

AN INTEGRATIVE APPROACH TO EVALUATING METABOLIC INDICATORS IN
MANED WOLVES (*CHRYSOCYON BRACHYURUS*)

by

Jane Braswell
A Thesis
Submitted to the
Graduate Faculty
of
George Mason University
in Partial Fulfillment of
The Requirements for the Degree
of
Master of Science
Environmental Science & Policy

Committee:

_____	Dr. Kathleen E. Hunt, Thesis Chair
_____	Dr. Rosana Nogueira de Moraes, Committee Member
_____	Dr. Elizabeth W. Freeman, Committee Member
_____	Dr. Younsung Kim, Department Chairperson
_____	Dr. Gerald L. R. Weatherspoon, Associate Dean for Undergraduate and Graduate Affairs, College of Science
_____	Dr. Cody W. Edwards, Dean, College of Science
Date: _____	Summer Semester 2024 George Mason University Fairfax, VA

An Integrative Approach to Evaluating Metabolic indicators in Maned Wolves
(*Chrysocyon brachyurus*)

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of
Science at George Mason University

by

Jane Braswell
Bachelor of Arts
Franklin and Marshall College 2018

Director: Dr. Kathleen Hunt, Associate Professor
Smithsonian-Mason School of Conservation &
Department of Biology, George Mason University

Summer Semester 2024
George Mason University
Fairfax, VA

Copyright 2024 Jane Braswell
All Rights Reserved

DEDICATION

To Santi, thank you for keeping me company along the way. I can't wait to meet you.

ACKNOWLEDGEMENTS

I would like to extend my deepest gratitude to the many individuals and organizations who have supported me throughout the course of my thesis.

First and foremost, I want to thank the project's sponsors, the Smithsonian's National Zoo and Conservation Biology Institute (NZCBI) and Medtronic Inc.

I am immensely grateful to my advisors, Dr. Rosana Nogueira de Moraes and Dr. Kathleen Hunt, for making this project possible. Your guidance and encouragement have provided me with invaluable opportunities for both personal and professional growth, and your expertise and patience have been essential to my development as a researcher.

I am also deeply grateful to my committee member, Dr. Elizabeth Freeman, for support throughout my degree. Your insight and advice have been essential to my progress and success.

This project would not have been possible without the dedication and hard work of NZCBI's carnivore team, including Marissa Gonzales, Chris Lemons, Jessica Kordell, and Ish Ganame. Thank you for your commitment to the success of my research and the welfare of the maned wolves. Additionally, I want to thank those in the endocrine lab, Niki Boisseau and Steve Paris, for sharing your expertise with me. It was truly invaluable to my success.

A special thank you to the Smithsonian-Mason School of Conservation's faculty and staff for providing me with the most amazing teaching assistantship. This opportunity introduced me to so many new areas of conservation to explore.

To everyone in the Hunt Lab, thank you for creating such a fun, collaborative, and supportive environment.

Lastly, I want to express my gratitude to my mother, Sue Braswell, and husband, Yosvany Rodriguez. Thank you for providing me with the extensive emotional support I have required over the past three years. I could not have reached this milestone without you.

TABLE OF CONTENTS

	Page
List of Tables	viii
List of Figures	ix
List of Abbreviations	x
Abstract	xi
Introduction.....	1
Materials and Methods.....	11
Study subjects.....	11
Biologging.....	13
Hair samples.....	14
Sample collection and processing.....	14
Hair T3 extraction and analysis	16
Additional determinants of metabolism	18
Astronomical season.....	18
Food intake	19
Body weight.....	20
Seasonal averages.....	20
Statistical analyses.....	20
Analytical validation of hT3.....	20
Seasonal patterns in metabolic activity	21
Results.....	24
Hair growth rate	24
Analytical validation of hT3	24
Seasonal patterns in metabolic activity	24
Discussion.....	32
Measuring T3 in hair.....	32
Seasonal patterns in metabolic activity	33
Appendix 1: Hair Growth Rate Estimate	39
Appendix 2: AIC-Based model selection	40
Appendix 3: Model Summaries	47

Appendix 4: Comparison of solvents for hair extraction.....	52
References.....	53

LIST OF TABLES

Table	Page
Table 1 Demographic information and data collection dates for maned wolves (n = 8) monitored via biologgers (Reveal LINQ™, Medtronic Inc., MN, USA) and shaved for seasonal analysis of hair triiodothyronine (T3). Maned wolves were located at the Smithsonian’s National Zoo and Conservation Biology Institute in Front Royal, VA, USA.....	12
Table 2 Estimated marginal means (EMM) and 95% confidence intervals (CI), along with estimates of continuous predictors from final models concerning daily average heart rate (HR), daily average heart rate variability (HRV), hair triiodothyronine (hT3), daily total activity, daily food intake, and monthly average body weight in maned wolves located at the Smithsonian National Zoo and Conservation Biology Institute in Front Royal, VA, USA. EMMs for models containing multiple categorical predictors were calculated using the emmean function within the emmeans R package. Colors indicate sex (blue = both sexes, purple = females, and green = males). Transparency indicates the value of EMMs, with higher values being more opaque. See Appendix 3 for full model summaries.	27

LIST OF FIGURES

Figure	Page
Figure 1 Estimated marginal means (EMMs) from final models concerning the influence of sex and astronomical season on daily average heart rate (A), daily average heart rate variability (B), hair triiodothyronine (hT3; C), daily total activity (D), daily food intake (E), and monthly average body weight (F) in maned wolves located at the Smithsonian National Zoo and Conservation Biology Institute in Front Royal, VA, USA. EMMs for models containing multiple categorical predictors were calculated using the emmean function within the emmeans R package. 95% confidence intervals are displayed as whiskers extending from EMM points. Colors indicate sex (blue = both sexes, purple = females, and green = males). See Appendix 3 for full model summaries.	28
Figure 2 Terplot for generalized additive model for location, scale, and shape testing the impact of daily average ambient temperature, daily food intake, and daily activity on daily average heart rate (HR) in maned woves located at the Smithsonian National Zoo and Conservation Biology Institute in Front Royal, VA, USA. Plots indicate partial effect of explanatory variables on HR. HR estimates are represented by blue trendlines [cubic spline (cs) smooth predictors] and their standard errors by blue shaded area. See A3-3 for full model summary.	29
Figure 3 Terplot for generalized additive model for location, scale, and shape testing the impact of daily average ambient temperature, daily food intake, and daily activity on daily average heart rate variability (HRV) in maned wolves located at the Smithsonian National Zoo and Conservation Biology Institute in Front Royal, VA, USA. Plots indicate partial effect of explanatory variables on HRV. HRV estimates are represented by blue trendlines [cubic spline (cs) smooth predictors] and their standard errors by blue shaded area. See A3-3 for full model summary.....	30
Figure 4 Predicted values of hair triiodothyronine (T3) in response to changes in ambient temperature in captive maned wolves located at the Smithsonian’s National Zoo and Conservation Biology Institute in Front Royal, VA, USA.	32

LIST OF ABBREVIATIONS

Akaike information criterion.....	AIC
Autonomic nervous system.....	ANS
Coefficient of variation	CV
Enzyme-linked immunosorbent assay	ELISA
Ethanol	EtOH
Generalized Additive Model for Location, Scale, and Shape.....	GAMLSS
Generalized Linear Mixed Model.....	GLMM
Heart rate.....	HR
Heart rate variability	HRV
Hypothalamic-Pituitary-Thyroid.....	HTP
Hair triiodothyronine	hT3
Linear Mixed Model	LMM
Methanol	MeOH
Smithsonian’s National Zoo and Conservation Biology Institute	NZCBI
Parasympathetic nervous system	PNS
Sympathetic nervous system.....	SNS
Thyroid hormone	TH
Triiodothyronine	T3

ABSTRACT

AN INTEGRATIVE APPROACH TO EVALUATING METABOLIC INDICATORS IN MANED WOLVES (*CHRYSOCYON BRACHYURUS*)

Jane Braswell M.S.

George Mason University, 2024

Thesis Director: Dr. Kathleen Hunt

Integrated datasets of physiology and behavior are critical to determining the impact of environmental threats on animal health. Continued advancements in biologging technology aid this effort; however, additional techniques, such as endocrinology, can help contextualize findings. Maned wolves (*Chrysocyon brachyurus*) experience broad-scale landscape alteration, presenting a need for reliable indices of metabolic health. I tested a protocol for long-term monitoring of metabolic health in free-ranging wildlife by integrating biologging and analysis of hair triiodothyronine (T3), a form of thyroid hormone, using captive maned wolves as a model. Hair samples were collected approximately every three months for just over one year from maned wolves (n=8) implanted with Reveal LINQ™ (Medtronic Inc., Minneapolis, MN) biologgers at the Smithsonian National Zoo and Conservation Biology Institute in Front Royal, Virginia, USA. Daily average heart rate (HR) and heart rate variability (HRV), and daily total activity were calculated from raw biologger data. T3 was measured in guard hair via a T3

enzyme-linked immunosorbent assay (Arbor Assays, MI), which passed analytical validations for maned wolf hair extract. To evaluate the feasibility of integrating these measures, I tested the impact of various determinants of metabolism (e.g. season, ambient temperature, activity, food intake, and body weight) on HR, HRV, and hair T3. I detected seasonal patterns in all metabolic indicators. Notably, HR increased and HRV decreased in winter, indicating increased activation of the sympathetic nervous system, and by inference, increased metabolic activity. Variation in HR and HRV was primarily explained by ambient temperature. Contrary to predictions, hair T3 was greatest in summer and lowest in winter and was positively impacted by ambient temperature. This may indicate that increased metabolic activity in winter was triggered by types of stressors that are not reflected in circulating thyroid hormone concentrations.

Alternatively, apparent hormone concentrations in hair may reflect different time periods than expected or may not reflect plasma concentrations consistently. Ultimately, I demonstrate the feasibility of combining biologging metrics with analysis of T3 in hair, but also highlight the need for further investigation of hair T3 as a potential proxy of plasma T3, and of T3, in turn, as an indicator of energetic state. Because hormones are incorporated into hair as it grows, may be a particularly valuable sample type for retrospective analysis of animals' physiological state over time. Thus, this integrated assessment of biologging parameters and endocrinology shows promise for application *in situ*, in combination with other ecological assessments, as the field aims to understand how free-ranging maned wolves' meet the energetic demands of altered landscapes.

INTRODUCTION

As wildlife species face unprecedented rates of environmental change, integrated datasets of animals' physiology and behavior are critical in determining the impact of potential threats on animal health and, ultimately, mitigating population declines (Madliger et al., 2018). Continued advancements in animal-borne data logging technology, also known as “biologgers,” assist research efforts in assessing physiology and behavior in free-living wildlife (Forin-Wiart et al., 2019). Biologgers are becoming increasingly applicable to a variety of species in different environments, as both wearable and small, implantable monitors are now available (Laske et al., 2018a). These instruments have been fitted with sensors to measure a suite of physiological, behavioral, and environmental parameters simultaneously, and provide fine-scale quantitative data for difficult-to-study species within their natural environments (Forin-Wiart et al., 2019). However, in some cases, supplemental approaches may be necessary to establish a more complete picture of how species respond to the suite of stressors encountered in altered landscapes (Madliger et al., 2018; Williams et al., 2019). Hormones in particular can offer useful additional insights into the physiological state of the animal since they directly mediate important physiological events such as changes in reproductive state, response to stress, and shifts in metabolism (McCormick & Romero, 2017). Here, I discuss the utility of integrating biologging and endocrinology – more specifically, heart

rate (HR), activity, and thyroid hormone (TH) concentrations within hair – for long-term monitoring of metabolic activity in free-ranging wildlife populations.

Metabolism refers to the biochemical processes by which cells break down nutrients and tissues to obtain energy for bodily functions (catabolism), utilizing that energy to construct larger molecules that form new tissues (anabolism; Koviri, 2023). Metabolism is impacted by various intrinsic and extrinsic factors such as sex, age, nutrition, physical activity, and temperature, often with interactive effects (Glazier & Gjoni, 2024). These cumulative effects influence an animal's metabolic balance (Koviri, 2023). When the sympathetic nervous system (SNS) is activated in response to environmental stimuli, catabolic reactions like cellular respiration increase and anabolic reactions are suppressed (Koviri, 2023). In the context of free-ranging wildlife populations, threats like habitat alteration, climate change, and human disturbance can lead to changes in food availability and quality, thermoregulation, and activity patterns, which may cause the energetic demand of an animal's environment to exceed available resources and result in metabolic stress (Ayres et al., 2012; DeRango et al., 2019; Rogers et al., 2021).

HR is one of the most common measures collected by biologists (Leimgruber et al., 2023) and can serve as an indicator of metabolic activity (Green, 2011). HR and metabolic rate reliably exhibit a statistically significant positive relationship, although the magnitude of this relationship is dependent on various factors (Green et al., 2009). By monitoring HR, we also are able to calculate heart rate variability (HRV), a particularly important metric in understanding animal stress (Moraes et al., 2021; Pohlin et al., 2017;

Støen et al., 2015). HRV is the fluctuation across time intervals between consecutive heart beats and reflects the balance between two competitive branches of the autonomic nervous system (ANS); the parasympathetic nervous system (PNS) that maintains homeostasis and decreases HR, and the SNS that responds to environmental stimuli by increasing HR (Carravieri et al., 2016). Because of the competitive nature of the PNS and SNS, HRV allows us to understand an animal's ability to respond to environmental stressful or energetically demanding stimuli and return to homeostasis. As a result, higher HRV is associated with positive health and welfare (Briefer et al., 2015; Carravieri et al., 2016).

Heart rate monitoring has allowed for a better understanding of wildlife stress within numerous taxonomic groups to date (birds: (Carravieri et al., 2016; Derose-Wilson et al., 2015; Viblanc et al., 2012); canids: Moraes et al., 2021; ursids:(Ditmer et al., 2015); pinnipeds: (Chaise et al., 2017); and ungulates: (Briefer et al., 2015; Leimgruber et al., 2023; Pohlin et al., 2017). Though most investigations have focused on the impact of acute, anthropogenic stressors on HR and HRV (Derose-Wilson et al., 2015; Ditmer et al., 2015, 2018; Evans et al., 2016; Græsli, Le Grand, et al., 2020; Laske et al., 2011; Le Grand et al., 2019; Støen et al., 2015; Viblanc et al., 2012), a number of studies also have addressed the impact of more prolonged, energetically demanding environmental and physiological conditions on HR and HRV (Laske et al., 2011, 2014; Pohlin et al., 2017; Thiel et al., 2022). HR and HRV are mediated by complex interactions between different physiological processes (Moraes et al., 2021). As a result, these metrics alone have limited capability of teasing apart the degree to which different ecological factors

contribute to animals' stress. This is particularly relevant with prolonged challenges like seasonal fluctuations in temperature and food availability (Ayres et al., 2012; Bechshøft et al., 2013; Bryan et al., 2014), which can be magnified by climate change and anthropogenic disturbance (Birnie-Gauvin et al., 2017). Therefore, it is important to recognize additional tools that can supplement this type of data to better understand the stress physiology of wildlife species.

Analyzing trends in HR alongside THs may offer the opportunity to isolate the relative influence of metabolic stressors on animals' cumulative stress profiles. Neuroendocrine pathways respond to internal and external stimuli by signaling target tissues to initiate physiological processes via hormones (McCormick & Romero, 2017). Consequently, some hormones, such as THs, can directly or indirectly impact HR and have the potential to aid interpretation of HR and HRV patterns observed in wildlife species (Mullur et al., 2014). Thyroid hormones, such as the most often studied biologically active form, triiodothyronine (T3), are regulated by the hypothalamic-pituitary-thyroid (HPT) axis and play a critical role in adjusting basal metabolic rate in response to various external and internal stimuli (Behringer et al., 2018), especially if those stimuli are prolonged (e.g. reduced prey availability; Ayres et al., 2012; cold temperatures; Thompson et al., 2017; pregnancy; Gesquiere et al., 2018). Compared to steroid hormones, THs are relatively understudied in wildlife (Behringer et al., 2018). Much of the existing literature has utilized THs to assess metabolic stress related to food availability and thermoregulation, often in the context of seasonality (Gesquiere et al., 2018; M. J. Myers et al., 2006; Thompson et al., 2017; Tomasi & Mitchell, 1994).

Generally, lower concentrations of THs can be indicative of nutritional stress, as the HTP axis decreases basal metabolic rate to preserve energetic resources (Behringer et al., 2018). Given adequate nutrition, however, THs tend to increase with the energetic demands of the environment (Kozłowski et al., 2020). For example, THs often increase during periods of ambient cold temperatures (Silva, 2005). Thus, TH analysis may provide a valuable tool to assess wildlife health as species attempt to meet the energetic demands of various environmental and anthropogenic influences.

As TH tends to respond to long-term changes in energetic demands, TH studies in wildlife are often approached via sample matrices that capture long-term fluctuations in metabolic state. For this reason, keratinized samples – e.g., hair, feather, claw, etc. – constitute an exciting opportunity. Because circulating hormones are incorporated into these tissues as they grow, a sample can reflect the TH secreted across a period of months, rather than minutes, hours, or days, facilitating long-term assessment of animals' physiological state *in-situ* (Gormally & Romero, 2020). In some cases, subsamples taken across the length of the sample (e.g. proximal vs. distal segments of a long hair) can represent distinct periods of time, with some particularly slow-growing keratin samples (baleen, claw, toenail, etc.) providing years-long endocrine profiles of individuals (Crain et al., 2021; Hunt et al., 2017, Rich et al., 2024). Additionally, steroid and thyroid hormones remain intact within keratinized tissues for extended periods at room temperature (Hunt et al., 2017; Koren et al., 2019). This makes transport and long-term storage of keratinized samples in a field setting considerably easier than more commonly

utilized sample types, such as feces, urine, or serum (Koren et al., 2002), which require freezing and may be impacted by repeated freeze-thaw cycles (Behringer et al., 2018).

Patterns in metabolic activity revealed by HR monitoring and TH analysis are even more valuable when assessed in combination with behavioral metrics, such as activity, which generally represents an energetic cost (Wilmers et al., 2015).

Accelerometers quantify activity by measuring an animal's velocity in three dimensions and are another common feature of biologgers (Brown et al., 2013). Given the metabolic demand associated with adapting to a changing environment (Birnie-Gauvin et al., 2017), the integration of activity data with indicators of metabolism is directly applicable to *in-situ* conservation efforts of wildlife species. However, it is necessary to validate these methods for a target species before applying them to free-ranging populations, which studies often accomplish within populations under human care (Houser et al., 2021; Leimgruber et al., 2023; Mondol et al., 2020; Moraes et al., 2021).

Maned wolves (*Chrysocyon brachyurus*) may represent an ideal study species for comparing these metrics within the context of studying free-moving individuals of a near-threatened species of conservation relevance. The maned wolf is the largest canid endemic to South America and is the only species within its genus, *Chrysocyon* (Fontoura-Rodrigues & Eizirik, 2014). The species is currently listed as “near threatened” by the IUCN, with an estimated free-ranging population of only 17,000 individuals remaining (Paula & DeMatteo, 2015). Maned wolves have experienced broad-scale landscape alteration due to agricultural expansion (Carvalho et al., 2009; Vynne et al., 2014), presenting a need for reliable indices of metabolic health for the species. Maned

wolves are found within central and eastern South America, though the extent of available habitat has decreased compared to historic conditions (Carravieri et al., 2016; Queirolo et al., 2011) resulting in reduced gene flow across their distribution (Rodriguez-Castro et al., 2022). Even where suitable habitat is available in southern regions, it appears local populations may no longer be present (Kasper et al., 2023). In Brazil, much of the species' range exists within the Brazilian Cerrado, the most biodiverse savannah ecosystem (N. Myers et al., 2000). Here, and throughout their range, maned wolves are considered a keystone species for their role in seed dispersal and germination (Reis et al., 2023; Motta-Junior et al., 2014), and controlling populations of small rodents (Consorte-McCrea, 2013). Maned wolves are multi-trophic omnivores, primarily nocturnal with peaks of activity at dusk and dawn. They are usually solitary, though they live in monogamous pairs during the breeding season, and display relatively high site fidelity (Dietz, 1984; May-Júnior et al., 2009); Motta-Junior et al., 2014). The primary threats to the species *in situ* include habitat loss and fragmentation (Paula & DeMatteo, 2015), disease spread from proximity to domestic animals (Curi et al., 2012; Fiori et al., 2023), and death from vehicle collisions (Barbosa et al., 2020). Continued research efforts aim to evaluate the severity of these threats and develop tools for long-term monitoring of maned wolves' physiology. Thus far, maned wolves appear to demonstrate moderate adaptability to agroecosystems and human development, as evidenced by density estimates and endocrine studies (Massara et al., 2012; Trolle et al., 2006; Vynne et al., 2014).

The recent application of biologgers in maned wolves *ex situ* shows promise for developing a more complete understanding of the impacts of environmental pressures on free-ranging individuals (Moraes et al., 2021). Specifically, Moraes et al. (2021) demonstrated feasibility of obtaining HR data from free-moving, unanesthetized maned wolves, and also revealed that behavior may not always accurately reflect maned wolves' internal physiological state. Yet, there is currently no validated method for TH analysis within hair from maned wolves. While endocrine studies typically include analytical validations (verification that hormone assays can accurately quantify the desired hormone), biological validations, which test whether the resulting hormone data truly represent the animals' physiological state, are often underreported (Koren et al., 2019). In other wildlife species, biological validation studies have compared TH concentrations to age (Behringer et al., 2018; Houser et al., 2021; M. J. Myers et al., 2006), body mass (Crocker et al., 2012; Gobush et al., 2014; Mustonen et al., 2006; Rimbach et al., 2017), metabolic rate (Rimbach et al., 2017; Tomasi & Mitchell, 1994), food availability or calorie intake (Crocker et al., 2012; Mustonen et al., 2006; Rimbach et al., 2017; Schaebs et al., 2016; Wasser et al., 2010), pregnancy (Gesquiere et al., 2018), and more generally season, as this can encompass multiple factors related to nutrition and thermoregulation (Gesquiere et al., 2018; M. J. Myers et al., 2006; Thompson et al., 2017; Tomasi & Mitchell, 1994). Often, biological validations of THs will incorporate more than one of these physiological and/or environmental variables, enabling results to be interpreted with greater confidence.

The present study intends to build on the successful deployment of implantable biologgers in maned wolves reported by Moraes et al. (2021) by modeling an integrated assessment of metabolic activity including HR, HRV, and activity (from biologgers), along with T3 (from hair). Specific aims include developing protocols to extract and quantify T3 from maned wolf hair using a commercially available enzyme-linked immunosorbent assay (ELISA), perform analytical validations, and perform preliminary biological validations by describing the relationship between seasonal patterns in HR, HRV, activity, hair T3 (hT3), and additional intrinsic and extrinsic determinants of metabolism when maintained in human care.

Given prior evidence that steroid hormones, such as glucocorticoids, are detectable within hair (Koren et al., 2019; Pereira et al., 2022; Roffler et al., 2022) and T3, specifically, is detectable within other keratinized tissues (Hunt et al., 2017), I predict that T3 will be quantifiable within hair samples collected from maned wolves. I hypothesize that winter will constitute a period of increased metabolic demand for maned wolves living within North American zoos due to colder temperatures (Silva, 2005) and possible associated increases in daily activity (Emmons, 2012). Thus, I predict that maned wolves' hT3 and HR will be elevated in winter relative to other seasons to maintain body temperature via adaptive thermogenesis (Silva, 2005). As a result of increased HR and SNS activation, I predict that HRV will be lowest in winter (Kazmi et al., 2016).

Evidence suggests that within their native range, maned wolves are consistently active at temperatures below 23°C – though exhibit decreased travel distances below

20°C – and become inactive at temperatures above 27-28°C (Emmons, 2012). However, it is relevant to note that some circumstances that may lead free-ranging maned wolves to exhibit increased activity and travel distances in cooler months (ex. limited access to food and water during the dry season; (Emmons, 2012) are not present for maned wolves within a zoo setting. Additionally, maned wolves within North American facilities experience much lower annual temperature averages and extremes than their free-ranging counterparts (Lawrimore, 2016). Thus, I predict that activity will be lowest in summer but make no predictions regarding differences in activity between the remaining seasons.

MATERIALS AND METHODS

STUDY SUBJECTS

Study subjects consisted of eight adult maned wolves (four male and four female) housed at the Smithsonian Conservation Biology Institute (NZCBI) in Front Royal, Virginia (Table 1). In accordance with the Association of Zoos and Aquariums' Maned Wolf Species Survival Plan (Fletcher et al., 1995), individuals were housed singly or in pairs of same-sex littermates in outdoor enclosures of appropriate size and structure, with access to indoor areas or resting boxes with supplemental heat and water *ad libitum*. Maned wolves were provided a daily diet of custom maned wolf chow (Mazuri, Land O' Lakes, Inc., Richmond, IN, USA) mixed with canola oil, variable produce (apple, pear, mango, papaya, banana, grapes, sweet potato, carrots, tomato, and green beans), and additional protein (egg, mice, fish, and Toronto Zoo Small Carnivore Diet prepared by Milliken Meat Products LTD., Ontario, Canada). Two females were also provided guinea pigs 2-3 times per week. The amount of chow and additional protein included in individual maned wolves' diets was adjusted over the study period depending on the animal's body weight and the amount of food they regularly consumed. Each animal was regularly provided environmental and behavioral enrichment, and subject to little human activity outside of their interactions with animal care staff, since the NZCBI's Front Royal location is closed to the public. All experimental procedures were approved by the NZCBI's Institutional Animal Care and Use Committee (IACUC # SI-21019).

Table 1. Demographic information and data collection dates for maned wolves (n = 8) monitored via biologgers (Reveal LINQ™, Medtronic Inc., MN, USA) and shaved for seasonal analysis of hair triiodothyronine (T3). Maned wolves were located at the Smithsonian’s National Zoo and Conservation Biology institute in Front Royal, VA, USA.

Subject	Studbook	Sex	Age (yr) ¹	Weight (kg) ²	Biologging		Hair collection
					Implantation	Final download	
Apollo	3681	M	2.8	24.9	12-02-2020	10-08-2022	08-02-2021
							11-18-2021
							02-11-2022
							06-10-2022
							09-22-2022
Zeus	3680	M	2.8	26.7	12-02-2020	10-08-2022	08-02-2021
							11-18-2021
							02-11-2022
							06-10-2022
							09-22-2022
Oceano	3643	M	3.0	27.1	01-20-2021	01-11-2023	08-30-2021
							*09-28-2021
							12-01-2021
							03-23-2022
							06-23-2022
Lume	3644	M	3.0	28.2	01-20-2021	*04-20-2022	08-30-2021
							11-17-2021
							03-23-2022
							06-23-2022
							09-25-2022
Avara	3525	F	5.3	25.1	04-14-2021	10-06-2022	08-31-2021
							*10-01-2021
							01-27-2022
							03-23-2022
							06-30-2022
Eliana	3583	F	3.8	24.3	03-10-2021	12-12-2022	08-27-2021
							11-29-2021
							03-23-2022
							06-30-2022
							10-08-2022
Layla	3438	F	6.4	24.8	05-20-2021	10-06-2022	08-27-2021
							11-08-2021
							05-19-2022
							10-06-2022
Terra	3646	F	3.3	24.8	*08-19-2021	10-8-2022	08-19-2021
							11-08-2021
							03-15-2022
							06-23-2022
							10-08-2022

* Indicates that the bilogger did not collect data through the entire study period (if in the *Bilogging* columns) or hair sample was collected one month after the previous collection to estimate growth rate and was not analyzed for hair T3 (if in *Hair collection* column).

¹ At the time of implantation

² Averaged across study period

BIOLOGGING

Subjects were implanted with subcutaneous Reveal LINQ™ (Medtronic Inc., Minneapolis, MN, USA) bi-loggers in the left peristernal region over the heart following procedures described in Moraes et al. (2021) between December 2020 and August 2021. In a previous study, Moraes et al. (2021) demonstrated that these devices can be successfully deployed in this species with little impact on animals' health and behavior following implantation, and overall good detection accuracy. Hair shaved from the surgical site was opportunistically collected during the procedures on all four female wolves for assay validation purposes, and was stored in a paper envelope until processing. At the time of implantation, bi-loggers were activated via transcutaneous telemetry (CareLink® Model 2090 Programmer) and programmed with custom software ('B-Ware; (Laske et al., 2018b) to calculate and record 2-minute HR averages (beats per minute; bpm), daily HRV (standard deviation of beat-to-beat intervals in sinus rhythm using the average of 5-min segment medians during a 24-h recording; SDANN), total activity every 15 minutes (total number of minutes the animal was active), in addition to several other parameters not utilized in this study. Raw data utilized for this study was downloaded via direct telemetry as was described in Moraes et al. (2021) while subjects were physically restrained in a squeeze crate approximately every three months (a routine

procedure for NSCBI maned wolves; for specific dates, see Table 1). When possible, downloads were scheduled during other husbandry or veterinary procedures requiring restraint (ex. shaving for identification purposes, preventative control of ectoparasites, etc.).

To account for subtle changes in the biologgers' internal clocks over time, parameters recorded at intervals less than 24 hours, including HR and activity, were time-corrected according to procedures described in Moraes et al. (2021). Daily mean HR was calculated by averaging all data points across the 24-hour diel cycle. Daily total activity was calculated by summing all 15-min total activity recordings across the 24-hour diel cycle.

HAIR SAMPLES

Sample collection and processing

Hair samples were collected from all subjects approximately every three months between August 2021 and October 2022 when animals were restrained for bilogger data downloads (Table 1). Canid species have compound hair follicles from which multiple hair shafts – some guard hairs (larger diameter) and majority undercoat hairs (smaller diameter) – emerge (Welle & Wiener, 2016). The canid hair cycle includes three primary stages, the active growth phase (anagen), the regression phase wherein the hair shaft separates from the follicle (catagen), and the quiescent phase before the hair is shed when there is no longer blood supply to the hair shaft (telogen; (Welle & Wiener, 2016). In domestic dogs, hair growth is not synchronized, meaning adjacent follicles can be in different stages of the hair cycle simultaneously (Welle & Wiener, 2016). In animals

adapted to cold climates (e.g. temperate zones, the Arctic, and alpine habitats), hairs synchronize their anagen phase to a specific time of year, typically fall; in tropical and subtropical species, however, hairs are typically not synchronized. In the absence of published studies concerning hair growth in maned wolves, my working assumption based on anecdotal information was that maned wolves experienced continuous unsynchronized hair growth, with periods of synchronized hair growth during seasonal shedding in fall and (to a greater degree) in spring.

The anatomical location from which samples were collected was standardized across subjects given evidence that hormone concentrations can vary by body region (Pereira et al., 2022). During sample collection, animal care staff shaved an approximately 5x5 cm area on the animal's left shoulder as close to the skin as possible with electric clippers. Additional hair samples were collected opportunistically if any region of the animal was shaved for other purposes (e.g. for identification or other medical procedures). Samples were immediately transferred to paper envelopes and stored at room temperature until processing.

One month after the first hair collection, I collected another hair sample from two individuals (Oceano and Avara) in roughly the same location to estimate the growth rate of guard hair in the shoulder region. An additional sample was collected from Oceano the following month from the opposite shoulder which had been shaved one month prior for identification purposes. After guard hair was isolated from each sample, I measured the length of all guard hairs in millimeters with digital calipers and calculated the average hair length for each sample and the average hair length across all three samples.

Before hormone extraction, guard hairs were isolated from the remaining samples, given evidence that hormone concentrations may vary across hair type and because the ratio of guard to undercoat hair is likely to vary across the annual cycle (Pereira et al., 2022). While maned wolves' undercoat hairs are very thin, wavy, and light in color, guard hairs were visually identified by their distinct thickness and straightness, as well as their color, which is light at the base, red/brown along the length of the hair, and black at the tip. If I was unable to confidently identify individual hairs as undercoat or guard hair (e.g. they met some, but not all characteristics of a guard hair), they were set aside along with undercoat hairs. I standardized the length of guard hairs analyzed; all guard hairs within a sample were cut at 5 cm from the proximal end with scissors, with the 5-cm-long proximal piece then retained for analysis, and the distal tip discarded. After sorting, the guard hairs from each sample were cut into ~ 1 cm pieces with scissors and all pieces were ground together in a ball mill (Resch Ball Mill MM200; 10 mL stainless steel grinding jar; 12 mm stainless steel grinding ball) for 5 min at 25 Hz.

Hair T3 extraction and analysis

Extraction methods were adjusted from (Bennett & Hayssen, 2010), as follows. For each sample, 100 mg of powdered hair was suspended in 2 mL of 100% methanol (MeOH), vortexed at room temperature for 20 hours (Multi-Pulse Vortexer, Glas-col, Terre Haute, IN, USA) in a capped 16x125 mm borosilicate glass tube, and centrifuged for 20 min at 2500 rpm. If a sample yielded less than 50 mg of powder, the entire powdered sample was extracted in a customized volume of MeOH so as to maintain a constant ratio of 1 mg powder to 0.02 mL of MeOH. The supernatant was pipetted into a

clean 16x125 mm borosilicate glass tube and evaporated under direct air. Dried extracts were stored at -20°C until analysis. Just before analysis, dried extracts were reconstituted in assay buffer (Arbor Assays Kit K056, Ann Arbor, MI) to produce concentration between x4 and x6 according to the specific volume recovered after centrifuging. For example, for most samples, I was able to recover 1.65 mL of supernatant and reconstitute dried extracts with 275 uL of assay buffer, i.e. a six-fold increase (x6) in concentration. Smaller volume samples were reconstituted at a x4-x5 concentration to produce a large enough volume of 1:1 extract for determination of T3 concentrations, with final data adjusted accordingly. After assay buffer was added, samples were then briefly sonicated.

I determined hT3 concentrations via commercial Triiodothyronine EIA kits (Arbor Assays Kit K056, Ann Arbor, MI). All assays were run with non-specific binding wells, blanks, a standard curve, and high- and low-binding controls in duplicate following the manufacturer's protocols. I added an additional high binding (low dose) standard to the curve, resulting in eight standards total.

Analytical validations and comparison of extraction protocols were performed using preliminary hair samples as follows. An aliquot of 1:1 hair extract was serially diluted in assay buffer to produce five dilutions of 1:1, 1:2, 1:4, 1:8, and 1:16, which were assayed alongside the standard curve to test for parallelism of linear portions of the two binding curves (i.e. serially diluted hair sample compared to hormone standards, with both curves plotted as percent-bound vs log[relative dose]). The purpose of this parallelism test is to assess whether the assay antibody's binding affinity for the putative T3 hair extract is identical to its binding affinity for pure T3; good parallelism is

considered strong evidence that the substance present in the sample is indeed the desired hormone (or a very similar metabolite). To test for assay accuracy (aka “matrix effect”), I assayed a set of five standards alongside 1:2 mixtures of the same standards combined with pooled 1:1 hair extract. The purpose of this validation is to test whether the assay has good mathematical accuracy across a range of concentrations, despite presence of the various other compounds in the sample matrix.

The remaining samples were run at 1:1 dilution. Raw T3 concentrations (pg/mL) were multiplied by the reconstitution volume of the extract (i.e., producing total pg of hormone extracted from the sample), then divided by the weight of the extracted hair, and divided by 1000 to convert to final units of ng of immunoreactive hormone per g of hair. Samples with percent-bindings that fell outside the standard curve or those with a coefficient of variation (CV) greater than 10% between wells were re-assayed if enough of the sample remained to extract. If samples could not be re-assayed, they were removed from the final dataset.

ADDITIONAL DETERMINANTS OF METABOLISM

In addition to activity data collected via the biologgers, I utilized the following types of data collected by animal care staff and external sources to understand the influence of intrinsic (e.g. sex, activity, food intake, body weight) and extrinsic factors (e.g. ambient temperature, season) on HR, HRV, and hT3:

Astronomical season

Astronomical seasons, i.e. spring, summer, winter, fall, were determined by the dates of the solstice and equinox within the northern hemisphere during the study period

(Summer solstice: Jun 21, Autumnal equinox: Sep 22, Winter solstice: Dec 22, Spring equinox: Mar 21).

Ambient temperature

Data regarding ambient temperature in Front Royal, Virginia during the study period was obtained from the National Ecological Observatory Network (NEON, 2024). Daily mean ambient temperature was calculated by averaging 30-min temperature recordings (°C) within a 24-hr period and converting to degrees Fahrenheit to improve model fitting.

Food intake

Daily, animal care staff recorded the weight of maned wolf chow (g) provided to subjects and the weight of chow remaining from the prior feeding (g). I calculated daily food intake (g) as the difference between the weight of chow provided and remaining, excluding days for which the amount of chow consumed could not be reliably measured (e.g. the subject regurgitated, the subject was scatter-fed, the remaining chow was saturated with water). For pairs of male maned wolves that were housed together, animal care staff recorded the total weight of chow provided and remaining for the pair as a whole. Daily food intake was divided in half to estimate each individual's food intake. It is important to note that this estimate of food intake does not include other elements of the maned wolves' diets (e.g. meats, produce, additional proteins, or prey items). However, animal care staff reported that the maned wolves readily consume the meats and proteins within their diet, and that typically only chow and/or produce are not consumed.

Body weight

Subjects were weighed periodically throughout the study period, typically once every 1-2 months when restrained for biollogger downloads or other husbandry-related purposes, however, frequency varied between individuals.

SEASONAL AVERAGES

I calculated seasonal averages of all parameters to compare to hT3 concentrations measured within hair samples. These seasonal averages corresponded to the estimated growth period of each hair sample collected from a given individual and were calculated by averaging daily values across the three months prior to hair collection dates (or between subsequent hair collection dates, if this period was less than three months), excluding data from collection dates themselves. The only exception was body weight, for which seasonal means were calculated by averaging weights (kg) for that individual across the same period, but included the weight recorded on the day of hair collection if applicable.

STATISTICAL ANALYSES

Analytical validation of hT3

Parallelism of hT3 was determined by comparing the difference in slope between the percent-bindings of serially diluted hair extract and the standard curve using an F test (Grotjan and Keel, 1996; Ezan and Grassi, 2000; Hunt et al., 2017). Accuracy was determined via linear regression of the observed concentration of hormone versus the known hormone concentration of standards and was considered satisfactory if r^2 was

greater than 0.95 and the slope was between 0.7 and 1.3 (Grotjan and Keel, 1996; Ezan and Grassi, 2000; Hunt et al., 2017).

Seasonal patterns in metabolic activity

I utilized a series of models to (1) assess the impact of astronomical season and additional determinants of metabolism on HR, HRV, and hT3, and (2) to determine the impact of astronomical season on said determinants of metabolism to compare to seasonal patterns in HR, HRV, and hT3. Prior to fitting models, I confirmed that continuous predictors did not exhibit collinearity, evidenced by Pearson's correlation coefficients less than 0.70. However, given that astronomical season and ambient temperature are directly related, I chose to not include these covariates in the same models. All models included a random intercept for subjects to account for repeated measures and any inherent variation between individual maned wolves. We determined the most appropriate fixed effects structure for each response variable by comparing models with various configurations of covariates, including an intercept-only model, and selecting the model with the lowest Akaike Information Criterion (AIC) value. If model selection indicated that sex was an important predictor in any of these models, I also tested model structures that included an interaction term between sex and remaining covariates. I evaluated whether final models met assumptions by plotting residuals against fitted values. For final models containing categorical covariates, I calculated estimated marginal means to facilitate pairwise comparisons.

Daily average HR and HRV followed seasonal waveform patterns. Thus, I followed modeling procedures previously described for biologging data by Leimgruber et

al. (2023), which utilized Generalized Additive Models for Location, Scale, and Shape (GAMLSS). I used GAMLSS with a Johnson's SU distribution to model the influence of metabolic determinants including sex, activity, food intake, ambient temperature, and astronomical season on daily average HR and HRV. Because body weight could only be averaged on a monthly scale, it was excluded from this analysis. I was interested in the impact of both astronomical season and ambient temperature on HR and HRV, thus I chose to fit two separate models for both response variables, one including fixed effects for sex and astronomical season, and another including fixed effects for sex, activity, food intake, and ambient temperature. Relevant GAMLSS were fit with cubic splines to allow for non-linear relationships between continuous predictors and the response variable.

I assessed the influence of metabolic determinants including sex, astronomical season, and seasonal averages of ambient temperature, activity, food intake, and body weight on hT3 using Generalized Linear Mixed Models (GLMM) fit with a Gamma distribution and log link function. Similar to HR and HRV models, I was interested in the impact of both astronomical season and ambient temperature on hT3 and chose to fit two separate models, one including fixed effects for sex and astronomical season, and another including fixed effects for sex, activity, food intake, and ambient temperature.

Continuous predictors were scaled to facilitate the analysis of models with multiple continuous predictors.

Finally, to facilitate comparison of seasonal patterns in HR, HRV, and hT3 to various determinants of metabolic activity, I assessed the influence of sex and season on

daily activity, daily food intake, and monthly average body weight. For models in which activity served as the response variable, I used GLMMs fit with a Poisson distribution and log link function because activity, measured as active min per day, is considered count data. Whereas, for models in which food intake or body weight served as the response variable, I utilized linear mixed models (LMMs).

All statistical analyses were performed in R version 4.3.2 (R Core Team, 2023) and R Studio (2023.12.1.402; Posit team, 2024). Models were run using the *gamlss* (Rigby et al., 2005) and *lme4* (Bates et al., 2015) packages and compared using the *stepGAIC* function within the *gamlss* package for GAMLSS models and the *AICcmodavg* package (Mazerolle, 2020) for GLMMs and LMMs. Estimated marginal means were calculated using the *emmean* function within the *emmeans* package (Lenth, 2023). R squared values were calculated using the *r2* function within the *performance* package for LMMs (Lüdecke et al., 2021), the *r.squaredGLMM* function within the *MuMIn* package for GLMMs (Bartoń, 2023), and the *Rsq* function within the *gamlss* package for GAMLSS (Rigby et al., 2005).

RESULTS

HAIR GROWTH RATE

Hair length after approximately one month of growth ranged from 0.55 cm to 7 cm between samples, with an average of 1.82 cm (Appendix 1-1). Based on this average, I estimated that 5 cm of hair represents roughly 2.75 months of growth. However, there is substantial variation between samples collected from different individuals and samples collected repeatedly from the same individual (Appendix 1-1).

ANALYTICAL VALIDATION OF hT3

T3 was consistently detectable in maned wolf hair, though at relatively low concentrations. I found no difference in slope between serial dilutions of hair extracts and the standard curve ($F = 0.75$, $P = 0.41$), indicating acceptable assay parallelism. Additionally, assay accuracy was deemed acceptable based on the linear relationship between observed vs known hT3 concentration (Slope = 0.95, $r^2 = 0.99$). Together, these results demonstrate that hT3 can be reliably measured with little interference in binding from other biological components within hair samples (Brown et al., 2004).

SEASONAL PATTERNS IN METABOLIC ACTIVITY

Model comparisons identified that season, ambient temperature, food intake, and activity were associated with HR (Appendix 2-1, 2-2, 3-1, 3-2) and HRV (Appendix 2-3, 2-4, 3-3, 3-4). As expected, HR and HRV displayed opposing seasonal patterns (Figure 1). Daily average HR was highest in winter at 86.98 bpm [95% confidence interval (CI), (86.78, 87.19)] and lowest in summer at 76.05 bpm [CI, (75.74, 76.36)]. Whereas, daily

average HRV was lowest in winter at 228.06 ms [CI, (226.67, 229.46)] and highest in summer at 267.40 ms [CI, (265.57, 269.23)]. Estimated marginal means for HR and HRV within all seasons are reported in Table 2. Sex did not provide additional explanatory information in HR and HRV models containing astronomical season nor those containing continuous predictors, and thus, was not retained in the final models. In the latter models containing continuous predictors, ambient temperature had the greatest influence, negatively correlating with HR (Figure 2) and positively correlating with HRV (Figure 3). For every 1 °F increase in ambient temperature, HR was expected to decrease by 0.62 bpm and HRV was expected to increase by 1.78 ms (Table 2). Food intake was positive correlated with both HR (Figure 2) and HRV (Figure 3), though to a minor degree. For every 1 g increase in food intake, HR was expected to increase by 0.028 bpm and HRV by 0.020 ms (Table 2). Finally, activity was positively correlated with HR (Figure 2) and negatively correlated with HRV (Figure 3), though again, to a very minor degree. For every 1 min increase in activity, HR was expected to increase by only 0.005 bpm and HRV was expected to decrease by 0.04 ms (Table 2). Final models containing astronomical season explained 42.6% and 49.0 % of the observed variation in HR and HRV respectively. Final models containing ambient temperature, food intake, and activity explained 67.6% and 50.5 % of the observed variation in HR and HRV respectively.

Table 2. Estimated marginal means (EMM) and 95% confidence intervals (CI), along with estimates of continuous predictors from final models concerning daily average heart rate (HR), daily average heart rate variability (HRV), hair triiodothyronine (hT3), daily total activity, daily food intake, and monthly average body weight in maned wolves located at the Smithsonian National Zoo and Conservation Biology Institute in Front Royal, VA, USA. EMMs for

models containing multiple categorical predictors were calculated using the *emmean* function within the *emmeans* R package. Colors indicate sex (blue = both sexes, purple = females, and green = males). Transparency indicates the value of EMMs, with higher values being more opaque. See Appendix 3 for full model summaries.

Response Variable	Covariates							
	Estimated Marginal Mean (2.5%, 97.5% CI)				Estimate			
	Winter	Spring	Summer	Fall	Ambient temp (F)	Activity (min)	Food intake (g)	Body weight (kg)
BOTH SEXES								
HR (bpm)	86.98 (86.78, 87.19)	83.79 (83.43, 84.14)	76.05 (75.74, 76.36)	80.47 (80.14, 80.80)	-0.6205	0.028	0.005	Not Tested
HRV (ms)	228.07 (226.67, 229.46)	233.34 (231.18, 235.51)	267.40 (265.57, 269.23)	258.12 (256.08, 260.17)	1.7779	0.020	-0.0449	Not Tested
T3 (ng/g)	0.33 (0.29, 0.38)	0.63 (0.57, 0.71)	0.68 (0.62, 0.74)	0.51 (0.46, 0.57)	0.1718	Dropped	Dropped	Dropped
FEMALES								
Activity (min)	355.98 (339.25, 373.54)	419.31 (399.41, 440.20)	401.41 (382.80, 420.93)	420.27 (400.39, 441.14)	--	--	--	--
Food intake (g)	281.20 (251.70, 310.70)	289.26 (259.76, 318.76)	234.44 (205.06, 263.82)	337.23 (307.73, 366.72)	--	--	--	--
Body weight (kg)	24.99 (24.46, 25.51)	24.44 (23.91, 24.96)	24.50 (23.98, 25.02)	25.19 (24.66, 25.72)	--	--	--	--
MALES								
Activity (min)	437.34 (416.74, 458.95)	515.13 (490.70, 540.78)	493.15 (470.32, 517.10)	516.32 (491.67, 542.21)	--	--	--	--
Food intake (g)	397.35 (367.85, 426.85)	445.98 (416.44, 475.53)	415.45 (386.06, 444.84)	454.11 (424.60, 483.62)	--	--	--	--
Body weight (kg)	27.03 (26.49, 27.56)	26.48 (25.94, 27.00)	26.54 (26.02, 27.06)	27.23 (26.70, 27.76)	--	--	--	--

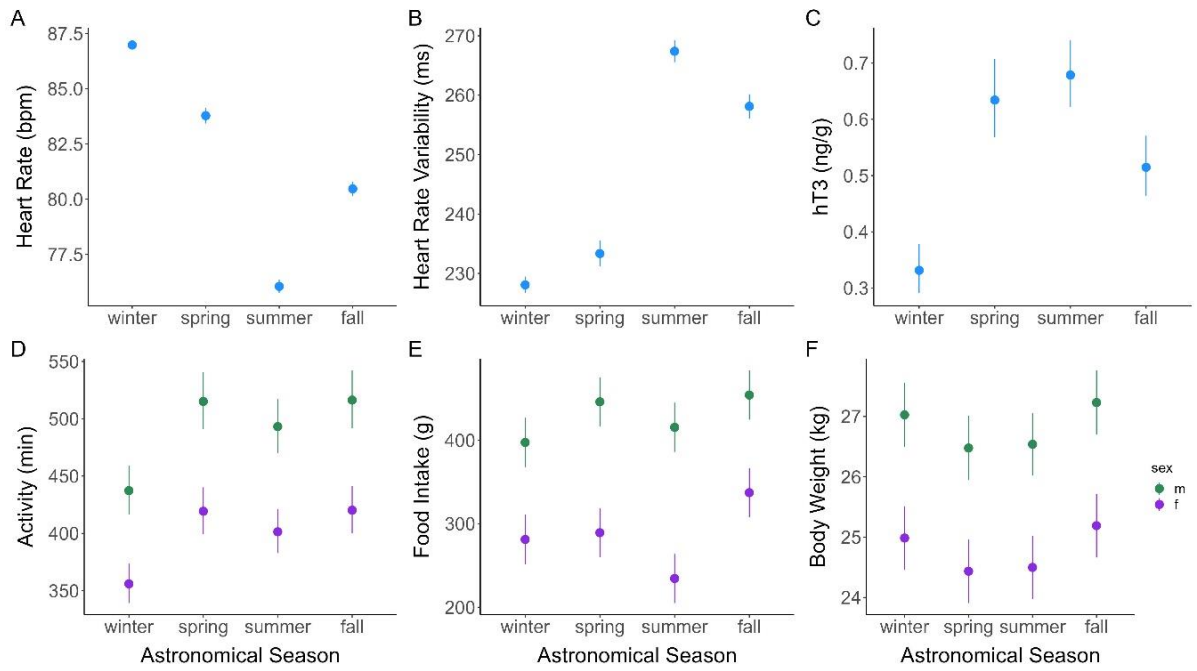


Figure 1. Estimated marginal means (EMMs) from final models concerning the influence of sex and astronomical season on daily average heart rate (A), daily average heart rate variability (B), hair triiodothyronine (hT3; C), daily total activity (D), daily food intake (E), and monthly average body weight (F) in maned wolves located at the Smithsonian National Zoo and Conservation Biology Institute in Front Royal, VA, USA. EMMs for models containing multiple categorical predictors were calculated using the *emmean* function within the *emmeans* R package. 95% confidence intervals are displayed as whiskers extending from EMM points. Colors indicate sex (blue = both sexes, purple = females, and green = males). See Appendix 3 for full model summaries.

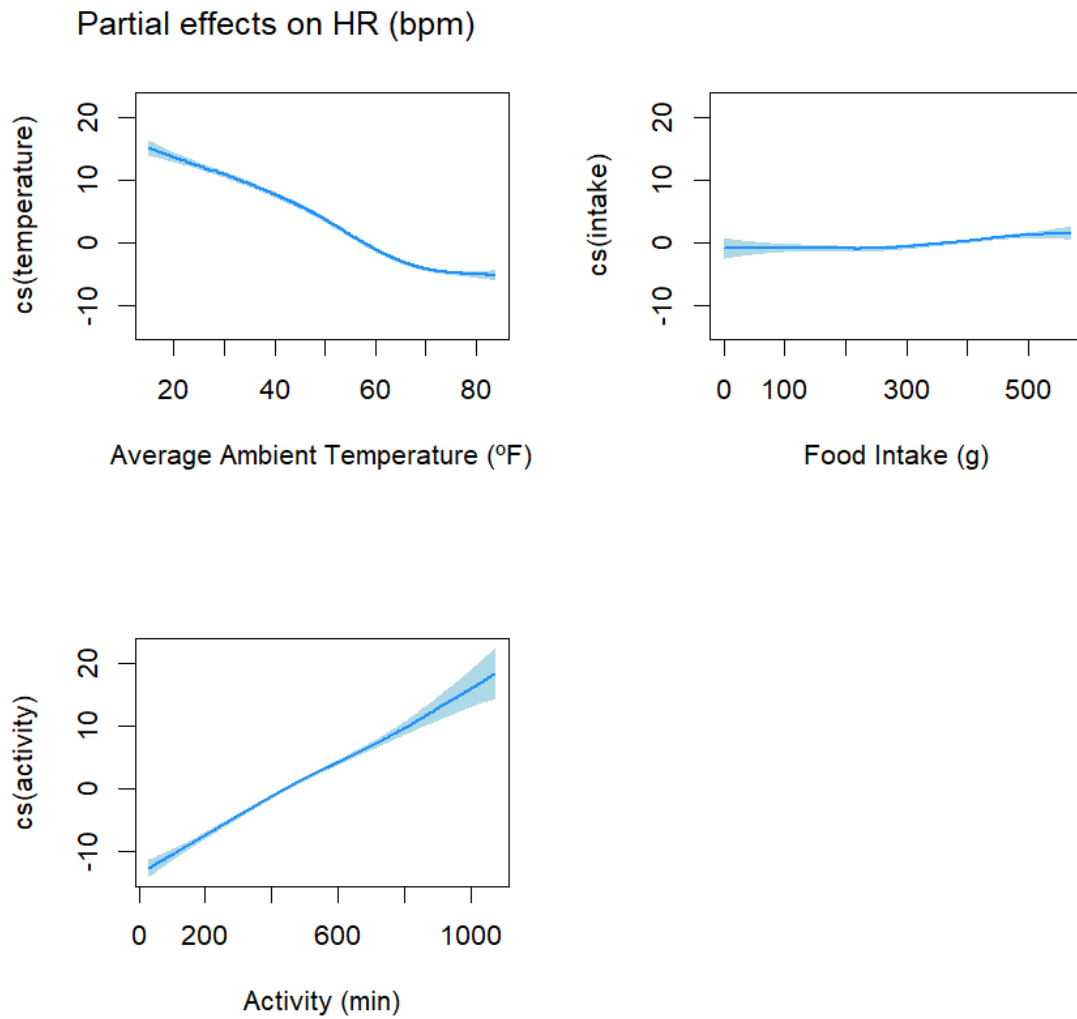


Figure 2. Termpart for generalized additive model for location, scale, and shape testing the impact of daily average ambient temperature, daily food intake, and daily activity on daily average heart rate (HR) in maned wolves located at the Smithsonian National Zoo and Conservation Biology Institute in Front Royal, VA, USA. Plots indicate partial effect of explanatory variables on HR. HR estimates are represented by blue trendlines [cubic spline (cs) smooth predictors] and their standard errors by blue shaded area. See Appendix 3-3 for full model summary.

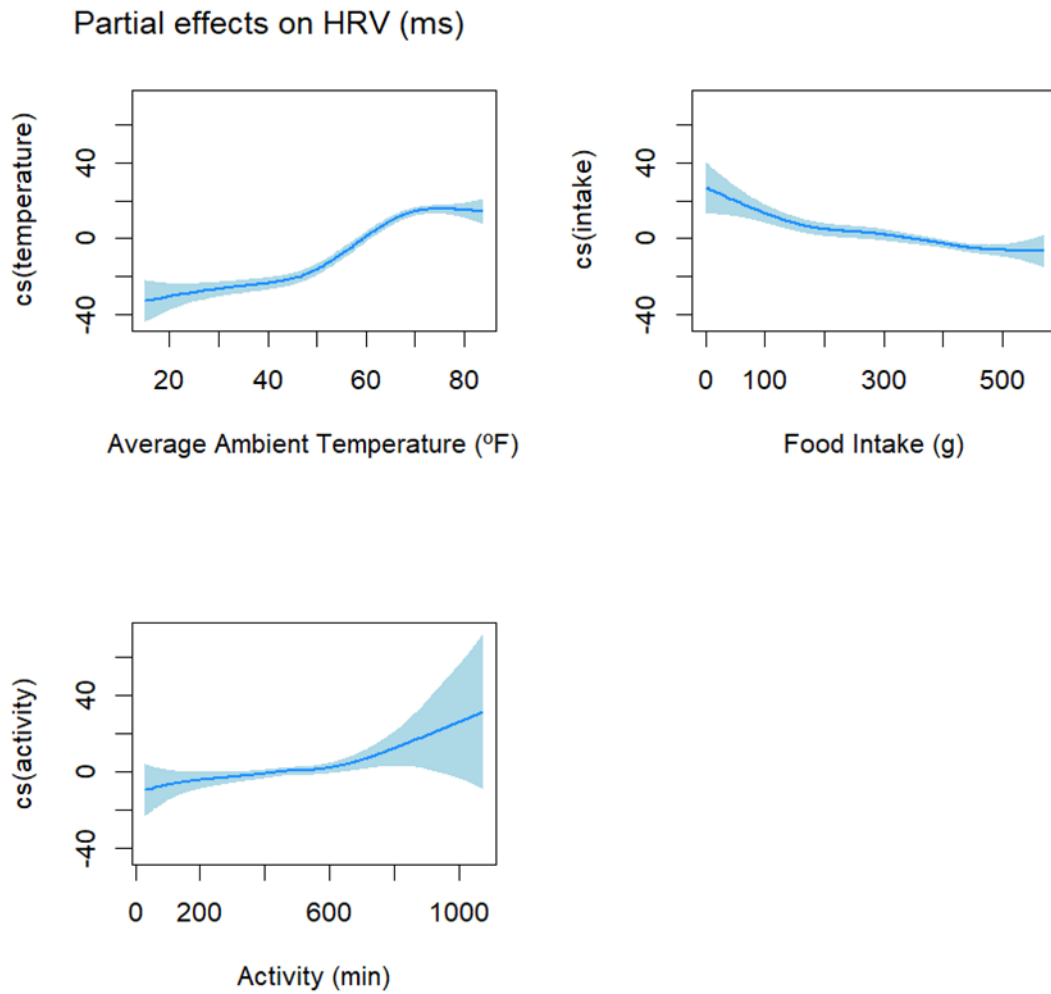


Figure 3. Termpart for generalized additive model for location, scale, and shape testing the impact of daily average ambient temperature, daily food intake, and daily activity on daily average heart rate variability (HRV) in maned wolves located at the Smithsonian National Zoo and Conservation Biology Institute in Front Royal, VA, USA. Plots indicate partial effect of explanatory variables on HRV. HRV estimates are represented by blue trendlines [cubic spline (cs) smooth predictors] and their standard errors by blue shaded area. See Appendix 3-3 for full model summary.

Not all hair samples were able to be analyzed for T3 concentrations due to limited sample sizes. However, even with the reduced sample size ($n = 30$), model comparisons indicated that astronomical season and ambient temperature significantly impacted hT3 (Appendix 2-5, 2-6, 3-5, 3-6). Hair T3 was lowest in winter at 0.33 ng/g [CI, (0.29, 0.38)] and highest in summer at 0.68 ng/g [CI, (0.62, 0.74)], followed by spring at 0.63 ng/g [CI, (0.57, 0.71)] and then fall at 0.51 ng/g [CI, (0.46, 0.57)]. Estimated marginal means for hT3 within all seasons are reported in Table 2. In the model containing continuous predictors, ambient temperature displayed a positive association with hT3 (Figure 4). For every 1°F increase in ambient temperature, hT3 was expected to increase by 0.17 ng/g (Table 2). Sex, food intake, and body weight did not provide enough explanatory information to remain in the final model. The final model containing only astronomical season explained 58.4% of the observed variation in hT3, whereas the final model containing only ambient temperature explained only 34.1% of the observed variation in hT3.

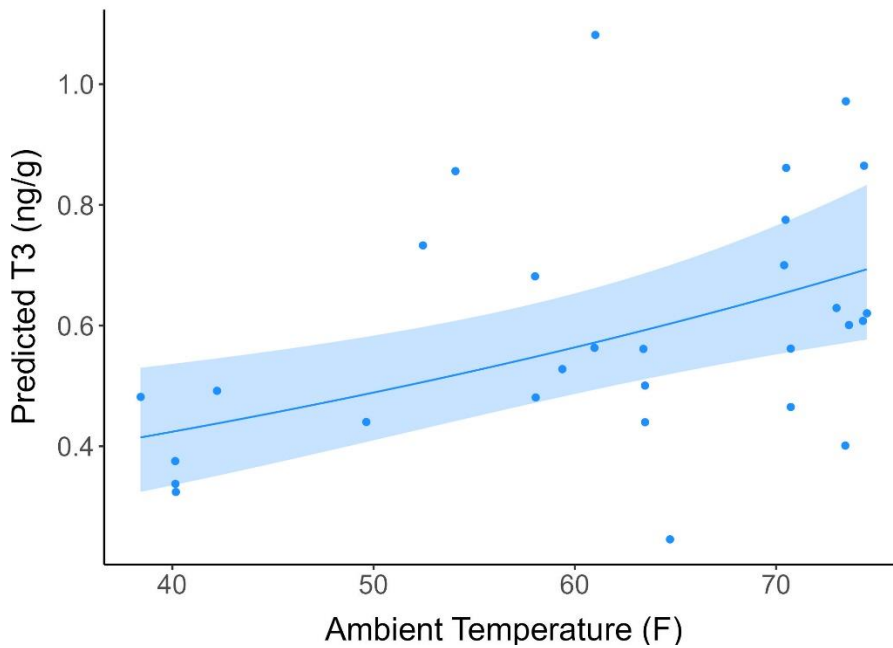


Figure 4. Predicted values of hair triiodothyronine (T3) in response to changes in ambient temperature in captive maned wolves located at the Smithsonian’s National Zoo and Conservation Biology Institute in Front Royal, VA, USA.

Model comparisons revealed that sex and astronomical season significantly influenced daily activity (Appendix 2-7 and 3-7), daily food intake (Appendix 2-8 and 3-8), and average monthly body weight (Appendix 2-9 and 3-9), with seasonal patterns in daily food intake differing significantly by sex (Figure 1; Appendix 3-8). Across all response variables, males displayed greater values than females. Estimated marginal means for all astronomical seasons, and sexes if relevant, are reported in Table 2.

DISCUSSION

The goal of this study was to provide additional methods – specifically, T3 analysis of hair – that when integrated with biologging data, could help identify factors impacting changes in metabolic activity in maned wolves.

MEASURING T3 IN HAIR

Hair growth rate is an important methodological consideration in endocrine studies and can vary substantially by species, body location, an animal's external environment, and its internal physiology (Koren et al., 2019). The average growth rate of maned wolf hair samples measured in this study was 1.82 cm per month. This estimated growth rate indicates that by only utilizing the first 5 cm of hair proximal to the animal's body, the hormone concentrations measured reflect approximately the most recent three months of growth as expected. However, the growth rate of samples differed substantially between individuals and between samples from the same individual collected in consecutive months (Appendix 1-1). While these data may provide a preliminary understanding of the growth rate of guard hair in maned wolves, hair growth rate is likely to vary by season and individual, among other factors, warranting further investigation utilizing significantly larger sample sizes.

Previous studies have quantified hormones, namely glucocorticoids, in keratinized tissues (e.g. hair, claws) from canid species (Accorsi et al., 2008; Bennett & Hayssen, 2010; Bryan et al., 2013; Grigg et al., 2017; Pereira et al., 2022; Roffler et al., 2022; Roth et al., 2016; Schell et al., 2017); however, this study is the first to report concentrations of

T3 in a canid keratin tissue. There also are few reports of T3 measurement in keratin samples more broadly in mammals (Hunt et al., 2017).

I successfully extracted and quantified T3 from maned wolf hair using a commercially available ELISA. Additionally, comparison of extraction protocols for maned wolf hair using 80% EtOH and 100% MeOH indicated no significant difference in hormone yield, suggesting both are appropriate extraction mediums.

SEASONAL PATTERNS IN METABOLIC ACTIVITY

Seasonal fluctuations in wildlife behavior and physiology are well-documented (Dalton et al., 2015; Dardente, 2012; Gesquiere et al., 2018; Houser et al., 2021; McClune et al., 2015; Shave et al., 2020; Thiel et al., 2022), and were evident in all physiological and behavioral parameters considered in this study. As expected, HR and HRV displayed opposing seasonal patterns (Figure 1). Notably, HR increased and HRV decreased in winter, supporting my prediction that winter would constitute a period of increased metabolic activity. When considering the potential impacts of ambient temperature, activity, and food intake, it appears that the influence of ambient temperature on HR and HRV primarily drives this trend, with ambient temperature exhibiting a negative association with HR and a positive association with HRV (Figure 2). These findings are consistent with controlled laboratory studies conducted on small mammals, which consistently observe increased heart rate in colder temperatures (Bernet et al., 1975; Swoap et al., 2004). In contrast, species native to high latitudes may show specific adaptations to preserve energetic resources in cold winters (e.g. hibernation as an extreme case, reduced basal metabolic rate in some other cases), and thus, some studies

demonstrate decreases in HR associated with cold temperatures (e.g. ruminants, ursids, and some birds; Græsli, Thiel, et al., 2020; Moen, 1978; Pohlin et al., 2017; Thiel et al., 2022; Wascher et al., 2018). My data suggest that tropical or subtropical species such as the maned wolf may, rather, show increases in HR in cold temperatures.

The impacts of activity and food intake on maned wolves' HR and HRV were relatively minimal (albeit statistically significant). However, maned wolves did show seasonal patterns in activity, food intake, and body weight, which differed by sex in the case of food intake (Figure 1). Seasonal activity patterns did not fully support my prediction that activity would be lowest in summer. Maned wolves' activity was markedly lower in winter compared to other seasons; however, summer did not show maximal activity, but rather, activity was still lower in summer compared to spring and fall (Figure 2.) As mentioned previously, maned wolves in human care do not experience the same environmental pressures as their free-ranging counterparts, which may be the cause of differing activity patterns. Taken together, findings do not suggest nutritional stress or metabolic imbalances of clinical significance in any season; even during periods of increased activity and food intake with a concurrent loss in body weight, the overall range of body weights was relatively narrow, e.g. no animals became emaciated and maximum fluctuation in body weight across the study period displayed by an individual was 2.6 kg (Apollo). Interestingly, activity, food intake, and body weight were greatest in fall for both sexes, which may be related to reproduction, which occurs from October to February for maned wolves within the northern hemisphere (Songsasen & Rodden, 2010).

Patterns in hT3 in this study were opposite to predictions. I observed a seasonal pattern of hT3 rising in summer (Figure 2), with hT3 exhibiting a positive correlation to ambient temperature, contrary to my prediction that hT3 would peak in winter. Thyroid hormones are thought to provide insight into basal metabolic requirements, which are influenced by prolonged physiological or environmental conditions (Behringer et al., 2018). Numerous studies detail the influence of seasonal changes in temperature and food availability on THs. In temperate regions, T3 concentrations typically increase in colder months and decrease in hotter months within temperate regions (Hunninck et al., 2020; Thompson et al., 2017; Tomasi & Mitchell, 1994). In tropical regions with wet/dry seasons, studies report decreased T3 concentrations in wildlife in the dry season and increased T3 in the wet season (Gesquiere et al., 2018; Thompson et al., 2017). When T3 is increased in warmer months it is typically explained by increased energetic requirements associated with reproductive and molting periods (Myers et al., 2006). Finally, there are also studies that report no detectable variation in T3 across seasons (Williams et al., 2019).

One possible explanation for my results is that the maned wolves in this study had continual access to temperature-controlled dens, which may have reduced the need for increased basal metabolic rate (adaptive thermogenesis) in times of cold ambient temperatures. This interpretation is supported by my finding that maned wolves display decreased activity in winter, potentially because they are spending a greater proportion of time in heated dens. It would therefore be informative to assess hT3 in wild maned wolves *in situ*.

The lack of correlation between seasonal peaks in HR and hT3 was unexpected. Assuming my interpretation of the time period represented by the hT3 data is correct (see next), one possible explanation for the winter pattern of HR increasing and HRV decreasing, yet without T3 deviating from baseline concentrations, could be if HR changes were related primarily to non-metabolic stressors (e.g. psychological). There may also have been additional factors not considered in this study that vary with season and temperature and influence this pattern. Further research utilizing much larger sample sizes, ideally including free-roaming animals *in situ*, could help to determine whether hT3 and HR normally show an inverse relationship in a field context. Additionally, incorporating analyses of hair glucocorticoids may help indicate if additional sources of stress are responsible for increased SNS activation.

My interpretation of hT3 data rest upon assumptions regarding the time period assumed to correspond with T3 concentrations of hair collected on a given date, i.e. when was T3 actually incorporated into the hair. These assumptions may be incorrect. In a controlled setting, the “shave-re-shave” method of hair collection – which involves shaving the hair from a specific area and then re-shaving the exact same location after a designated amount of time to ensure collection of only anagen hairs – is the gold standard for collection of samples for hormone analyses (Koren et al., 2019). I initially set out to employ this method; however, it was logistically challenging to repeatedly shave the identical location on non-sedated maned wolves with precision. Thus, unavoidable deviations in the exact area shaved may have resulted in quiescent hairs (i.e., in the telogen stage) that had been grown at prior times (possibly months before collection), and

that therefore may contain hormones from different time periods. If this were the case, hT3 concentrations may represent cumulative concentrations over longer periods of time than originally estimated.

Additionally, there are many other characteristics of hair samples that may impact hormone concentrations, such as color, body region, hair length, and even method of collection, among others (Acker et al., 2018; Baier et al., 2019; Bennett & Hayssen, 2010; Braun et al., 2017; Heimbürge et al., 2020; LeBeau et al., 2011), and some of these possible explanatory variables could not be assessed in the present study. For instance, shorter hair samples may contain lower concentrations of hormones regardless of duration of growth (Baier et al., 2019) and hair growth may be slower in winter (Diaz et al., 2004). Future studies on hair hormone concentrations of maned wolves could address some of these potentially important factors, e.g., comparing short to long hairs, or comparing regrowth rate of shaved regions in winter vs, summer.

In conclusion, all variables assessed in this study – HR, HRV, activity, food intake, body weight, and hT3 – showed significant seasonal variation, and significant associations with each other, in eight maned wolves housed in *ex situ*, with HR and HRV in particular demonstrating the expected relationships with ambient cold temperatures. Although hT3 patterns did not match my predictions, hT3 did show significant seasonal variation, and could therefore be an informative supplement to biollogger-derived data. However, I recommend additional studies with larger sample sizes that focus on understanding growth rate of hair in this species (between and across individuals, and in relation to other factors such as season, ambient temperature, animal health, etc.).

Nevertheless, I demonstrate that we can quantify T3 in maned wolf hair and observe seasonal variation, which in the context of additional data could help to determine the impact of metabolic stressors on cardiac patterns. Successful validation of these methods will provide a novel framework for long-term monitoring of maned wolves' metabolic activity. By capitalizing on the value of hair for retrospective endocrine analysis concerning species *in situ*, these methods will be directly applicable to free-ranging populations responding to environmental change.

APPENDIX 1: HAIR GROWTH RATE ESTIMATE

A1-1: Average hair length within hair samples (n = 3) used to estimate hair growth rate in maned wolves.

Subject	Collection date	Location	Hair count	Ave length (cm)
Oceano	2021-08-30	Left shoulder	170	1.58
Oceano	2021-09-28	Right shoulder	147	2.26
Avara	2021-10-01	Left shoulder	220	1.62

APPENDIX 2: AIC-BASED MODEL SELECTION

A2-1: AIC-based model selection to determine the best fit for generalized additive models for location, scale, and shape assessing the impact of sex and astronomical season on daily average heart rate in captive maned wolves ($n = 8$) housed at the Smithsonian's National Zoo and Conservation Biology Institute between June 2021 and October 2022.

Covariates	AIC	dAIC
intercept + season + (1 subject)	35726	0.00
intercept + sex + season + (1 subject)	35728	2.00
intercept + sex + (1 subject)	36208	482.00
intercept + sex + season	37436	1710.00

A2-2. AIC-based model selection to determine the best fit for generalized additive models for location, scale, and shape assessing the impact of sex, ambient temperature, activity, and food intake on daily average heart rate in captive maned wolves ($n = 8$) housed at the Smithsonian’s National Zoo and Conservation Biology Institute between June 2021 and October 2022. Continuous predictors were fit with cubic splines (cs) using the *cs* function of the *gamlss* package in R.

Covariates	AIC	dAIC
intercept + cs (ambient temp) + cs (activity) + cs (food intake) + (1 subject)	18706.00	0.00
intercept + sex + cs (ambient temp) + cs (activity) + cs (food intake) + (1 subject)	18708.00	2.00
intercept + sex + cs (ambient temp) + cs (activity) + (1 subject)	18743.00	37.00
intercept + sex + cs (ambient temp) + cs (activity) + cs (food intake)	19392.00	686.00
intercept + sex + cs (ambient temp) + cs (food intake) + (1 subject)	20039.00	1333.00
intercept + sex + cs (activity) + cs (food intake) + (1 subject)	20794.00	2088.00

A2-3: AIC-based model selection to determine the best fit for generalized additive models for location, scale, and shape assessing the impact of sex and astronomical season on daily average heart rate variability in captive maned wolves (n = 8) housed at the Smithsonian’s National Zoo and Conservation Biology Institute between June 2021 and October 2022.

Covariates	AIC	dAIC
intercept + season + (1 subject)	35726.00	0.00
intercept + sex + season + (1 subject)	35728.00	2.00
intercept + sex + (1 subject)	36208.00	482.00
intercept + sex + season	37436.00	1710.00

A2-4. AIC-based model selection to determine the best fit for generalized additive models for location, scale, and shape assessing the impact of sex, ambient temperature, activity, and food intake on daily average heart rate variability in captive maned wolves (n = 8) housed at the Smithsonian’s National Zoo and Conservation Biology Institute between June 2021 and October 2022. Continuous predictors were fit with cubic splines (cs) using the *cs* function of the *gamlss* package in R.

Covariates	AIC	dAIC
intercept + cs (ambient temp) + cs (activity) + cs (food intake) + (1 subject)	28891.00	0.00
intercept + sex + cs (ambient temp) + cs (activity) + cs (food intake) + (1 subject)	28893.00	2.00
intercept + sex + cs (ambient temp) + cs (food intake) + (1 subject)	28905.00	14.00
intercept + sex + cs (ambient temp) + cs (activity) + (1 subject)	28914.00	23.00
intercept + sex + cs (activity) + cs (food intake) + (1 subject)	29367.00	476.00
intercept + sex + cs (ambient temp) + cs (activity) + cs (food intake)	30006.00	1115.00

A2-5: AIC-based model selection to determine the best fit for generalized linear mixed model assessing the impact of sex and astronomical season on seasonal hair triiodothyronine (hT3) in captive maned wolves (n = 8) housed at the Smithsonian's National Zoo and Conservation Biology Institute between June 2021 and October 2022.

Covariates	AIC	dAIC
intercept + season + (1 subject)	-18.23	0.00
intercept + sex + season + (1 subject)	-15.12	3.11
intercept + (1 subject)	-6.53	11.70
intercept + sex + (1 subject)	-4.10	14.13

A2-6. AIC-based model selection to determine the best fit for generalized linear mixed model assessing the impact of sex, ambient temperature, activity, food intake, and body weight on seasonal hair triiodothyronine (hT3) in captive maned wolves (n = 8) housed at the Smithsonian’s National Zoo and Conservation Biology Institute between June 2021 and October 2022. Continuous predictors were standardized using the *scale* function in base R.

Covariates	AIC	dAIC
intercept + ambient temp + (1 subject)	-12.36	0.00
intercept + ambient temp + activity + (1 subject)	-9.75	2.61
intercept + ambient temp + body weight + (1 subject)	-9.58	2.78
intercept + ambient temp + food intake + (1 subject)	-9.52	2.84
intercept + sex + ambient temp + (1 subject)	-9.52	2.84
intercept + ambient temp + activity + body weight + (1 subject)	-6.61	5.75
intercept + ambient temp + food intake + activity + (1 subject)	-6.54	5.82
intercept + ambient temp + food intake + body weight + (1 subject)	-6.38	5.98
intercept + (1 subject)	-6.37	5.99
intercept + ambient temp + activity + food intake + body weight + (1 subject)	-3.15	9.21
intercept + activity + food intake + body weight + (1 subject)	-1.35	11.02
intercept + sex + ambient temp + activity + food intake + body weight + (1 subject)	0.65	13.02

A2-7: AIC-based model selection to determine the best fit for generalized linear mixed model assessing the impact of sex and astronomical season on daily total activity in captive maned wolves (n=8) housed at the Smithsonian’s National Zoo and Conservation Biology Institute between June 2021 and October 2022.

Covariates	AIC	dAIC
intercept + sex + season + (1 subject)	39757.42	0.00
intercept + season + (1 subject)	39761.78	4.36
intercept + sex + (1 subject)	39851.03	93.61
intercept + (1 subject)	39855.17	97.76
intercept + sex * season + (1 subject)	model would not converge	

A2-8: AIC-based model selection to determine the best fit for linear mixed model assessing the impact of sex and astronomical season on daily food intake in captive maned wolves (n = 8) housed at the Smithsonian’s National Zoo and Conservation Biology Institute between June 2021 and October 2022.

Covariates	AIC	dAIC
intercept + sex * season + (1 subject)	38638.59	0.00
intercept + sex + season + (1 subject)	38766.22	127.63
intercept + season + (1 subject)	38773.34	134.75
intercept + sex + (1 subject)	39211.50	572.90
intercept + (1 subject)	39218.50	579.91

A2-9: AIC-based model selection to determine the best fit for linear mixed model assessing the impact of sex and astronomical season on monthly average body weight in captive maned wolves (n = 8) housed at the Smithsonian’s National Zoo and Conservation Biology Institute between June 2021 and October 2022.

Covariates	AIC	dAIC
intercept + sex + season + (1 subject)	183.67	0.00
intercept + sex * season + (1 subject)	185.32	1.65
intercept + season + (1 subject)	187.98	4.30
intercept + sex + (1 subject)	198.39	14.72
intercept + (1 subject)	202.830.00	19.16

APPENDIX 3: MODEL SUMMARIES

A3-1: Summary of generalized additive model for location, scale, and shape (Johnson’s SU distribution, mu identity link) assessing the impact of astronomical season on daily average heart rate (HR; bpm) in captive maned wolves (n = 8; Formula: $HR \sim \text{season} + \text{re}(\text{random} = \sim 1|\text{subject})$); Generalized R^2 [Coxsnell] = 0.375) housed at the Smithsonian’s National Zoo and Conservation Biology Institute between June 2021 and October 2022.

Covariate	Estimate	Std. Error	t	P
Intercept	86.98	0.2646	328.69	< 0.001
Spring	-3.21	0.3866	-8.32	< 0.001
Summer	-10.95	0.3362	-32.57	< 0.001
Fall	-6.53	0.3932	-16.61	< 0.001

A3-2: Summary of generalized additive model for location, scale, and shape (Johnson’s SU distribution, mu identity link) assessing the impact of ambient temperature, activity, and food intake on daily average heart rate (HR; bpm) in captive maned wolves (n = 8; Formula: $HR \sim \text{cs}(\text{ambient temp}) + \text{cs}(\text{activity}) + \text{cs}(\text{food intake}) + \text{re}(\text{random} = \sim 1|\text{subject})$); Generalized R^2 [Coxsnell] = 0.676) housed at the Smithsonian’s National Zoo and Conservation Biology Institute between June 2021 and October 2022.

Continuous predictors were fit with cubic splines using the *cs* function of the *gamlss* package in R.

Covariate	Estimate	Std. Error	t	P
Intercept	75.53	0.3727	202.68	< 0.001
cs (ambient temperature)	-0.62	0.0116	-53.52	< 0.001
cs (activity)	0.005	0.0008	6.45	< 0.001
cs (food intake)	0.028	0.0007	43.45	< 0.001

A3-3: Summary of generalized additive model for location, scale, and shape (Johnson’s SU distribution, mu identity link) assessing the impact of astronomical season on daily average heart rate variability (HRV; ms) in captive maned wolves (n = 8; Formula: HRV ~ season + re (random = ~1|subject); Generalized R² [Coxsnell] = 0.468) housed at the Smithsonian’s National Zoo and Conservation Biology Institute between June 2021 and October 2022.

Covariate	Estimate	Std. Error	t	P
Intercept	228.07	1.436	158.79	< 0.001
Spring	5.27	2.203	2.39	0.02
Summer	39.33	1.797	21.89	< 0.001
Fall	30.06	2.252	13.35	< 0.001

A3-4: Summary of generalized additive model for location, scale, and shape (Johnson’s SU distribution, mu identity link) assessing the impact of ambient temperature, activity, and food intake on daily average heart rate variability (HRV; ms) in captive maned wolves (n = 8; Formula: HRV ~ cs (ambient temp) + cs (activity) + cs (food intake) + re (random = ~1|subject); Generalized R² [Coxsnell] = 0.505) housed at the Smithsonian’s National Zoo and Conservation Biology Institute between June 2021 and October 2022. Continuous predictors were fit with cubic splines using the *cs* function of the *gamlss* package in R.

Covariate	Estimate	Std. Error	t	P
Intercept	228.92	2.8803	79.478	< 0.001
cs (ambient temperature)	1.78	0.0808	21.996	< 0.001
cs (activity)	-0.04	0.0063	-7.095	< 0.001
cs (food intake)	0.02	0.0052	3.909	< 0.001

A3-5: Summary of generalized linear mixed model (Gamma distribution, log link) assessing the impact of astronomical season on hair triiodothyronine (hT3; ng/h) in captive maned wolves (n = 8; Formula: $hT3 \sim \text{season} + (1|\text{subject})$; Marginal R^2 [trigamma] = 0.48) housed at the Smithsonian’s National Zoo and Conservation Biology Institute between June 2021 and October 2022.

Covariate	Estimate	Std. Error	t	P
Intercept	-1.10	0.1309	-8.44	< 0.001
Spring	0.65	0.1434	4.53	< 0.001
Summer	0.72	0.1272	5.63	< 0.001
Fall	0.44	0.1404	3.14	0.002

A3-6: Summary of generalized linear mixed model (Gamma distribution, log link) assessing the impact of ambient temperature on hair triiodothyronine (hT3; ng/g) in captive maned wolves (n = 8; Formula: $hT3 \sim \text{ambient temp} + (1|\text{subject})$; Marginal R^2 [trigamma] = 0.48) housed at the Smithsonian’s National Zoo and Conservation Biology Institute between June 2021 and October 2022. Continuous predictors were standardized using the *scale* function in base R.

Covariate	Estimate	Std. Error	t	P
Intercept	-0.55	0.07476	-7.41	< 0.001
Ambient temperature	0.17	0.05250	3.27	0.001

A3-7: Summary of generalized linear mixed model (negative binomial distribution, log link) assessing the impact of sex and astronomical season on daily total activity (min) in captive maned wolves (n = 8; Formula: activity ~ sex + season + (1|subject); Marginal R² [trigamma] = 0.48) housed at the Smithsonian’s National Zoo and Conservation Biology Institute between June 2021 and October 2022.

Covariate	Estimate	Std. Error	t	P
Intercept	5.87	0.0481	122.03	< 0.001
Male	0.21	0.0663	3.10	0.002
Spring	0.16	0.0190	8.61	< 0.001
Summer	0.12	0.0159	7.58	< 0.001
Fall	0.17	0.0192	8.65	< 0.001

A3-8: Summary of linear mixed model assessing the impact of sex and astronomical season on daily food intake (g) in captive maned wolves (n = 8; Formula: food intake ~ sex * season + (1|subject); Marginal R² = 0.432) housed at the Smithsonian’s National Zoo and Conservation Biology Institute between June 2021 and October 2022.

Covariate	Estimate	Std. Error	t	CI (2.5%, 97.5%)
Intercept	281.20	29.4993	9.53	221.90, 337.13
Male	116.15	41.7174	2.78	41.26, 200.38
Spring	8.06	5.4537	1.48	-3.56, 20.37
Summer	-46.76	4.7409	-9.86	-56.52, -36.33
Fall	56.03	5.4224	10.33	45.00, 67.36
Male:Spring	40.57	7.8641	5.16	24.39, 56.16
Male:Summer	64.86	6.7530	9.60	51.84, 77.99

A3-9: Summary of linear mixed model assessing the impact of sex and astronomical season on monthly average body weight (kg) in eight captive maned wolves (n = 8; Formula: body weight ~ sex + season + (1|subject); Marginal R² = 0.439) housed at the Smithsonian’s National Zoo and Conservation Biology Institute between June 2021 and October 2022.

Covariate	Estimate	Std. Error	t	CI (2.5%, 97.5%)
Intercept	24.99	0.5279	47.33	23.86, 26.01
Male	2.04	0.7289	2.80	0.42, 3.51
Spring	-0.55	0.1952	-2.82	-0.93, -0.17
Summer	-0.49	0.1710	-2.85	-0.81, -0.16
Fall	0.20	0.1964	1.04	-0.16, 0.56

APPENDIX 4: COMPARISON OF SOLVENTS FOR HAIR EXTRACTION

To compare the efficacy of 100% MeOH and 80% ethanol (EtOH) for T3 extraction from maned wolf hair samples, I assayed serial dilutions (1:1, 1:2, 1:4, and 1:8) of pooled MeOH hair extract and pooled EtOH hair extract (extracted using the same protocol described for MeOH) from the same hair samples, and compared the apparent hormone concentration detected with 100% MeOH extraction vs 80% EtOH (20% distilled water) extraction. I selected these two solvents for comparison as they are the most common extraction solvents reported in the literature for endocrine studies of non-plasma samples, with 100% MeOH having the advantage of low cost, rapid evaporation throughput, while 80% EtOH often can improve extraction efficiency of thyroid hormones, but also involves higher cost and a greatly reduced throughput due to slower evaporation time. A paired t-test was used to determine whether hair extraction in 100% MeOH or 80% EtOH yielded higher concentrations of hT3 by comparing hT3 concentrations across serial dilutions. Results revealed no significant difference in T3 concentrations across serial dilutions of hair extracted in 100% MeOH and 80% EtOH. Therefore, all subsequent extractions utilized 100% MeOH due to advantages of cost and throughput.

REFERENCES

- Accorsi, P. A., Carloni, E., Valsecchi, P., Viggiani, R., Gamberoni, M., Tamanini, C., & Seren, E. (2008). Cortisol determination in hair and faeces from domestic cats and dogs. *General and Comparative Endocrinology*, *155*(2), 398–402.
<https://doi.org/10.1016/j.ygcen.2007.07.002>
- Acker, M., Mastromonaco, G., & Schulte-Hostedde, A. I. (2018). The effects of body region, season and external arsenic application on hair cortisol concentration. *Conservation Physiology*, *6*(1). <https://doi.org/10.1093/conphys/coy037>
- Ayres, K. L., Booth, R. K., Hempelmann, J. A., Koski, K. L., Emmons, C. K., Baird, R. W., Balcomb-Bartok, K., Hanson, M. B., Ford, M. J., & Wasser, S. K. (2012). Distinguishing the Impacts of Inadequate Prey and Vessel Traffic on an Endangered Killer Whale (*Orcinus orca*) Population. *PLoS ONE*, *7*(6), e36842.
<https://doi.org/10.1371/journal.pone.0036842>
- Baier, F., Grandin, T., Engle, T., & Edwards-Callaway, L. (2019). Evaluation of hair characteristics and animal age on the impact of hair cortisol concentration in feedlot steers. *Frontiers in Veterinary Science*, *6*, 323.
<https://doi.org/10.3389/fvets.2019.00323>
- Barbosa, P., Schumaker, N. H., Brandon, K. R., Bager, A., & Grilo, C. (2020). Simulating the consequences of roads for wildlife population dynamics. *Landscape and Urban Planning*, *193*.
<https://doi.org/10.1016/j.landurbplan.2019.103672>

- Bartoń K (2023). *_MuMIn: Multi-Model Inference_*. R package version 1.47.5, <<https://CRAN.R-project.org/package=MuMIn>>.
- Bechshøft, T. Ø., Sonne, C., Rigét, F. F., Letcher, R. J., Novak, M. A., Henchey, E., Meyer, J. S., Eulaers, I., Jaspers, V. L. B., Covaci, A., & Dietz, R. (2013). Polar bear stress hormone cortisol fluctuates with the North Atlantic Oscillation climate index. *Polar Biology*, *36*(10), 1525–1529. <https://doi.org/10.1007/s00300-013-1364-y>
- Behringer, V., Deimel, C., Hohmann, G., Negrey, J., Schaebs, F. S., & Deschner, T. (2018). Applications for non-invasive thyroid hormone measurements in mammalian ecology, growth, and maintenance. *Hormones and Behavior*, *105*, 66–85. <https://doi.org/10.1016/j.yhbeh.2018.07.011>
- Bennett, A., & Hayssen, V. (2010). Measuring cortisol in hair and saliva from dogs: Coat color and pigment differences. *Domestic Animal Endocrinology*, *39*(3), 171–180. <https://doi.org/10.1016/j.domaniend.2010.04.003>
- Bernet, F., Collache, M., & Denimal, J. (1975). Influence de la température ambiante sur le métabolisme et la fréquence cardiaque du rat albinos, au repos. *Archives Internationales de Physiologie et de Biochimie*, *83*(4), 633–645. <https://doi.org/10.3109/13813457509081883>
- Birnie-Gauvin, K., Peiman, K. S., Raubenheimer, D., & Cooke, S. J. (2017). Nutritional physiology and ecology of wildlife in a changing world. *Conservation Physiology*, *5*(1). <https://doi.org/10.1093/conphys/cox030>

- Braun, U., Michel, N., Baumgartner, M. R., Hässig, M., & Binz, T. M. (2017). Cortisol concentration of regrown hair and hair from a previously unshorn area in dairy cows. *Research in Veterinary Science*, *114*, 412–415.
<https://doi.org/10.1016/j.rvsc.2017.07.005>
- Briefer, E. F., Oxley, J. A., & McElligott, A. G. (2015). Autonomic nervous system reactivity in a free-ranging mammal: Effects of dominance rank and personality. *Animal Behaviour*, *110*, 121–132. <https://doi.org/10.1016/j.anbehav.2015.09.022>
- Brown, D. D., Kays, R., Wikelski, M., Wilson, R., & Klimley, A. P. (2013). Observing the unwatchable through acceleration logging of animal behavior. *Animal Biotelemetry*, *1*(1), 20. <https://doi.org/10.1186/2050-3385-1-20>
- Bryan, H. M., Adams, A. G., Invik, R. M., Wynne-Edwards, K. E., & Smits, J. E. (2013). Hair as a meaningful measure of baseline cortisol levels over time in dogs. *Journal of the American Association for Laboratory Animal Science*, *52*(2).
- Bryan, H. M., Darimont, C. T., Paquet, P. C., Wynne-Edwards, K. E., & Smits, J. E. G. (2014). Stress and reproductive hormones reflect inter-specific social and nutritional conditions mediated by resource availability in a bear-salmon system. *Conservation Physiology*, *2*(1), cou010–cou010.
<https://doi.org/10.1093/conphys/cou010>
- Carravieri, A., Müller, M. S., Yoda, K., Hayama, S., & Yamamoto, M. (2016). Dominant parasympathetic modulation of heart rate and heart rate variability in a wild-caught seabird. *Physiological and Biochemical Zoology*, *89*(4), 263–276.
<https://doi.org/10.1086/686894>

- Carvalho, F. M. V., De Marco, P., & Ferreira, L. G. (2009). The Cerrado into-pieces: Habitat fragmentation as a function of landscape use in the savannas of central Brazil. *Biological Conservation*, *142*(7), 1392–1403.
<https://doi.org/10.1016/j.biocon.2009.01.031>
- Chaise, L. L., Paterson, W., Laske, T. G., Gallon, S. L., McCafferty, D. J., Théry, M., Ancel, A., & Gilbert, C. (2017). Implantation of subcutaneous heart rate data loggers in southern elephant seals (*Mirounga leonina*). *Polar Biology*, *40*, 2307–2312. <https://doi.org/10.1007/s00300-017-2144-x>
- Consorte-McCrea, A., Queirolo, D., & Arruda Bueno, A. (2014). Relationships between the maned wolf and people. In Consorte-McCrea, A., Ferraz Santos, E. (Ed.), *Ecology and conservation of the maned wolf* (pp. 35–52). CRC Press.
- Crain, D. D., Karpovich, S. A., Quakenbush, L., & Polasek, L. (2021). Using claws to compare reproduction, stress and diet of female bearded and ringed seals in the Bering and Chukchi seas, Alaska, between 1953–1968 and 1998–2014. *Conservation Physiology*, *9*(1), coaa115. <https://doi.org/10.1093/conphys/coaa115>
- Crocker, D. E., Ortiz, R. M., Houser, D. S., Webb, P. M., & Costa, D. P. (2012). Hormone and metabolite changes associated with extended breeding fasts in male northern elephant seals (*Mirounga angustirostris*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *161*(4), 388–394.
<https://doi.org/10.1016/j.cbpa.2011.12.013>
- Curi, N. H. D. A., Coelho, C. M., Malta, M. D. C. C., Magni, E. M. V., Sábato, M. A. L., Araújo, A. S., Lobato, Z. I. P., Santos, J. L. C., Santos, H. A., Ragozo, A. A. M.,

- & Souza, S. L. P. D. (2012). Pathogens of wild maned wolves (*Chrysocyon brachyurus*) in Brazil. *Journal of Wildlife Diseases*, 48(4), 1052–1056.
<https://doi.org/10.7589/2011-10-304>
- Dalton, A. J. M., Rosen, D. A. S., & Trites, A. W. (2015). Resting metabolic rate and activity: Key components of seasonal variation in daily energy expenditure for the northern fur seal (*Callorhinus ursinus*). *Canadian Journal of Zoology*, 93(8), 635–644. <https://doi.org/10.1139/cjz-2014-0313>
- Dardente, H. (2012). Melatonin-dependent timing of seasonal reproduction by the *Pars Tuberalis*: Pivotal roles for long daylengths and thyroid hormones. *Journal of Neuroendocrinology*, 24(2), 249–266. <https://doi.org/10.1111/j.1365-2826.2011.02250.x>
- DeRango, E. J., Prager, K. C., Greig, D. J., Hooper, A. W., & Crocker, D. E. (2019). Climate variability and life history impact stress, thyroid, and immune markers in California sea lions (*Zalophus californianus*) during El Niño conditions. *Conservation Physiology*, 7(1), coz010. <https://doi.org/10.1093/conphys/coz010>
- Derose-Wilson, A., Fraser, J. D., Karpanty, S. M., & Hillman, M. D. (2015). Effects of overflights on incubating Wilson’s plover behavior and heart rate. *The Journal of Wildlife Management*, 79(8), 1246–1254. <https://doi.org/10.1002/jwmg.959>
- Diaz, S. F., Torres, S. M. F., Dunstan, R. W., & Lekcharoensuk, C. (2004). An analysis of canine hair re-growth after clipping for a surgical procedure. *Veterinary Dermatology*, 15(1), 25–30. <https://doi.org/10.1111/j.1365-3164.2004.00356.x>

- Dietz, J. M. (1984). Ecology and social organization of the maned wolf (*Chrysocyon brachyurus*). *Smithsonian Contributions to Zoology*, 392, 1–51.
<https://doi.org/10.5479/si.00810282.392>
- Ditmer, M. A., Rettler, S. J., Fieberg, J. R., Iaizzo, P. A., Laske, T. G., Noyce, K. V., & Garshelis, D. L. (2018). American black bears perceive the risks of crossing roads. *Behavioral Ecology*, 29(3), 667–675.
<https://doi.org/10.1093/beheco/ary020>
- Ditmer, M. A., Vincent, J. B., Werden, L. K., Tanner, J. C., Laske, T. G., Iaizzo, P. A., Garshelis, D. L., & Fieberg, J. R. (2015). Bears show a physiological but limited behavioral response to unmanned aerial vehicles. *Current Biology*, 25(17), 2278–2283. <https://doi.org/10.1016/j.cub.2015.07.024>
- Emmons, L. H. (2012). The maned wolves of Noel Kempff Mercado National Park. *Smithsonian Contributions to Zoology*, 639, 1–133.
- Evans, A. L., Singh, N. J., Fuchs, B., Blanc, S., Friebe, A., Laske, T. G., Frobert, O., Swenson, J. E., & Arnemo, J. M. (2016). Physiological reactions to capture in hibernating brown bears. *Conservation Physiology*, 4(1).
<https://doi.org/10.1093/conphys/cow061>
- Fiori, F., De Paula, R. C., Navas-Suárez, P. E., Boulhosa, R. L. P., & Dias, R. A. (2023). The sarcoptic mange in maned wolf (*Chrysocyon brachyurus*): Mapping an emerging disease in the largest South American canid. *Pathogens*, 12(6), 830.
<https://doi.org/10.3390/pathogens12060830>

- Fletcher, N. B., Zoo, J. B., Rodden, M., Conservation, N. Z., Taylor, S., & Zoo, L. (1995). Husbandry manual for the maned wolf. In (*Unpublished technical report prepared and distributed by the Association of Zoo and Aquariums Maned Wolf Species Survival Plan*).
- Fontoura-Rodrigues, M. L., & Eizirik, E. (2014). Evolutionary and conservation genetics of the maned wolf. In Consorte-McCrea, A., Ferraz Santos, E. (Ed.), *Ecology and conservation of the maned wolf* (pp. 77–86). CRC Press.
- Forin-Wiart, M., Enstipp, M. R., Le Maho, Y., & Handrich, Y. (2019). Why implantation of bio-loggers may improve our understanding of how animals cope within their natural environment. *Integrative Zoology*, *14*(1), 48–64.
<https://doi.org/10.1111/1749-4877.12364>
- Gesquiere, L. R., Pugh, M., Alberts, S. C., & Markham, A. C. (2018). Estimation of energetic condition in wild baboons using fecal thyroid hormone determination. *General and Comparative Endocrinology*, *260*, 9–17.
<https://doi.org/10.1016/j.ygcen.2018.02.004>
- Glazier, D. S., & Gjoni, V. (2024). Interactive effects of intrinsic and extrinsic factors on metabolic rate. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *379*(1896), 20220489. <https://doi.org/10.1098/rstb.2022.0489>
- Gobush, K. S., Booth, R. K., & Wasser, S. K. (2014). Validation and application of noninvasive glucocorticoid and thyroid hormone measures in free-ranging Hawaiian monk seals. *General and Comparative Endocrinology*, *195*, 174–182.
<https://doi.org/10.1016/j.ygcen.2013.10.020>

- Gormally, B. M. G., & Romero, L. M. (2020). What are you actually measuring? A review of techniques that integrate the stress response on distinct time-scales. *Functional Ecology*, *34*(10), 2030–2044. <https://doi.org/10.1111/1365-2435.13648>
- Græsli, A. R., Le Grand, L., Thiel, A., Fuchs, B., Devineau, O., Stenbacka, F., Neumann, W., Ericsson, G., Singh, N. J., Laske, T. G., Beumer, L. T., Arnemo, J. M., & Evans, A. L. (2020). Physiological and behavioural responses of moose to hunting with dogs. *Conservation Physiology*, *8*(1), coaa122. <https://doi.org/10.1093/conphys/coaa122>
- Græsli, A. R., Thiel, A., Fuchs, B., Singh, N. J., Stenbacka, F., Ericsson, G., Neumann, W., Arnemo, J. M., & Evans, A. L. (2020). Seasonal hypometabolism in female moose. *Frontiers in Ecology and Evolution*, *8*. <https://doi.org/10.3389/fevo.2020.00107>
- Grigg, E. K., Nibblett, B. M., Robinson, J. Q., & Smits, J. E. (2017). Evaluating pair versus solitary housing in kennelled domestic dogs (*Canis familiaris*) using behaviour and hair cortisol: A pilot study. *Veterinary Record Open*, *4*(1), e000193. <https://doi.org/10.1136/vetreco-2016-000193>
- Heimbürge, S., Kanitz, E., Tuchscherer, A., & Otten, W. (2020). Is it getting in the hair? – Cortisol concentrations in native, regrown and segmented hairs of cattle and pigs after repeated ACTH administrations. *General and Comparative Endocrinology*, *295*, 113534. <https://doi.org/10.1016/j.ygcen.2020.113534>

- Houser, D. S., Champagne, C. D., Wasser, S. K., Booth, R. K., Romano, T., & Crocker, D. E. (2021). Influence of season, age, sex, and time of day on the endocrine profile of the common bottlenose dolphin (*Tursiops truncatus*). *General and Comparative Endocrinology*, 313. <https://doi.org/10.1016/j.ygcen.2021.113889>
- Hunninck, L., Jackson, C. R., May, R., Røskaft, E., Palme, R., & Sheriff, M. J. (2020). Triiodothyronine (T3) levels fluctuate in response to ambient temperature rather than nutritional status in a wild tropical ungulate. *Conservation Physiology*, 8(1), coaa105. <https://doi.org/10.1093/conphys/coaa105>
- Hunt, K. E., Lysiak, N. S., Robbins, J., Moore, M. J., Seton, R. E., Torres, L., & Buck, C. L. (2017). Multiple steroid and thyroid hormones detected in baleen from eight whale species. *Conservation Physiology*, 5. <https://doi.org/10.1093/conphys/cox061>
- Kasper, C. B., da Fontoura Rodrigues, M. L., Severo, M. M., Cherem, J. J., Azambuja, R. B., Queirolo, D., de Souza, P., Bretos Moro, M. E., da Silva, F. P., Carlos, I. R., Montes, M. A., & da Silva, L. G. (2023). The last and endangered maned wolves (*Chrysocyon brachyurus*) from southern Brazil and Uruguay: New records and spatial distribution modeling. *Studies on Neotropical Fauna and Environment*. <https://doi.org/10.1080/01650521.2023.2285870>
- Kazmi, S. Z. H., Zhang, H., Aziz, W., Monfredi, O., Abbas, S. A., Shah, S. A., Kazmi, S. S. H., & Butt, W. H. (2016). Inverse correlation between heart rate variability and heart rate demonstrated by linear and nonlinear analysis. *PLOS ONE*, 11(6). <https://doi.org/10.1371/journal.pone.0157557>

- Koren, L., Bryan, H., Matas, D., Tinman, S., Fahlman, Å., Whiteside, D., Smits, J., & Wynne-Edwards, K. (2019). Towards the validation of endogenous steroid testing in wildlife hair. *Journal of Applied Ecology*, *56*(3), 547–561.
<https://doi.org/10.1111/1365-2664.13306>
- Koren, L., Mokady, O., Karaskov, T., Klein, J., Koren, G., & Geffen, E. (2002). A novel method using hair for determining hormonal levels in wildlife. *Animal Behaviour*, *63*(2), 403–406. <https://doi.org/10.1006/anbe.2001.1907>
- Koviri, D. (2023). Animal metabolism: How animals convert food into energy, processes, importance and factors affecting it. *Research & Reviews: Journal of Veterinary Sciences*, *7*(2), 12–13.
- Kozłowski, C. P., Clawitter, H., Guglielmino, A., Schamel, J., Baker, S., Franklin, A. D., Powell, D., Coonan, T. J., & Asa, C. S. (2020). Factors affecting glucocorticoid and thyroid hormone production of island foxes. *The Journal of Wildlife Management*, *84*(3), 505–514. <https://doi.org/10.1002/jwmg.21808>
- Laske, T. G., Evans, A. L., Arnemo, J. M., Iles, T. L., Ditmer, M. A., Fröbert, O., Garshelis, D. L., & Iaizzo, P. A. (2018a). Development and utilization of implantable cardiac monitors in free-ranging American black and Eurasian brown bears: System evolution and lessons learned. *Animal Biotelemetry*, *6*(13).
<https://doi.org/10.1186/s40317-018-0157-z>
- Laske, T. G., Evans, A. L., Arnemo, J. M., Iles, T. L., Ditmer, M. A., Fröbert, O., Garshelis, D. L., & Iaizzo, P. A. (2018b). Development and utilization of implantable cardiac monitors in free-ranging American black and Eurasian brown

- bears: System evolution and lessons learned. *Animal Biotelemetry*, 6(1), 13.
<https://doi.org/10.1186/s40317-018-0157-z>
- Laske, T. G., Garshelis, D. L., & Iaizzo, P. A. (2011). Monitoring the wild black bear's reaction to human and environmental stressors. *BMC Physiology*, 11(13).
<https://doi.org/10.1186/1472-6793-11-13>
- Laske, T. G., Garshelis, D. L., & Iaizzo, P. A. (2014). Big data in wildlife research: Remote web-based monitoring of hibernating black bears. *BMC Physiology*, 14.
<https://doi.org/10.1186/s12899-014-0013-1>
- Lawrimore, J. H., Ray, R., Applequist, S., Korzeniewski, B., Menne, M. J. (2016) Global Summary of the Year (GSOY), Version 1. [1995–2016]. NOAA National Centers for Environmental Information. <https://doi.org/10.7289/JWPF-Y430> (last accessed 15 March 2024)
- Le Grand, L., Thorsen, N. H., Fuchs, B., Evans, A. L., Laske, T. G., Arnemo, J. M., Sæbø, S., & Støen, O.-G. (2019). Behavioral and physiological responses of Scandinavian brown bears (*Ursus arctos*) to dog hunts and human encounters. *Frontiers in Ecology and Evolution*, 7. <https://doi.org/10.3389/fevo.2019.00134>
- LeBeau, M. A., Montgomery, M. A., & Brewer, J. D. (2011). The role of variations in growth rate and sample collection on interpreting results of segmental analyses of hair. *Forensic Science International*, 210(1–3), 110–116.
<https://doi.org/10.1016/j.forsciint.2011.02.015>
- Leimgruber, P., Songsasen, N., Stabach, J. A., Horning, M., Reed, D., Buk, T., Harwood, A., Layman, L., Mathews, C., Vance, M., Marinari, P., Helmick, K. E., Delaski,

- K. M., Ware, L. H., Jones, J. C., Silva, J. L. P., Laske, T. G., & Moraes, R. N. (2023). Providing baseline data for conservation–heart rate monitoring in captive scimitar-horned oryx. *Frontiers in Physiology, 14*, 1079008.
<https://doi.org/10.3389/fphys.2023.1079008>
- Lenth R (2023). emmeans: Estimated marginal means, aka least-squares means. R package version 1.8.8, <<https://CRAN.R-project.org/package=emmeans>>. Performance
- Lüdtke et al., (2021). performance: An R package for assessment, comparison and testing of statistical models. *Journal of Open Source Software, 6*(60), 3139.
<https://doi.org/10.21105/joss.03139>
- Madliger, C. L., Love, O. P., Hultine, K. R., & Cooke, S. J. (2018). The conservation physiology toolbox: Status and opportunities. *Conservation Physiology, 6*(1).
<https://doi.org/10.1093/conphys/coy029>
- Massara, R. L., De Oliveira Paschoal, A. M., Hirsch, A., & Chiarello, A. G. (2012). Diet and habitat use by maned wolf outside protected areas in Eastern Brazil. *Tropical Conservation Science, 5*(3), 284–300.
<https://doi.org/10.1177/194008291200500305>
- May-Júnior, J. A., Songsasen, N., Azevedo, F. C., Santos, J. P., Paula, R. C., Rodrigues, F. H. G., Rodden, M. D., Wildt, D. E., & Morato, R. G. (2009). Hematology and blood chemistry parameters differ in free-ranging maned wolves (*Chrysocyon brachyurus*) living in the Serra da Canastra National Park versus adjacent

farmlands, Brazil. *Journal of Wildlife Diseases*, 45(1), 81–90.

<https://doi.org/10.7589/0090-3558-45.1.81>

McClune, D. W., Kostka, B., Delahay, R. J., Montgomery, W. I., Marks, N. J., & Scantlebury, D. M. (2015). Winter is coming: Seasonal variation in resting metabolic rate of the european badger (*Meles meles*). *PLOS ONE*, 10(9), e0135920. <https://doi.org/10.1371/journal.pone.0135920>

McCormick, S. D., & Romero, L. M. (2017). Conservation endocrinology. *BioScience*, 67(5), 429–442. <https://doi.org/10.1093/biosci/bix026>

Moen, A. N. (1978). Seasonal changes in heart rates, activity, metabolism, and forage intake of white-tailed deer. *The Journal of Wildlife Management*, 42(4), 715–738. <https://doi.org/10.2307/3800763>

Moraes, R. N., Laske, T. G., Leimgruber, P., Stabach, J. A., Marinari, P. E., Horning, M. M., Laske, N. R., Rodriguez, J. V., Eye, G. N., Kordell, J. E., Gonzalez, M., Eyring, T., Lemons, C., Helmick, K. E., Delaski, K. M., Ware, L. H., Jones, J. C., & Songsasen, N. (2021). Inside out: Heart rate monitoring to advance the welfare and conservation of maned wolves (*Chrysocyon brachyurus*). *Conservation Physiology*, 9(1), coab044. <https://doi.org/10.1093/conphys/coab044>

Motta-Junior, J. C., Queirolo, D., & Arruda Bueno, A. (2014). Feeding ecology: A review. In Consorte-McCrea, A., Ferraz Santos, E. (Ed.), *Ecology and conservation of the maned wolf* (pp. 87–98). CRC Press.

- Mullur, R., Liu, Y.-Y., & Brent, G. A. (2014). Thyroid hormone regulation of metabolism. *Physiological Reviews*, *94*, 355–382.
<https://doi.org/10.1152/physrev.00030.2013>
- Mustonen, A.-M., Pyykönen, T., Puukka, M., Asikainen, J., Hänninen, S., Mononen, J., & Nieminen, P. (2006). Physiological adaptations to fasting in an actively wintering canid, the Arctic blue fox (*Alopex lagopus*). *Journal of Experimental Zoology Part A: Comparative Experimental Biology*, *305A*, 32–46.
<https://doi.org/10.1002/jez.a.241>
- Myers, M. J., Rea, L. D., & Atkinson, S. (2006). The effects of age, season and geographic region on thyroid hormones in Steller sea lions (*Eumetopias jubatus*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *145*, 90–98. <https://doi.org/10.1016/j.cbpa.2006.05.004>
- Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, *403*(6772), 853–858. <https://doi.org/10.1038/35002501>
- NEON (National Ecological Observatory Network). Single aspirated air temperature (DP1.00002.001), RELEASE-2024. <https://doi.org/10.48443/rr7n-7d52>. Dataset accessed from <https://data.neonscience.org/data-products/DP1.00002.001/RELEASE-2024> on March 7, 2024.
- Paula, R. C., DeMatteo, K. (2015). *Chrysocoyon brachyurus*. IUCN Red List Threat Species 8235. doi:dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T4819A82316878.en.

- Pereira, P., Fandos Esteruelas, N., Nakamura, M., Rio-Maior, H., Krofel, M., Di Blasio, A., Zoppi, S., Robetto, S., Llana, L., García, E., Oleaga, Á., López-Bao, J. V., Fayos Martinez, M., Stavenow, J., Ågren, E. O., Álvares, F., & Santos, N. (2022). Hair cortisol concentration reflects the life cycle and management of grey wolves across four European populations. *Scientific Reports*, *12*(1).
<https://doi.org/10.1038/s41598-022-09711-x>
- Pohlin, F., Brabender, K., Fluch, G., Stalder, G., Petit, T., & Walzer, C. (2017). Seasonal variations in heart rate variability as an indicator of stress in free-ranging pregnant Przewalski's horses (*E. ferus przewalskii*) within the Hortobágy National Park in Hungary. *Frontiers in Physiology*, *8*. <https://doi.org/10.3389/fphys.2017.00664>
- Queirolo, D., Moreira, J. R., Soler, L., Emmons, L. H., Rodrigues, F. H. G., Pautasso, A. A., Cartes, J. L., & Salvatori, V. (2011). Historical and current range of the near threatened maned wolf *Chrysocyon brachyurus* in South America. *Oryx*, *45*(2), 296–303. <https://doi.org/10.1017/S0030605310000372>
- Reis, S. C. D., Dias, J. H. P., Sousa, L. D. O. E., Chiarello, A. G., Sá, M. E. D., & Ramos, I. P. (2023). Germination of fruits eaten by the maned wolf *Chrysocyon brachyurus* (Illiger, 1815) (Carnivora, Canidae). *Biota Neotropica*, *23*(3).
<https://doi.org/10.1590/1676-0611-bn-2022-1413>
- Rich, G., Stennett, R., Galloway, M., McClure, M., Riley, R., Freeman, E.W., Hunt, K.E. 2024. Nailing it: Investigation of elephant toenails for retrospective analysis of adrenal and reproductive hormones. *Conservation Physiology*, in press. Accepted July 2024.

- Rimbach, R., Pillay, N., & Schradin, C. (2017). Both thyroid hormone levels and resting metabolic rate decrease in African striped mice when food availability decreases. *Journal of Experimental Biology*, *220*, 837–843.
<https://doi.org/10.1242/jeb.151449>
- Rodriguez-Castro, K. G., Lemos, F. G., Azevedo, F. C., Freitas-Junior, M. C., Desbiez Arnaud, L. J., & Galetti Jr., P. M. (2022). Human highly modified landscapes restrict gene flow of the largest neotropical canid, the maned wolf. *Biodiversity and Conservation*, *31*(4), 1229–1247.
<https://doi.org/10.1007/s10531-022-02385-x>
- Roffler, G. H., Karpovich, S., Charapata, P., & Keogh, M. J. (2022). Validation and measurement of physiological stress and reproductive hormones in wolf hair and claws. *Wildlife Society Bulletin*, *46*. <https://doi.org/10.1002/wsb.1330>
- Rogers, S. A., Robbins, C. T., Mathewson, P. D., Carnahan, A. M., van Manen, F. T., Haroldson, M. A., Porter, W. P., Rogers, T. R., Soule, T., & Long, R. A. (2021). Thermal constraints on energy balance, behaviour and spatial distribution of grizzly bears. *Functional Ecology*, *35*(2), 398–410. <https://doi.org/10.1111/1365-2435.13727>
- Roth, L. S. V., Faresjö, Å., Theodorsson, E., & Jensen, P. (2016). Hair cortisol varies with season and lifestyle and relates to human interactions in German shepherd dogs. *Scientific Reports*, *6*(1), 19631. <https://doi.org/10.1038/srep19631>
- Schaebs, F. S., Wolf, T. E., Behringer, V., & Deschner, T. (2016). Fecal thyroid hormones allow for the noninvasive monitoring of energy intake in capuchin

- monkeys. *Journal of Endocrinology*, 231(1), 1–10. <https://doi.org/10.1530/JOE-16-0152>
- Schell, C. J., Young, J. K., Lonsdorf, E. V., Mateo, J. M., & Santymire, R. M. (2017). Investigation of techniques to measure cortisol and testosterone concentrations in coyote hair. *Zoo Biology*, 36(3), 220–225. <https://doi.org/10.1002/zoo.21359>
- Shave, J. R., Cherry, S. G., Derocher, A. E., & Fortin, D. (2020). Seasonal and inter-annual variation in diet for gray wolves *Canis lupus* in Prince Albert National Park, Saskatchewan. *Wildlife Biology*, 2020(3), 1–9. <https://doi.org/10.2981/wlb.00695>
- Silva, J. E. (2005). Thyroid hormone and the energetic cost of keeping body temperature. *Bioscience Reports*, 25(3–4), 129–148. <https://doi.org/10.1007/s10540-005-2882-9>
- Songsasen, N., & Rodden, M. D. (2010). The role of the Species Survival Plan in maned wolf *Chrysocyon brachyurus* conservation. *International Zoo Yearbook*, 44(1), 136–148. <https://doi.org/10.1111/j.1748-1090.2009.00093.x>
- Støen, O.-G., Ordiz, A., Evans, A. L., Laske, T. G., Kindberg, J., Frøbert, O., Swenson, J. E., & Arnemo, J. M. (2015). Physiological evidence for a human-induced landscape of fear in brown bears (*Ursus arctos*). *Physiology & Behavior*, 152, 244–248. <https://doi.org/10.1016/j.physbeh.2015.09.030>
- Swoap, S. J., Overton, M., & Garber, G. (2004). Effect of ambient temperature on cardiovascular parameters in rats and mice: A comparative approach. *American*

Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 287, R391–R396. <https://doi.org/10.1152/ajpregu.00731.2003>

Thiel, A., Giroud, S., Hertel, A. G., Friebe, A., Devineau, O., Fuchs, B., Blanc, S., Støen, O.-G., Laske, T. G., Arnemo, J. M., & Evans, A. L. (2022). Seasonality in biological rhythms in Scandinavian brown bears. *Frontiers in Physiology*, 13. <https://doi.org/10.3389/fphys.2022.785706>

Thompson, C. L., Powell, B. L., Williams, S. H., Hanya, G., Glander, K. E., & Vinyard, C. J. (2017). Thyroid hormone fluctuations indicate a thermoregulatory function in both a tropical (*Alouatta palliata*) and seasonally cold-habitat (*Macaca fuscata*) primate. *American Journal of Primatology*, 79. <https://doi.org/10.1002/ajp.22714>

Tomasi, T. E., & Mitchell, D. A. (1994). Seasonal shifts in thyroid function in the cotton rat (*Sigmodon hispidus*). *Journal of Mammalogy*, 75(2), 520–528. <https://doi.org/10.2307/1382576>

Trolle, M., Noss, A. J., De Lima, E. S., & Dalponte, J. C. (2006). Camera-trap studies of maned wolf density in the Cerrado and the Pantanal of Brazil. In D. L. Hawksworth & A. T. Bull (Eds.), *Vertebrate Conservation and Biodiversity* (Vol. 5, pp. 371–378). Springer Netherlands. https://doi.org/10.1007/978-1-4020-6320-6_24

Viblanc, V. A., Smith, A. D., Gineste, B., & Groscolas, R. (2012). Coping with continuous human disturbance in the wild: Insights from penguin heart rate response to various stressors. *BMC Ecology*, 12(10). <https://doi.org/10.1186/1472-6785-12-10>

- Vynne, C., Booth, R. K., & Wasser, S. K. (2014). Physiological implications of landscape use by free-ranging maned wolves (*Chrysocyon brachyurus*) in Brazil. *Journal of Mammalogy*, 95(4), 696–706. <https://doi.org/10.1644/12-MAMM-A-247>
- Wascher, C. A. F., Kotrschal, K., & Arnold, W. (2018). Free-living greylag geese adjust their heart rates and body core temperatures to season and reproductive context. *Scientific Reports*, 8(1), 2142. <https://doi.org/10.1038/s41598-018-20655-z>
- Wasser, S. K., Azkarate, J. C., Booth, R. K., Hayward, L., Hunt, K., Ayres, K., Vynne, C., Gobush, K., Canales-Espinosa, D., & Rodríguez-Luna, E. (2010). Non-invasive measurement of thyroid hormone in feces of a diverse array of avian and mammalian species. *General and Comparative Endocrinology*, 168, 1–7.
- Welle, M. M., & Wiener, D. J. (2016). The hair follicle: A comparative review of canine hair follicle anatomy and physiology. *Toxicologic Pathology*, 44(4), 564–574. <https://doi.org/10.1177/0192623316631843>
- Williams, C. T., Chmura, H. E., Zhang, V., Dillon, D., Wilsterman, K., Barnes, B. M., & Buck, C. L. (2019). Environmental heterogeneity affects seasonal variation in thyroid hormone physiology of free-living arctic ground squirrels (*Urocitellus parryii*). *Canadian Journal of Zoology*, 97(9), 783–790. <https://doi.org/10.1139/cjz-2018-0302>

BIOGRAPHY

Jane Braswell received her Bachelor of Arts in Biological Foundations of Behavior from Franklin and Marshall College in 2018. After receiving her Masters of Science in Environmental Science and Policy from George Mason University in 2024, she will continue pursuing a career in wildlife conservation and management. Currently, she is employed by the Human-Beaver Coexistence Fund.