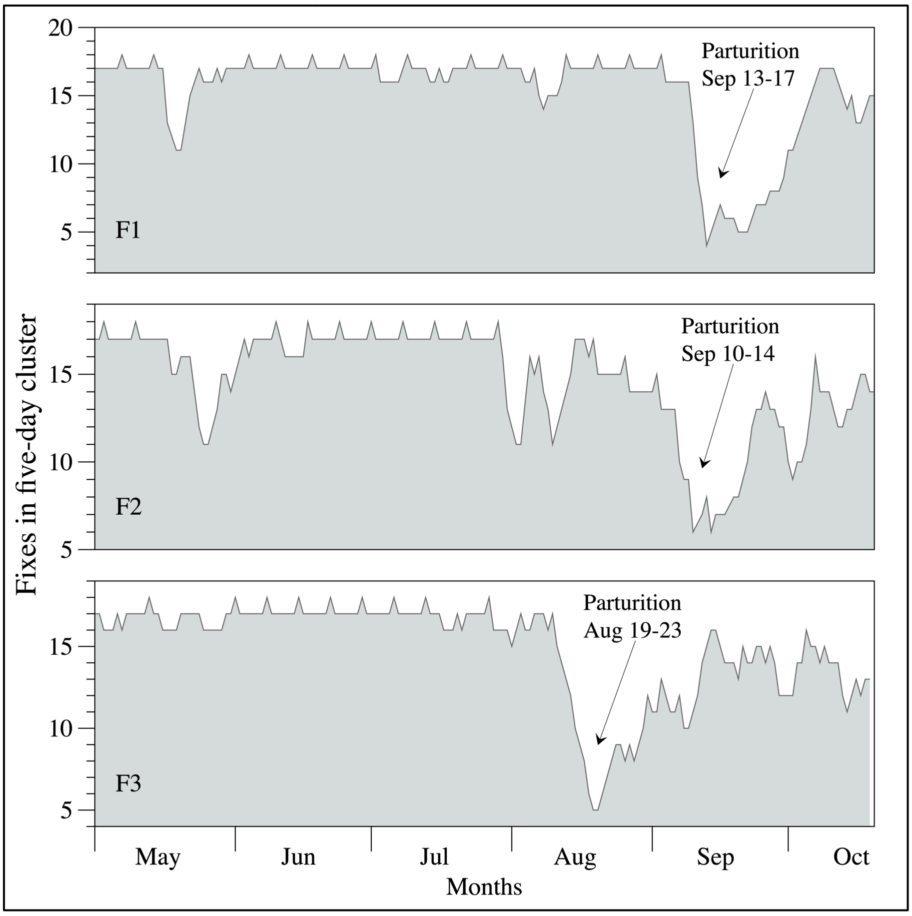


Table 2 – Model coefficients of spatial parameters in hoary foxes (*Lycalopex vetulus*) with the intercept and the interaction with each of the four harmonic vectors.

		Value	**Std. Error**	**DF**	**t-value**	**p-value**
BiwHR	Intercept	0.72	0.30	177	2.41	0.02
	S1	0.30	0.07	177	4.27	0.00
	C1	-0.00	0.09	177	-0.04	0.97
	S2	-0.05	0.07	177	-0.66	0.51
	C2	0.08	0.07	177	1.02	0.31
Shelter Displacement	Intercept	5.01	0.16	1776	32.16	0.00
	S1	0.25	0.07	1776	3.49	0.00
	C1	0.01	0.08	1776	0.18	0.86
	S2	0.06	0.07	1776	0.92	0.36
	C2	0.35	0.07	1776	5.20	0.00
Individual Overlap	Intercept	0.81	0.01	166	81.40	0.00
	S1	-0.01	0.01	166	-0.79	0.43
	C1	-0.01	0.01	166	-1.56	0.12
	S2	0.00	0.01	166	0.26	0.80
	C2	0.02	0.01	166	2.97	0.00
Couple Overlap	Intercept	0.74	0.02	44	36.44	0.00
	S1	-0.02	0.02	44	-1.35	0.19
	C1	-0.12	0.03	44	-3.57	0.00
	S2	-0.04	0.02	44	-2.22	0.03
	C2	-0.04	0.02	44	-2.09	0.04

Figure 6 – Successful GPS fixes collection of females hoary foxes (*Lycalopex vetulus*) monitored with a schedule of 1 location every 7 hours.



3.4. DISCUSSION

Knowing the HR of a species is essential to understand its biological and ecological needs, such as how much area is necessary to assure long-term species survival. In addition, understanding how the animal uses its space and which rhythmic processes regulate this use can facilitate identifying the animals' responses to environmental changes. This data can help design conservation and management plans for endangered species such as the hoary fox.

There are few references of HR studies for hoary foxes. The first estimated HR areas of individuals from southwest of Bahia state (2.08 km^2 and 3.85 km^2, N=2), using the Minimum Convex Polygon (MCP) estimator (JUAREZ; MARINHO-FILHO, 2002). COURTENAY et al., 2006 estimated the HR of a couple in the western of Minas Gerais state (4.2 km^2 and 4.56 km^2, N=2), through the Restricted Polygon method. DALPONTE et al., 2018 studied hoary foxes in eastern Mato Grosso, estimating an average HR of 2.01 km^2 (1.4 km^2 - 2.4 km^2 range, N=4) with MCP. The study with the largest sample of monitored hoary foxes was carried in Limoeiro region, the same area of the present study and estimated an average HR of 2.68 km^2 (0.36 km^2 – 7.39 km^2 range, N=23), estimated with AKDE (LEMOS, 2016).

All these studies were carried on private farms within human-modified landscapes, and all monitored animals with VHF telemetry. Here, we present the first HR estimates from GPS tracking for hoary foxes. Total HRs ranged from 0.52 km^2 to 3.13 km^2 (mean = 1.59 km^2, N=8), which is within values previously described for the species. There was a considerable variation in HR size between couples, with differences of up to 502% between them. However, HRs of individuals within each couple were similar, and there were no significant differences regarding sex. Therefore, this similarity of HR sizes within individuals of the same couple and the heterogeneity between couples corroborate the hypothesis that hoary foxes are monogamic and share their ranges, which may vary between couples and different season of the couple as suggested by LEMOS, 2016.

The HR size of an animal may vary depending on individual characteristics, such as body size and sex (GITTLEMAN; HARVEY, 1982), or due to environmental conditions, such as habitat productivity and prey availability (HARESTAD; BUNNEL, 1979). There is no sexual dimorphism in hoary foxes, and both sexes have similar body mass (mean = 3.5kg) (LEMOS, 2016). Body mass were also similar between different couples of this study. Therefore, HR heterogeneity may be related to resource heterogeneity between areas, such as food and shelter availability. Thus, we highlight the importance of further studies to explore underlying factors that drive HR sizes, since they varied significantly even within a relatively small study region (58 km^2).

Home ranges represent the spatial expression of behaviors that animals perform to survive and reproduce (BURT, 1943), and thus, studies on HRs constitute part of the basic knowledge of a species. However, most studies focus on establishing the limits of stable HRs during a given period, assuming that the use of different portions of these areas is homogeneous over time. Through the metrics calculated here and the proposed modeling approach we demonstrated that even for stabilized resident individuals of a species with small HRs, it is possible to observe consistent differences on how animals use their total HR over time. Besides, results showed that these variations on space use throughout the year are not random among individuals. Fluctuations detected by the models suggest that, regardless the differences in HR size between individuals, spatial metrics varies consistently on specific periods of the year, evidencing an underlying rhythmic process that is aligned with the hoary fox annual reproductive cycle.

From the mating phase through gestation, the size of the used area, expressed by the BiwHR, progressively decreased, reaching its minimum values during parturition and first care phases. After birth, hoary foxes' puppies remain exclusively inside the den for approximately 30 days and require intensive parental care for at least two months (COURTENAY et al., 2006; DALPONTE, 2009). Although parents stay in different dens from the puppies, they frequently visit them, the female for nursing and the male for guarding and caring for them, reducing the parents foraging time (COURTENAY et al., 2006). Hence, the significant reduction on BiwHR during this period probably results from the demand of being around the offspring den. Once puppies start to leave the den and explore further, the BiwHR progressively increases, reaching its maximum values on offspring dispersal, when parents could freely explore their total HR without parental care activities.

These intensive requirements during the first care phase could also explain the marked decrease in shelter displacement during this period. The most common shelters are dense bushes and armadillo burrows (LEMOS, 2016). These burrows represent an essential resource for hoary foxes' reproduction since they are used as parturition dens (COURTENAY et al., 2006; DALPONTE, 2009). The dynamics of shelters are still little studied for hoary foxes. Other small canid species such as the swift fox (*Vulpes velox*), kit fox (*Vulpes macrotis*), and arctic fox (*Vulpes lagopus*) also shelter in dens (TANNERFELDT; ELMHAGEN; ANGERBJÖRN, 2002). Swift fox dens are usually clustered, and the distances between successive dens are around 200 and 500m (MOEHRENSCHLAGER; MOEHRENSCHLAGER, 1999). Kit foxes use between 4-5 dens per month, and average displacement between successive dens is about 700m (RALLS et al., 1990). There are usually 2-3 dens of potential parity within an arctic fox territory and many smaller ones (satellites) (TANNERFELDT; ELMHAGEN; ANGERBJÖRN, 2002). Our study accessed hoary foxes' shelter dynamics by calculating the displacement between shelters of two consecutive days. Models predicted the longest displacements to occur in June (~ 210m), peak of the mating phase, and between December and January (~ 220m), when puppies are no longer restricted to the den. Therefore, the parents are able to change the sleeping point more frequently. Higher fidelity to the shelter occurred in September (~ 80m), around parturition events. We should consider that when animals are inside burrows, errors related to GPS locations increase. Therefore, predicted displacements during parturition and first care should be even smaller than we predict.

GPS tracking data has been used to study reproductive behaviors and calving on several species (DEMARS et al., 2013; PICARDI et al., 2020; WIESEL; KARTHUN-STRIJBOS; JÄNECKE, 2019). These events often result in changes on animals' movement behavior (EDELHOFF; SIGNER; BALKENHOL, 2016; GURARIE et al., 2016; WRIGHT et al., 2010), generally a drop in the movement rate (BONAR et al., 2018; DEMARS et al., 2013). However, for animals with calving and first care inside dens, movement assessment is inaccurate due to a marked loss of GPS fixes in this period (WALTON; MATTISSON, 2021). Although this missing data made it difficult to explore the hoary fox movements during this period, we used these gaps during data collection as a source of information to identify parturition events. By calculating the GPS success in five-day clusters, we were able to identify consistent missing patterns related to the parturition events, and we could estimate the delivery dates of all monitored females that reproduced during the study. It is challenging to detect parturition events in the field accurately, and it has not been made before for hoary foxes. We found two of these litters (F1 and F3) between October and November, and in December, we saw that F2 was lactating, but we could not observe offspring in the area. Hence, this report contributes to the species' reproductive knowledge, increasing our knowledge of the natural history of hoary foxes. We encourage that future studies use the same method to increase the number of reported birthdates since knowing the reproductive aspects of an endangered species is essential to design conservation and management plans in situ and ex-situ.

However, although animals concentrated their use around puppies’ den during parturition and first care, reducing the BiwHR sizes and shelter displacements, it does not mean that they were using exactly the same regions during this period. Individual overlap expressed how much animals moved their BiwHR from one fortnight to another, detailing displacements on space use occurred within the animals’ total HR. In general, the model estimated high values of individual overlap throughout the year (0.77 - 0.84 range), expected for a species with relatively small and stable HR. However, it consistently varied through seasons. Highest values occurred at the mating phase, followed by a reduction at first care. It means that, during first care, even though animals were using smaller areas, centered at the puppies’ dens, these areas were slightly shifting on space. It could be explained because once the animals cannot forage in extensive areas during this period, they could improve resources gathering by dislocating their foraging area around dens. Nevertheless, we should consider in our interpretation that since animals have smaller BiwHRs during this phase, small displacements would lead to higher differences in the individual overlap. That is a different scenario from the end of the parental care phase (March) when the second drop in individual overlap was associated with the highest BiwHRs. It means that after a long period of exploring the areas around the birth den, the parents not only covered larger areas, but also increased the displacements of these explored areas along the fortnights once puppies have grown. After offspring dispersal, they progressively reduced this BiwHR movement, increasing individual overlap values and restarting the cycle at mating phase.

We also explored how hoary foxes shared their HRs with their pairs. They presented similar HR contours and a high total couple overlap (mean = 0.89, range = 0.87 – 0.93). No sexual dimorphism, similar HR sizes between sexes, and high HR overlap are common characteristics among monogamous canids (DOBSON; WAY; BAUDOIN, 2010). Unlike most monogamous species that practice seasonal monogamy, with a short-time pairing, canids often maintain long-term cooperative and affiliative pair bonds and typically remain with the same partner until the death of one of them (MACDONALD et al., 2019). Hoary foxes had stable pairs throughout the year, and the couple intensively shared their HRs. However, our results on biweekly couple overlap showed that this intensity on HR sharing varied through the year. Although the model predicted high couple overlaps during most of the year, it markedly decreased during the parental care phase, when overlap values reduced by up to 35% between December and January.

That is the period when hoary foxes' puppies are weaned and start to forage, mainly with the male. Then, the time invested by the female in raising the puppies decreases while the male's investment remains relatively constant throughout parental care phase (COURTENAY et al., 2006). The female needs to reestablish the energy expended during pregnancy and breastfeeding the litter, which would be difficult sharing the same foraging areas with the male and the grown offspring. Therefore, the decrease on couple overlap during this phase is probably because females were exploring different foraging areas. Hence, both parents play fundamental roles throughout parental care, and both are essential for the offspring's success. Paternal care is rare among mammals, occurring in only 5-10% of species, but it is practically the rule in canids (MACDONALD et al., 2019). Despite this cooperative behavior, hoary fox couples are rarely observed foraging together (CANDEIAS, 2021; LEMOS; FACURE; AZEVEDO, 2011), leading them to be recurrently classified as a solitary species (DALPONTE, 2009). However, we should emphasize that although they are solitary foragers, our data regarding space use and parental care highlight the intensive social interaction between foxes in a couple as suggested by LEMOS, 2016.

Here, we bridged a little explored frontier between the spatial ecology and chronobiology on a poor-known Neotropical canid, the hoary fox. Although our results carry the limitations of a reduced number of individuals on a single species, our methodological approaches brought insights that could expand our understanding of canids ecological behavior. We have shown that under apparently stable HRs, there are dynamic and rhythmic processes regarding space use and social interactions of hoary foxes. Therefore, we encourage that future studies replicate the methods proposed here to improve our understanding of the underlying processes regarding HRs. BÖRGER et al., 2019 signalized that new HR studies should go beyond obtaining a metric and focus on answering why animals move the way they move by investigating how behavioral and ecological processes influence the movement of animals. In the present study, we contributed to this direction by describing detailed rhythmic variations on hoary foxes' movement behavior and demonstrating how it is closely related to the species' reproductive biology.

Understanding how animals interact with their essentially unstable environment will facilitate the identification of critical points for species conservation. Although environmental instability has ever been part of nature, climate change, agriculture intensification, and urbanization are leading to accelerated changes in natural biological rhythms in the last decades. Thus, the human footprint on planet, a mark of the Anthropocene, can disrupt the behavior synchronization of animals with their environment. Therefore, exploring animal behavior rhythms is critical to understand and predict the species responses to these changes. Hence, integrative approaches like we used in this study can subsidize conservation strategies that mitigate negative impacts to wildlife and facilitate coexistence in a fast-changing world.

BIBLIOGRAPHY

BONAR, M. et al. Implementing a novel movement-based approach to inferring parturition and neonate caribou calf survival. **PLOS ONE**, v. 13, n. 2, p. e0192204, 21 Feb. 2018.

BÖRGER, L. et al. An integrated approach to identify spatiotemporal and individual-level determinants of animal home range size. **American Naturalist**, v. 168, n. 4, p. 471–485, 2006.

BÖRGER, L. et al. Animal Home Ranges: Concepts, Uses, and Estimation. In: MURRAY, D. L.; SANDERCOCK, B. K. (Eds.). . **Population Ecology in Practice**. First Edit ed. [s.l.] John Wiley & Sons Ltd., 2019. p. 315–332.

BÖRGER, L.; DALZIEL, B. D.; FRYXELL, J. M. Are there general mechanisms of animal home range behaviour? A review and prospects for future research. **Ecology Letters**, v. 11, n. 6, p. 637–650, 2008.

BURT, W. H. Territoriality and Home Range Concepts as Applied to Mammals. **Journal of Mammalogy**, v. 24, n. 3, p. 346, 1943.

CALENGE, C. The package “adehabitat” for the R software: A tool for the analysis of space and habitat use by animals. **Ecological Modelling**, v. 197, n. 3–4, p. 516–519, 2006.

CANDEIAS, Í. Z. DAS et al. First assessment of hoary fox (Lycalopex vetulus) seasonal ovarian cyclicity by non-invasive hormonal monitoring technique. **Conservation Physiology**, v. 8, n. 1, p. 1–12, 2020.

CANDEIAS, Í. Z. DAS. **The time on hoary foxes clock: Uncovering daily and seasonal fine-scale behavior rhythms of a wild canid**. [s.l.] Universidade de São Paulo, 2021.

COLLI, G. R.; VIEIRA, C. R.; DIANESE, J. C. Biodiversity and conservation of the Cerrado: recent advances and old challenges. **Biodiversity and Conservation**, v. 29, n. 5, p. 1465–1475, 2020.

COURTENAY, O. et al. First observations on South America’s largely insectivorous canid: The hoary fox (Pseudalopex vetulus). **Journal of Zoology**, v. 268, n. 1, p. 45–54, 2006.

DALPONTE, J. C. Lycalopex vetulus (carnivora: Canidae). In: **Mammalian Species**. n. 847, p. 1–7, 2009.

DALPONTE, J. C. et al. Home range and spatial organization by the Hoary Fox Lycalopex vetulus (Mammalia: Carnivora: Canidae): Response to social disruption of two neighboring pairs. **Journal of Threatened Taxa**, v. 10, n. 6, p. 11703–11709, 2018.

DARWIN, C. **On the Origin of Species by Means of Natural Selection, or Preservation of Favoured Races in the Struggle for Life**. London, UK:

[s.n.].

DEMARS, C. A. et al. Inferring parturition and neonate survival from movement patterns of female ungulates: a case study using woodland caribou. **Ecology and Evolution**, v. 3, n. 12, p. 4149–4160, 23 Oct. 2013.

DOBSON, F. S.; WAY, B. M.; BAUDOIN, C. Spatial dynamics and the evolution of social monogamy in mammals. **Behavioral Ecology**, v. 21, n. 4, p. 747–752, 2010.

DOMINONI, D. M. et al. Methods in field chronobiology. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 372, n. 1734, 2017.

EDELHOFF, H.; SIGNER, J.; BALKENHOL, N. Path segmentation for beginners: an overview of current methods for detecting changes in animal movement patterns. **Movement Ecology**, v. 4, n. 1, p. 21, 1 Dec. 2016.

FIEBERG, J.; KOCHANNY, C. O. Quantifying Home-Range Overlap: The Importance of the Utilization Distribution. **The Journal of Wildlife Management**, v. 69, n. 4, p. 1346–1359, 12 Sep. 2005.

FLEMING, C. H. et al. Rigorous home range estimation with movement data: a new autocorrelated kernel density estimator. **Ecology**, v. 96, n. 5, p. 1182–1188, May 2015.

FLEMING, C. H.; CALABRESE, J. M. A new kernel density estimator for accurate home-range and species-range area estimation. **Methods in Ecology and Evolution**, v. 8, n. 5, p. 571–579, 28 May 2017.

FORESTER, J. D. et al. State-space models link elk movement patterns to landscape characteristics in Yellowstone National Park. **Ecological Monographs**, v. 77, n. 2, p. 285–299, 2007.

GITTLEMAN, J. L.; HARVEY, P. H. Carnivore home-range size, metabolic needs and ecology. **Behavioral Ecology and Sociobiology**, v. 10, n. 1, p. 57–63, Feb. 1982.

GRASSEL, S. M.; RACHLOW, J. L.; WILLIAMS, C. J. Spatial interactions between sympatric carnivores: asymmetric avoidance of an intraguild predator. **Ecology and Evolution**, v. 5, n. 14, p. 2762–2773, 19 Jul. 2015.

GURARIE, E. et al. What is the animal doing? Tools for exploring behavioural structure in animal movements. **Journal of Animal Ecology**, v. 85, n. 1, p. 69–84, 23 Jan. 2016.

GUTIÉRREZ, E. E. et al. Climate change and its potential impact on the conservation of the Hoary Fox, Lycalopex vetulus (Mammalia: Canidae). **Mammalian Biology**, v. 98, n. August, p. 91–101, 2019.

HARESTAD, A. S.; BUNNEL, F. L. Home Range and Body Weight-A Reevaluation. **Ecology**, v. 60, n. 2, p. 389–402, 1979.

HELM, B. et al. Two sides of a coin: Ecological and chronobiological perspectives of timing in the wild. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 372, n. 1734, 2017.

JOHNSON, R. R. et al. Delineating grassland bird conservation areas in the U.S. Prairie pothole region. **Journal of Fish and Wildlife Management**, v. 1, n. 1, p. 38–42, 2010.

JUAREZ, K. M.; MARINHO-FILHO, J. Diet, Habitat Use, and Home Ranges of Sympatric Canids in Central Brazil. **Journal of Mammalogy**, v. 83, n. 4, p. 925–933, 2002.

KERNOHAN, B. J.; GITZEN, R. A.; SPAUGH, J. J. M. I. Analysis of animal space use and movements. In: MILLSPAUGH, J. J.; MARZLUFF, J. M. (Eds.). . **Radio tracking animal populations**. San Diego, California, USA: Academic Press, 2001. p. 125–166.

KRONFELD-SCHOR, N. et al. Chronobiology of interspecific interactions in a changing world. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 372, n. 1734, 2017.

LEMOS, F. G. **ECOLOGIA E COMPORTAMENTO DA RAPOSA-DO-CAMPO PSEUDALOPEX VETULUS E DO CACHORRO- DO-MATO CERDOCYON THOUS EM ÁREAS DE FAZENDAS NO BIOMA CERRADO**. [s.l.] Universidade Federal de Uberlândia, 2007.

LEMOS, F. G. **Ecologia e conservação da raposa-do-campo (Lycalopex vetulus) e interações com canídeos simpátricos em áreas antropizadas do Brasil**. [s.l.] Universidade Federal de Uberlândia, 2016.

LEMOS, F. G.; FACURE, K. G. Seasonal variation in foraging group size of crab-eating foxes and hoary foxes in the Cerrado Biome, Central Brazil. **Mastozoología Neotropical**, v. 18, n. 2, p. 239–245, 2011.

LEMOS, F. G.; FACURE, K. G.; COSTA, A. N. Canid News Interference competition between the crab-eating fox and the hoary. p. 2002–2005, 2007.

LEMOS, F. G.; FACURE, K. G.; DE AZEVEDO, F. C. A first approach to the comparative ecology of the hoary fox and the crab-eating fox in a fragmented human altered landscape in the Cerrado Biome at Central Brazil. **Middle-Sized Carnivores in Agricultural Landscapes**, n. February, p. 143–160, 2011.

MACDONALD, D. W. et al. Monogamy: Cause, Consequence, or Corollary of Success in Wild Canids? **Frontiers in Ecology and Evolution**, v. 7, n. September, p. 341, 2019.

MCLOUGHLIN, P. D.; FERGUSON, S. H.; MESSIER, F. Intraspecific Variation in Home Range Overlap with Habitat Quality: A Comparison among Brown Bear Populations. **Evolutionary Ecology**, v. 14, n. 1, p. 39–60, Jan. 2000.

MOEHRENSCHLAGER, C.; MOEHRENSCHLAGER, A. **Canadian swift fox**

(Vulpes velox) population assessment: winter, 1999. Edmonton, Alberta: [s.n.].

MORELLET, N. et al. Seasonality, weather and climate affect home range size in roe deer across a wide latitudinal gradient within Europe. **Journal of Animal Ecology**, v. 82, n. 6, p. 1326–1339, Nov. 2013.

MORENO, C. R. C. The recognition of chronobiology in science. **Sleep Science**, v. 11, n. 1, p. 1, 2018.

PICARDI, S. et al. Analysis of movement recursions to detect reproductive events and estimate their fate in central place foragers. **Movement Ecology**, v. 8, n. 1, p. 24, 3 Dec. 2020.

POWELL, R. A.; MITCHELL, M. S. What is a home range? **Journal of Mammalogy**, v. 93, n. 4, p. 948–958, 2012.

RALLS, K. et al. **Kit fox— coyote relationships in the Carrizo Plain Natural Area**. Report nr Permit PRT 702631: [s.n.].

SIKES, R. S.; GANNON, W. L. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. **Journal of Mammalogy**, v. 92, n. 1, p. 235–253, 16 Feb. 2011.

TANNERFELDT, M.; ELMHAGEN, B.; ANGERBJÖRN, A. Exclusion by interference competition? The relationship between red and arctic foxes. **Oecologia**, v. 132, n. 2, p. 213–220, 2002.

TRIGUEIRO, W. R.; NABOUT, J. C.; TESSAROLO, G. Uncovering the spatial variability of recent deforestation drivers in the Brazilian Cerrado. **Journal of Environmental Management**, v. 275, n. December 2019, 2020.

VAN DER VEEN, D. R. et al. Flexible clock systems: Adjusting the temporal programme. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 372, n. 1734, 2017.

WALTON, Z.; MATTISSON, J. Down a hole: missing GPS positions reveal birth dates of an underground denning species, the red fox. **Mammalian Biology**, n. 0123456789, 2021.

WHITE, G.; GARROTT, R. **Analysis of Wildlife Radio-Tracking Data**. [s.l.] Academic Press, 1990.

WIESEL, I.; KARTHUN-STRIJBOS, S.; JÄNECKE, I. The Use of GPS Telemetry Data to Study Parturition, Den Location and Occupancy in the Brown Hyaena. **African Journal of Wildlife Research**, v. 49, n. 1, 8 Feb. 2019.

WORTON, B. J. Kernel Methods for Estimating the Utilization Distribution in Home-Range Studies. **Ecology**, v. 70, n. 1, p. 164–168, Feb. 1989.

WRIGHT, H. W. Y. et al. Mating tactics and paternity in a socially monogamous canid, the bat-eared fox (Otocyon megalotis). **Journal of Mammalogy**, v. 91, n. 2, p. 437–447, 2010.

YAN, W. B. et al. Seasonal variation and sexual difference of home ranges by takins. **Journal of Wildlife Management**, v. 81, n. 5, p. 938–942, 2017.

ZOELLICK, B. W. .; SMITH, N. S. . Size and Spatial Organization of Home Ranges of Kit Foxes in Arizona. **American Society of Mammalogists**, v. 73, n. 1, p. 83–88, 1992.

4. CONCLUSÃO

Ao longo desta tese pudemos aprofundar nossa compreensão sobre os processos rítmicos que envolvem a biologia comportamental da raposa-do-campo (*Lycalopex vetulus*). Comprovamos nossas hipóteses, apontando variações significativas no padrão comportamental da espécie ao longo do tempo, em diferentes escalas. Através dos dados detalhados oriundos de observação direta dos animais no campo, demonstramos não apenas que a espécie é noturna, mas também que concentram comportamentos específicos em determinados horários dentro deste período de atividade. Assim, destacamos a importância de considerarmos escalas mais finas de tempo em estudos ecológicos sobre nicho temporal. Mostramos também que esta rotina diária de comportamentos sofre alterações ao longo do ano, a depender da fase reprodutiva.

De forma complementar, através do monitoramento por transmissores GPS, comprovamos a existência de importantes processos rítmicos em relação ao uso do espaço e às interações sociais da raposa-do-campo. Assim, demonstramos que mesmo que vivam em áreas de vida aparentemente estáveis, a espécie possui uma relação dinâmica e cíclica na forma com que usa o meio. Além disso, as variações sazonais encontradas estão fortemente relacionadas às fases do ciclo reprodutivo da espécie. Esta sazonalidade reprodutiva, por sua vez, está sincronizada com as condições ambientais, como a disponibilidade de alimentos.

Assim, aprofundamos nosso conhecimento sobre uma espécie emblemática, o único canídeo endêmico do território brasileiro e um dos menos estudados do mundo. No entanto, acreditamos que a contribuição desta tese não se limita à espécie estudada. Nossos resultados trouxeram *insights* importantes que poderão ser considerados para outras espécies, especialmente carnívoros. Além dos resultados, acreditamos que as métricas e os métodos de análise propostos aqui representam um avanço importante para o estreitamento entre a ecologia e a cronobiologia. Sugerimos fortemente que estudos futuros repliquem estes métodos em outras espécies, realizando as adaptações e aprimoramentos necessários, para que possamos consolidar ferramentas de trabalho robustas que explorarem esta fronteira do conhecimento.

Apesar de nossos avanços, reconhecemos que apenas arranhamos a superfície de um sistema extremamente complexo. O comportamento animal resulta da relação do estado interno do indivíduo com fatores ambientais, como luminosidade, temperatura, disponibilidade de recursos e presença de predadores e competidores. O estado interno é composto por diversos ritmos endógenos que são finamente sincronizados, e essa flutuação na função endócrina leva a uma variação temporal pronunciada nos comportamentos dos animais, especialmente nos comportamentos mediados por hormônios. Portanto, além de identificar e descrever os padrões rítmicos no comportamento das espécies, sugerimos que estudos futuros investiguem também a regulação fisiológica desses ritmos e os gatilhos ambientais a eles relacionados.

Estes conhecimentos auxiliarão na compreensão das possíveis respostas destes animais a ecossistemas sob acelerada pressão antrópica. As mudanças climáticas e ecossistêmicas que vivemos no Antropoceno podem levar a um desalinhamento neste sistema complexo e sensível de regulação temporal entre os seres vivos e seu meio, impactando negativamente a conservação das espécies. Não existem soluções simples para problemas complexos. Devemos abandonar abordagens de conservação que busquem a preservação de estados fixos da natureza. Na natureza, a mudança é a regra. O fluxo interdependente é tudo o que existe. Precisamos pensar em abordagens integrativas, que busquem a conservação de processos. A conservação da vida é, na verdade, a conservação do fluxo da vida. O movimento de corpos. No espaço. E no tempo.

REFERÊNCIAS

BICKLEY, S. M. et al. Human perceptions of and interactions with wild canids on cattle ranches in central Brazil. **Oryx**, v. 54, n. 4, p. 546–553, 17 jul. 2020.

BÖRGER, L. et al. Animal Home Ranges: Concepts, Uses, and Estimation. In: MURRAY, D. L.; SANDERCOCK, B. K. (Eds.). . **Population Ecology in Practice**. First Edit ed. [s.l.] John Wiley & Sons Ltd., 2019. p. 315–332.

CANDEIAS, Í. Z. DAS et al. First assessment of hoary fox (Lycalopex vetulus) seasonal ovarian cyclicity by non-invasive hormonal monitoring technique. **Conservation Physiology**, v. 8, n. 1, p. 1–12, 2020.

CHIARELLO, A. G. et al. Mamíferos Ameaçados de Extinção no Brasil. In: MACHADO, A. B. M.; DRUMMOND, G. M.; PAGLIA, A. P. (Eds.). . **Livro Vermelho da Fauna Brasileira Ameaçada de Extinção**. Brsília: Fundação Biodiversitas, Belo Horizonte, 2008. p. 680–880.

COLLI, G. R.; VIEIRA, C. R.; DIANESE, J. C. Biodiversity and conservation of the Cerrado: recent advances and old challenges. **Biodiversity and Conservation**, v. 29, n. 5, p. 1465–1475, 2020.

COURTENAY, O. et al. First observations on South America’s largely insectivorous canid: The hoary fox (Pseudalopex vetulus). **Journal of Zoology**, v. 268, n. 1, p. 45–54, 2006.

DALPONTE, J. C. **História natural, comportamento e conservação da raposa-do-campo, Pseudalopex vetulu (Canidae)**. [s.l.] Universidade de Brasilia, 2003.

DALPONTE, J. C. Lycalopex vetulus (Carnivora: Canidae). **Mammalian Species**, n. 847, p. 1–7, 2009.

GIENAPP, P.; REED, T. E.; VISSER, M. E. Why climate change will invariably alter selection pressures on phenology. **Proceedings of the Royal Society B: Biological Sciences**, v. 281, n. 1793, p. 20141611, 22 Oct. 2014.

GÜLDÜR, T.; OTLU, H. G. Circadian rhythm in mammals: time to eat & time to sleep. **Biological Rhythm Research**, v. 48, n. 2, p. 243–261, 2017.

GUTIÉRREZ, E. E. et al. Climate change and its potential impact on the conservation of the Hoary Fox, Lycalopex vetulus (Mammalia: Canidae). **Mammalian Biology**, v. 98, n. August, p. 91–101, 2019.

HELM, B. et al. Two sides of a coin: Ecological and chronobiological perspectives of timing in the wild. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 372, n. 1734, 2017.

HOFFMANN, A. A.; SGRÒ, C. M. Climate change and evolutionary adaptation. **Nature**, v. 470, n. 7335, p. 479–485, 23 Feb. 2011.

JUAREZ, K. M.; MARINHO-FILHO, J. Diet, Habitat Use, and Home Ranges of Sympatric Canids in Central Brazil. **Journal of Mammalogy**, v. 83, n. 4, p. 925–933, 2002.

KRONFELD-SCHOR, N. et al. Chronobiology of interspecific interactions in a changing world. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 372, n. 1734, 2017.

LEMOS, F. et al. Avaliação do risco de extinção da Raposa-do-campo Lycalopex vetulus no Brasil. n. January, 2013.

LEMOS, F. G. **Ecologia e conservação da raposa-do-campo (Lycalopex vetulus) e interações com canídeos simpátricos em áreas antropizadas do Brasil**. [s.l.] Universidade Federal de Uberlândia, 2016.

LEMOS, F. G.; FACURE, K. G.; DE AZEVEDO, F. C. A first approach to the comparative ecology of the hoary fox and the crab-eating fox in a fragmented human altered landscape in the Cerrado Biome at Central Brazil. **Middle-Sized Carnivores in Agricultural Landscapes**, n. February, p. 143–160, 2011.

MORENO, C. R. C. The recognition of chronobiology in science. **Sleep Science**, v. 11, n. 1, p. 1, 2018.

NELSON, R. J. Biological Rhythms. In: NELSON, R. J. (Ed.). . **Behavioral Endocrinology**. third ed. [s.l.] Sinauer Associates, 2005. p. 587–667.

POWELL, R. A.; MITCHELL, M. S. What is a home range? **Journal of Mammalogy**, v. 93, n. 4, p. 948–958, 2012.

ROTENBERG, L.; MARQUES, N.; MENNA-BARRETO, L. História e perspectivas da cronobiologia. In: **Cronobiologia: Princípios e Aplicações**. [s.l.] EDUSP, 2003.

SCHWARTZ, W. J.; HELM, B.; GERKEMA, M. P. Wild clocks: Preface and glossary. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 372, n. 1734, 2017.

WEINERT, D. The temporal order of mammals. Evidence for multiple central and peripheral control mechanisms and for endogenous and exogenous components: some implications for research on aging. **Biological Rhythm Research**, v. 36, n. 4, p. 293–308, out. 2005.

VISSER, M. E. Keeping up with a warming world; assessing the rate of adaptation to climate change. **Proceedings of the Royal Society B: Biological Sciences**, v. 275, n. 1635, p. 649–659, 22 Mar. 2008.