Ambassador Animal Welfare:
Impact of Education Programs on Behavioral and Physiological Wellbeing

Shao Doyon-Degroote

A Thesis

In the Department of

Biology

Presented in Partial Fulfillment of the Requirements

For the Degree of

Master of Science (Biology)

At Concordia University

Montreal, Quebec, Canada

August 2023

Concordia University

School of Graduate Studies

This is to certify that the thesis is prepared by **Shao Doyon-Degroote**

Entitled: **Ambassador Animal Welfare: Impact of Education Programs on Behavioral and Physiological Wellbeing**

And submitted in partial fulfillment of the requirements for the degree of

Master of Science (Biology)

And complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the final examining committee:

______________________________________ Chair
Dr. Grant Brown

______________________________________ External Examiner
Dr. Emma Despland

______________________________________ Examiner
Dr. Grant Brown

______________________________________ Examiner
Dr. Sarah Turner

______________________________________ Thesis Supervisor
Dr. Robert Weladji

Approved by

____
Dr. Robert Weladji, Graduate program Director

____
Dr. Pascale Sicotte, Dean of Faculty

Date ____

Abstract

Ambassador Animal Welfare: Impact of Education Programs on Behavioral and Physiological Wellbeing

Shao Doyon-Degroote

The welfare of ambassador animals – zoo animals that have direct contact with the public as part of education programs – is a priority issue, since these animals are potentially exposed to more stressors than exhibited animals. This study investigated if education programs generated more stress in eight ambassador species at the Zoo de Granby and the Ecomuseum Zoo in Québec, Canada. We compared activity treatments involving handling and/or transport to a baseline treatment without programmed activities and used fecal glucocorticoid metabolite (FGM) levels and activity budgets to measure physiological and behavioral stress, respectively. Overall, we found in camels (*Camelus bactrianus*) and opossums (*Monodelphis domestica*) that animal rides and zoo workshops, respectively, had higher FGM levels exceeding the baseline thresholds. The FGM levels varied depending on the ambassador's total participation in programmed activities, so less used animals experienced acute stress sooner than most used ones. Undesirable behaviors, such as pacing and interaction with transparent boundaries, in some camels, armadillos, skinks, and pythons represented over 10 % of an individual's activity budget, but the species' mean did not exceed the threshold. Finally, the activity treatments significantly affected resting behaviors in birds, reptiles, and camelids without changing the activity rate between treatments. Overall, education programs did not significantly affect ambassadors' welfare, but targeted individuals among the species were stressed. Our findings will benefit the zoological community for example, by providing baseline FGM levels for each ambassador species and contribute to improving the understanding of repetitive and stereotyped behaviors across multiple species.

Acknowledgments

First, I would like to thank my supervisor, Dr. Robert Weladji, for his continuous support and always keeping his door open when I needed guidance. I also want to thank my committee members, Dr. Grant Brown and Dr. Sarah Turner, for their helpful feedback on my research. Moreover, I would like to thank my sources of funding: Zoo de Granby, Concordia University, MITACS, Natural Sciences and Engineering Research Council (NSERC), Québec Center for Biodiversity Science (QCBS), and Hydro Québec.

Within the Zoo de Granby, I thank Patrick Paré for his guidance and for giving me advice during my fieldwork. I also want to thank Marie-Andrée Hunter and the zoo staff from the Education Department for helping with the sample collection and the routine updates of the ambassador animals. A special thanks to my good friend Mélissa Loiseau, who kindly hosted me at her house during the summer and encouraged me during the difficult times of my data collection period. Additionally, I would like to thank Louis Lazure, for his role in the collaboration between the University and the different departments of the Zoo de Granby. Louis also helped me numerous times, from the literature review, the fieldwork, and the writing process to the final presentation of the findings. Thank you for always finding solutions to my problems and answering my questions.

Within the Ecomuseum Zoo of Montréal, I would like to thank Patricia Presseau and Caroline Gagné for their interest in my project and for letting me include the ambassador birds from their institution in my research. Again, the zoo staff from the Ecomuseum Zoo was amazing to work with, and I thank everyone I met for helping me throughout the summer. I also want to thank the Toronto Zoo laboratory for taking the time to analyze my samples. The success of this thesis would not have been possible without the collaboration of all these different zoological institutions, and I am grateful for all the new connections I made.

To continue, I would like to thank all my current and past lab friends, especially Alex and Laura, for their support over the last few years. Finally, I would like to thank my family and friends, who believed in me and pushed me to achieve this big step of graduate studies. Thank you to my boyfriend Ruslan and best friend Océane, who encouraged me every day and helped me in the most challenging moments, reminding me I could do anything. Furthermore, thank you to my mother and sister, who were always there for me and saw the best in me when I could not. I also want to finish by thanking my beloved angel Chance, who rests in peace now but still gave me the willpower to realize this dream until the end. This is for you.

Contribution of Authors

Shao Doyon-Degroote[1], Robert B. Weladji[1], Patrick Paré[2], Louis Lazure[1,2]

[1]Department of Biology, Concordia University, 7141 Sherbrooke West, Montreal, QC, H4B 1R6, Canada

[2]Zoo de Granby, 1050 David-Bouchard N, Granby, QC, J2H 0Y6, Canada

I would like to acknowledge here the contribution of the following collaborators on the project:

- Conception and design of the study: S, R, P, L
- Data collection: S, L
- Analysis and interpretation of data: S, R, L
- Drafting of manuscript: S
- Critical revision: S, R, L

Table of Content

List of Tables

List of Figures

Resting up, RUMI: Ruminating, SELF: Self-directed, SOCIAL: Social interaction, UND: Undesirable. Significance levels are indicated with stars. (Appendix D).

Figure 1.4. Activity budgets of three-banded armadillos according to A) the time of the day and B) the presence of environmental enrichment. EXP: Exploration, FEED: Feeding and drinking, LOCO: Locomotion, NOTV: Not visible, NVPR: Not visible, presumed resting, OTHER: Other active, PSHELT: Preparing shelter, REST: Resting, SELF: Self-directed, UND: Undesirable. Significance levels are indicated with stars. (Appendix E).

Figure 1.5. Activity budgets of grey short-tailed opossums according to A) the time of the day and B) the presence of environmental enrichment. EXP: Exploration, FEED: Feeding and drinking, LOCO: Locomotion, NOTV: Not visible, NVPR: Not visible, presumed resting, OTHER: Other active, REST: Resting, WHEEL: Running wheel, SELF: Self-directed, UND: Undesirable. Significance levels are indicated with stars. (Appendix F).

Figure 1.6. Activity budgets of blue-tongued skinks according to A) the time of the day and B) the presence of environmental enrichment. EXP: Exploration, FEED: Feeding and drinking, LOCO: Locomotion, NOTV: Not visible, NVPR: Not visible, presumed resting, OTHER: Other active, REST: Resting, SELF: Self-directed, UND: Undesirable. Significance levels are indicated with stars. (Appendix G).

Figure 1.7. Activity budgets of ball pythons according to A) the time of the day and B) the presence of environmental enrichment. EXP: Exploration, FEED: Feeding and drinking, LOCO: Locomotion, NOTV: Not visible, NVPR: Not visible, presumed resting, OTHER: Other active, PERIS: Periscoping, REST: Resting, SELF: Self-directed, UND: Undesirable. Significance levels are indicated with stars. (Appendix H).

Introduction

Zoos and aquariums are key venues to interact with wildlife species, wherein educators and scientists convey the importance of protecting biodiversity to the public. Several zoos carry out education programs that rely on live animals called "ambassador animals". The Association of Zoos and Aquariums (AZA) defines an *ambassador animal* as "an animal whose role includes handling and/or training by staff or volunteers for interaction with the public and in support of institutional education and conservation goals." (AZA, 2015). These animals include captive mammals (Acaralp-Rehnberg et al., 2020; Baskir et al., 2020; Kozlowski et al., 2021; Powell et al., 2020; Whitehouse-Tedd et al., 2021), reptiles (van Heerbeek et al., 2021), amphibians (Rommel et al., 2016), and birds (Caudell & Riddleberger, 2001; Saiyed et al., 2019). However, compared to exhibited animals, ambassadors can live in different living areas and are often transported and manipulated by educators to have direct or close contact with the public (Baird et al., 2016; Powell et al., 2020).

Designated ambassador animals will interact with the public in three main ways: on grounds inside their exhibit/enclosure (e.g., animal feeding, rides, or touch tanks), on grounds outside their exhibit/enclosure for zoo workshops (e.g., raptors on the glove, programmed shows), or off grounds of the zoo for outside events (e.g., school workshops, media, or fund-raising events) (AZA, 2015). Interacting with ambassador animals from education programs can be beneficial to increase empathy toward animals, raise knowledge and awareness on endangered species, promote engagement for conservation issues, and overall change the visitors' attitude toward wildlife (Clifford-Clarke et al., 2022; Luebke et al., 2016; Povey, 2002; Povey & Rios, 2002; Rank et al., 2021; Swanagan, 2000; Yerke & Burns, 1991). However, a study by Spooner et al. (2021) stated that ambassador animal encounters with high-intensity visitor contact and long-term exposure could be detrimental to animal welfare. Although the purpose of this study is not to compare the welfare of captive animals in zoos with that of free-ranging animals in the wild, the use of ambassador animals for programmed activities should not cause poorer welfare than when they are at rest. Not all encounters with ambassadors have adverse outcomes and some interactions with humans can represent a positive enrichment for some species (Spooner et al., 2021). Nevertheless, to justify the use of ambassadors, it is crucial to ensure their welfare.

Animal welfare has become one of the top priorities, along with conservation and education in zoos. The research on the welfare of exhibited animals has been increasing over the past years for the long-term effects of captivity (Borgmans et al., 2021), the zoo visitor's effects (Davey, 2007), the benefits of enrichment (Bashaw et al., 2016; Clark & Melfi, 2012; Swaisgood & Shepherdson, 2005), the presence of stereotypic or repetitive behavior (Mason & Latham, 2004; Rose et al., 2017), etc. Yet, little research focuses on ambassador animals, let alone on the impacts of programmed activities on their welfare (Binding et al., 2020). In addition, the results of these studies are often species- or situation-specific and biased to very few taxa (Melfi, 2009; Powell et al., 2020), which makes it difficult to generalize among all ambassador animals used in education programs. For example, Baskir et al. (2020) found that chinchilla (*Chinchilla lanigera*) ambassadors at the Saint Louis Zoo responded differently to the sight of handling gloves than cleaning gloves. However, other ambassador animal species might also have behavioral reactions to specific signals or disturbances. Therefore, to improve ambassador animal welfare, it is crucial to understand the impacts of education programs on a broader range of species.

Animal welfare has several definitions according to the approach used by the researcher to describe it and what is being measured. Therefore, the chosen variables may influence the interpretation of what is good or poor welfare and suggest different conclusions (Mason & Mendl, 1993). For example, it can measure several behavioral, physical, or physiological indicators to understand the quality of life from the animals' perspective (Binding et al., 2020; Bracke & Hopster, 2006; Brando & Buchanan-Smith, 2018; Sejian et al., 2011). For our research, it is defined according to Donald Broom – an animal welfare professor with multiple refereed scientific papers – as "the state of an animal in relation to its environment" and how the individual tries to cope with it (Broom, 2001). This state combines the animal's efforts to cope with its environment, the relevance of feelings associated with coping, like sufferance, and if those attempts lead to success or failure. When an animal's need for a particular resource or stimulus is unmet, it may negatively affect behavioral and physiological states, which can generate stress, fear, pain, or an overall reduced fitness by reduced growth, reproductive or survival rates in captivity (Broom, 1991). All animals have different backgrounds and responses to captivity as some are hand-reared, captive-bred, or wild-born. Hence, comparative studies using multiple measures of welfare are needed to investigate baseline wellbeing indicators (Mason, 2010). In addition to abiotic environmental and confinement-specific stressors experienced by exhibited animals (Morgan & Tromborg, 2007), ambassadors are exposed to more handling and transport through their programmed activities. Thus, identifying stressors is essential for a broader range of captive animals, including ambassadors, to prevent poor welfare and improve the current management practices.

A stressor represents a challenge to the organism's equilibrium state and causes a cascade of physiological events to cope with the challenge and return to stability. This response to acute stress can result in short-term behavior and physiologic changes such as increased vigilance and excretion of stress hormones through the HPA axis (hypothalamic-pituitary-adrenal axis) (Morgan & Tromborg, 2007). In chronic stress, the consequences of unbearable conditions can generate behavior modifications and impair in one or many ways an individual's activation of the HPA axis, including the intensity, the frequency, or the delay of the response, compared to an acute stress responses (Mormède et al., 2007). This can be observed as increasing undesirable behaviors such as abnormal repetitive behavior (Carlstead & Brown, 2005; Schouten & Wiegant, 1997), aggressive behavior (Bartolomucci et al., 2004; Mineur et al., 2003), and fear behavior (Boissy et al., 2001) while decreasing behavioral complexity (Rutherford et al., 2004) and exploratory behavior (Carlstead et al., 1993; Carlstead & Brown, 2005). Overall, the stress intensity and the stressor experienced by animals will vary depending on their species, sex, and age (Fischer & Romero, 2018; Shepherdson & Carlstead, 2001).

It is important to use a combination of non-invasive methods, such as physiological measurements and behavioral analyses in addition to health indicator documentation to measure stress without generating it and to interpret the results correctly (Gormally & Romero, 2020; Hill & Broom, 2009). For physiologic indicators, glucocorticoids are steroid hormones produced by the adrenal glands through the HPA axis to overcome stressful situations in both positive and negative affective states (Ralph & Tilbrook, 2016). Glucocorticoids have a wide range of metabolic effects on the organism and can be measured in plasma, saliva, hair, feathers, urine, and feces samples (Möstl, 2014). Specifically, fecal glucocorticoid metabolites (FGM) are ideal in zoo settings since they are non-invasive, species-specific, easy to collect and store, and they provide information on the total amount of excreted hormones through the intestinal passage since the last defecation (Gormally & Romero, 2020; Möstl, 2014). For example, maximum peak concentrations can be found about 12 h after a stressful event in ruminants, after 24 h in ponies, or after 48 h in pigs (Möstl & Palme, 2002). This lag time can vary from less than 30 minutes to more than one day and will also change according to the activity rhythm of animals, meaning it may be shorter (e.g., in birds) or longer (e.g., in large carnivores) (Palme, 2019; Touma & Palme, 2005). Touma & Palme

(2005) also mentioned that there can be individual variation within a species due to diurnal variation, seasonal variation or life history stage, so each animal should act as its control (Möstl, 2014). Fecal samples are also a great alternative to blood samples and tell the same story (Sheriff et al., 2010). FGM are sensitive to long-term stressors lasting hours to weeks, which can help understand both acute and chronic stress knowing that peak values will return to normal baseline values within 72h in many species (Baird et al., 2016; Gormally & Romero, 2020; Nagel et al., 2019; Powell et al., 2020; Thiel et al., 2005; Washburn et al., 2003; Wasser & Hunt, 2005). For all the previously mentioned reasons, studying FGM concentration variations can be a great physiologic indicator of stress.

Behavior is another indicator used to investigate stress and improve the welfare of captive zoo animals (Kleiman, 1992). In addition to activity budgets that provide an overview of how much time is dedicated to various behaviors by an individual, such as feeding, resting, and locomoting, undesirable behavior and activity rates from hypo- or hyper-activity post-stress can also be relevant to record compared to normal habits (Gormally & Romero, 2020; Watters et al., 2009). In this study, we considered undesirable behavior as either abnormal behavior, repetitive behavior or ones that could be harmful to the animal's health (Baird et al., 2016). Abnormal behavior is the performance of unnatural behavioral patterns compared to those in the wild, inappropriate use of behavior in specific contexts, or unhealthy behavior for an animal (Warwick et al., 2013). Furthermore, abnormal repetitive behaviors are a stress consequence observed in many captive wild species, whether kept in farms, laboratories, zoos, or aquariums (Mason & Latham, 2004). They are defined by repetitive, unvarying, and apparently functionless behavior patterns to the animal and split into two broad categories: 1) the impulsive or compulsive behaviors, which are repeated and goal-oriented, like self-mutilation for fur-chewing or feather plucking, and 2) the stereotypic behaviors performed by repeated motor function in linear or circular motions (Mason & Latham, 2004; Rose et al., 2017). It has been suggested that the performance of stereotypic behavior in more than 5 % of a population or more than 10 % of an animal's time indicates negative welfare (Broom, 1991). Unfortunately, in 2004 it was estimated that stereotypic behaviors were displayed by over 85 million animals worldwide, and many studies have found an interaction between the increase in stereotypic behavior with the decrease in captive animal welfare (Mason & Latham, 2004). Although there are complex links between these two factors and stereotypic behaviors are not always associated with poor welfare, they have become an important indicator to assess the behavioral well-being and monitor warning signs of potential suffering of animals in complement to other tools. Species differ significantly in how they display stereotypic behavior; besides pacing and circling observed in many species, there is feather-plucking in birds, oral stereotypy like bar-mouthing – licking, biting or playing with the lips on the enclosure's metal bars – in camelids, or self-harm and escape behaviors with transparent glass barriers in reptiles (Mason, 2010; Padalino et al., 2014; Warwick et al., 2013). Hence, the importance of fully understanding species-specific behavioral patterns and studying daily activity budgets.

Handling and transport are known stressors for wildlife and most studies focus on the consequences after a rare occurring event rather than handling and transport as part of a daily routine like for ambassadors when they participate in school workshops. Animals forced into transport are exposed to a wide range of stressors including unfamiliar noise, vehicle vibrations, restricted space in containers, and a complete change of their previous environment conditions (Suchy et al., 2007). The impacts of transport on zoo animal welfare have not been studied in ambassador animals, but they were studied in wild animals, circus animals, and captive animals and ultimately cause poor welfare (Dembiec et al., 2004; Iossa et al., 2009; Pohlin et al., 2021). To date, a few important findings have been published concerning the impacts of handling on ambassadors, and the results are not unanimous. Baird et al. (2016) found no significant difference between ambassadors used in education programs or not and that handling had no effect on welfare measures. However, they explained the amount of handling that an animal experienced was positively correlated with FGM concentrations and associated with several behaviors, such as undesirable behaviors, resting, and self-directed behaviors. Another study by Hogan et al. (2011) demonstrated that captive wombats (*Lasiorhinus latifrons*) had increased FGM levels and reactive behaviors after handling by the staff. A more recent publication from Powell et al. (2020) showed that the amount of handling ambassador guinea pigs (*Cavia porcellus*) received did not affect FGM but that there was significant individual variation. Thus, Van Heerbeek et al. (2021) mentioned, it is important to assess stress not only at the species level but also at the individual level to investigate potential stressors.

Ambassadors are powerful providers of conservation education to the public, so much so that over 75 % of global zoos offer the opportunity to interact with at least 90 different taxonomic orders of ambassador animals (D'Cruze et al., 2019). The Zoo de Granby and the Ecomuseum Zoo, both located in the province of Québec, in Canada, are accredited AZA (Association of Zoos and Aquariums) and CAZA (Canada's Accredited Zoos and Aquariums) zoological institutions with education programs involving the use of ambassador animals. They all participate in either zoo workshops, school workshops, or animal rides, which involve handling and/or transport regularly. To understand the impacts of programmed activities on a wide range of ambassador species, we chose to include species from the animal classes of mammals, reptiles, and birds, including the three-banded armadillo (*Tolypeutes matacus*), the grey short-tailed opossum (*Monodelphis domestica*), the Bactrian camel (*Camelus bactrianus*), the dromedary (*Camelus dromedarius*), the ball python (*Python regius*), the blue-tongued skink (*Tiliqua scincoides*), the great-horned owl (*Bubo virginianus*) and the American kestrel (*Falco sparverius*). We were interested in the physiological and behavioral impacts of ambassadors participating in activities compared to a baseline period without potential stressors of handling and transport.

We hypothesized that handling and transport during educational activities would generate greater stress in ambassadors than when they were not exposed to these at rest. Our objectives in this study were to: 1) find potential sources of stress (e.g., handling and transport) across ambassador animals used in education programs, 2) determine the effects of behavioral and physiological stress indicators after participating in activities, 3) establish a baseline and thresholds indicators of wellbeing for the studied species, and 4) assess variations of stress responses within and between mammals, reptiles, and birds. The findings will allow us to propose improvements to current management practices to enhance ambassador animals' welfare. Moreover, we predicted that ambassadors' participation in programmed activities would: 1) increase the secretion of FGM levels as compared to an established baseline; 2) show higher frequencies of undesirable behaviors; and 3) change the activity rate from the activity budget during a baseline period compared to a post-activity period.

Methods

Subjects, husbandry, and facilities

The methods and the animals used in this study were reviewed and approved by the Conservation Department and the Education Department at the Zoo de Granby, the Animal Care Department and the Ethics and Animal Welfare Committee at the Ecomuseum Zoo. The main observer (Shao Doyon-Degroote) also successfully passed the Fieldwork Training from the Animal Research Program at Concordia University and the training modules from the Canadian Council on Animal Care.

This research took place during summer 2022 at the Zoo de Granby and the Ecomuseum Zoo, located in the province of Québec, Canada. Four mammal species, two reptile species, and two bird species used in education programs were studied for a total of 27 subjects (Table 1, see Table 1.1. in Appendix A for more details on each individual). The ambassador species from the Zoo de Granby were the three-banded armadillo (*Tolypeutes matacus*), the grey short-tailed opossum (*Monodelphis domestica*), the Bactrian camel (*Camelus bactrianus*), the dromedary (*Camelus dromedarius*), the ball python (*Python regius*) and the blue-tongued skink (*Tiliqua scincoides*). In opossums and dromedaries, one individual did not participate in activities or rides during the data collection period and these animals were used as a control for analysis. Ambassador bird species from the Ecomuseum Zoo were the great-horned owl (*Bubo virginianus*) and the American kestrel (*Falco sparverius*). At least two individuals representing each species were studied. Data on animal class, species, sex, age, light cycle, number of years involved in education programs, enclosure settings, environmental enrichment, and absence of conspecifics for subjects in shared enclosures were recorded. For instance, zookeepers occasionally switched camels and dromedaries from their adjacent enclosure which allowed new space use opportunities. For bird species, all individuals had access to an indoor enclosure within the animal care facility and an outdoor enclosure with fenced barriers. Birds were, therefore, in contact with wildlife through the barriers. Information on activity outings, such as the date, start and end times, duration, and activity type (zoo workshop, school workshop) were recorded when available. Special events such as enclosure changes and medical procedures were noted when they occurred.

All species participated in programmed activities involving handling and/or transport, except for camelids, whose principal activities were camel rides. We compared four different treatments: baseline (no potential stressor experienced), workshops at the zoo (activity with handling and minimal transport experienced inside the zoo), workshops in schools (activity with handling and transport experienced outside the zoo), and camel ride days for the camels' and the dromedaries' activity treatment. The baseline treatment was defined as no handling or transport experienced for at least three consecutive days. In contrast, the activity treatment for zoo and school workshops was defined as the three consecutive days following any workshop event. The activity treatment consisted of three consecutive days following ride days at the zoo for the camel and the dromedary, which did not participate in workshops. This time-framed period allowed peak concentrations of cortisol or corticosterone in feces to be reached and return to normal following a stressful event, depending on the species' gut transit time (Baird et al., 2016; Nagel et al., 2019; Powell et al., 2020; Sid-Ahmed et al., 2013; Wasser & Hunt, 2005).

Behavioral data collection

Before beginning behavioral data collection, all ambassador animals were observed *ad libitum* to determine the peak activity hours for each species and compile all observed behaviors. This project incorporated "ZooMonitor", a web app developed by Lincoln Park Zoo, to collect instantaneous fecal sampling data systematically (Wark et al., 2019). We performed seven 20-min focal observation sessions at 15-sec intervals daily. The focal individuals and species used for observation were chosen randomly every day. Observation sessions were made according to each species' daily activity during their most active period to account for the species' different light cycles. Camelids were observed between 8:00 and 17:00, armadillos between 9:00 and 0:00, opossums between 16:00 and 0:00, both reptile species between 8:00 and 22:00, kestrels between 8:00 and 19:00 and owls between 15:00 and 5:00. Thus, contact time with ambassador animals was scheduled to start at 8:00 in the morning and to finish at 23:59 at night for in-person focal observations at the Zoo de Granby and between 8:00 in the morning and 17:00 in the evening at the Ecomuseum Zoo. For bird species, individuals were observed from a different room using live or past recordings with infrared continuous recording cameras. This protocol was meant to avoid behavior changes in birds because of the observer's presence. At each 15-sec interval, the focal animal's behavior was recorded using an exhaustive ethogram (Table 2). Observation sessions were not made during feeding time in both pythons and skinks because they rarely occurred, and the individuals would hide to consume their food for over 20 minutes. Thus, only drinking was recorded for reptiles' "Feeding and drinking" behavior. Pythons were not observed during shedding, as they would spend consecutive days not visible in their shelter box and were not used for activities until it was over. Resting behavior was modified for camels and dromedaries to separate resting while standing up or lying down. In both bird species, we differentiated "Rest passive" and "Rest Active" to separate vigilance and hunting behavior from actual resting. To calculate activity rates, we combined Resting, Not visible, presumed resting, Rest up, Rest down, and Rest passive behaviors into the "Passive" behavior category. All other behaviors of the ethogram except "Not visible" were combined into the "Active" behavior category. Undesirable behaviors were calculated as a percentage of Active behaviors.

Fecal sample collection and processing

A total of 269 fecal samples were collected from October 2021 to September 2022 for all species. Fresh fecal samples (<12 h old) were collected by zoo employees during opening hours (7:00-17:00) when available and placed in *Nasco* plastic bags labelled with animal ID, date, and time. Urates were separated from feces in bird and reptile samples. The samples were immediately stored in a freezer at -20 ºC and later shipped on dry ice to Toronto Zoo (Ontario, Canada) for laboratory analyses. Methanol was used as a solvent for hormone extraction in all species. A wet fecal extraction method was used for mammal species, whereas a dry extraction method was used for reptiles and birds. The hormone extracted was cortisol for armadillos and opossums and corticosterone for the other species. FGM concentrations were calculated on an ng/g feces basis. For all species, parallelism, recovery, and intra-assay variability were used for assay validation (Palme, 2019).

Statistical analysis

Behavior analysis

For all observation sessions, the effects of treatment, individual (for species with an ambassador and exhibited animals), time of day (AM, PM, Evening, Night - based on each species' light cycle), presence or absence of environmental enrichment, presence or absence of conspecific (for camels and dromedaries) and enclosure location (for species housed indoor and outdoor) were recorded.

We calculated the number of observations of each behavior in the ethogram over the total number of intervals in the observation period to get the count data of all behaviors in each species. Then, we analyzed three sets of generalized linear mixed models (GLMM) with the variables mentioned above as predictors in the models. The first GLMMs tested the effect of predictors on the count number of each behavior in the ethogram for each species separately. The second GLMM was focused on the proportion of undesirable behaviors for each species and individual. The third GLMM was interested in activity rates, specifically, the proportion of active behaviors (in mammals and birds) or passive behaviors (in reptiles) for each species and individual. The second and third GLMMs were also used between animal classes (mammals, reptiles, and birds) and among all eight ambassador species with the species as a random factor to understand the treatment effect. A negative binomial distribution and a logit link function were used in all models, with the individual ID added as a random factor to account for pseudoreplication. Age, sex, enclosure dimensions, and number of years in education programs were not included in statistical analysis because of the small sample size or insufficient variation between the subjects within the species. We did not include the "Not visible" behavior in our analyses since the behavior performed by the animal could not be identified by the observer, but the results of this behavior were included in the activity budget figures. We studied pairwise comparisons using a Tukey-Kramer correction to find statistical differences between groups in predictor variables. In all GLMMs, we selected the final model based on which one had the lowest Akaike information criterion (AIC). When multiple models within 2 AIC were the lowest, we selected the model with the least predictors (Burnham & Anderson, 2002).

To establish behavior thresholds, we did a literature review and consulted the representatives of the parties involved in this study, including the director and the coordinator of the Zoo de Granby's Research Department, the director of the Zoo de Granby's Education Department, the director of the Animal Care Department from the Ecomuseum Zoo, as well as the thesis supervisor from Concordia University. We considered that spending more than 10 % in undesirable behavior during its awaken time or presenting a variation greater than 50 % in activity rate was indicative of problematic welfare (Benn et al., 2019; Broom, 1991; Mason & Latham, 2004). Although the specific threshold of 50 % in activity rates had not been validated in the literature previously to qualify for poor welfare, it was defined *a priori* and allowed us to quantify variations in activity rates and to remain conservative in relation to changes that can occur without necessarily implying poor welfare for the animals. All tests used a significance level with a p-value < 0.05 and were performed using R 4.2.2 statistical software (R Core Team, 2022).

Fecal sample analysis

For all fecal samples collected, treatment effects, hours since last activity, days since last activity, number of activities in the last 30 days, mean time of activities, and the total number of hours out in the last 30 days were recorded as predictors in GLMMs. In camels and dromedaries, we used the number of days participating in rides in the last 30 days, the mean number of rides per day, and the total number of rides in the last 30 days. FGM concentrations were log-transformed to obtain a normal distribution. The individual ID and the hour of the sample collection time were used as random factors to control for hourly variation in FGM concentrations within a day. The FGM concentration GLMM was also used between animal classes (mammals, reptiles, and birds) and among all eight ambassador species with the species as a random factor to understand the treatment effect. The same protocol from the behavior analysis was used to select the final model with the lowest AIC. We used the Tukey-Kramer correction to test for pairwise significance between treatments. We used a generalized additive model (GAM) when significant predictors were continuous variables to study overall trends and peaks. The GAMs were validated using diagnostic plots as well as *gam.check* and *k.check* functions in R.

To establish baseline FGM thresholds, we followed an iterative process described by Brown et al. (1999) in which we calculated the FGM concentration mean and excluded the samples exceeding the mean + 2 SD. That step was repeated with recalculated means until all samples were below that threshold. FGM concentrations from activity treatments were considered normal if all samples were below the baseline threshold, potentially problematic if the average of all samples did not exceed the baseline threshold but a few samples did, and elevated if the average of all samples exceeded the baseline threshold (Baird et al., 2016; Bashaw et al., 2016; Brown et al., 1999; Howell-Stephens et al., 2012). Baseline FGM thresholds were calculated at the species and the individual levels for analysis with a significance level with a p-value < 0.05 (Menchetti et al., 2021).

<u>Results</u>

Behavior results

Activity budgets

In camelid species, the dominant behaviors were Resting up, Resting down, Ruminating, and Feeding (Fig. 1A). After participating in rides, camels spent significantly less time Resting up ($F_{1,436} = 4.632$ $p < 0.05$) than during baseline. Although it was not significantly different, camels spent more time Resting down after rides as well. Both treatment and absence of conspecific decreased Social interactions (Treatment: $F_{1,435} = 3.878$, $p < 0.05$; Absence of conspecific: $F_{1,435} = 4.469$, $p < 0.05$), although they were not observed frequently during the study (< 3 % of activity budget) (see Figure 1.1. in Appendix B for more details on each variable). In baseline treatment for both dromedaries, the ambassador animal spent less time in Locomotion ($F_{1,184} = 5.784$, $p < 0.05$) and more time in Ruminating ($F_{1,183} = 12.253$, $p < 0.001$), Not visible ($F_{1,184} = 22.594$, $p < 0.001$) and Other active behaviors ($F_{1,183} = 8.019$, $p < 0.01$) than the exhibited animal (see Figure 1.2. in Appendix C for more details on each variable). For the ambassador animal, the participation in rides significantly decreased Feeding ($F_{1,118} = 12.879$, $p < 0.001$), Locomotion ($F_{1,118} = 4.122$, p <

0.05), and Other active behaviors ($F_{1, 118} = 4.404$, $p < 0.05$) while increasing time spent Resting down ($F_{1, 116} = 7.158$, $p < 0.01$) (Fig. 1B) in the 24 h post-activity treatment. However, Resting down was also influenced by the enclosure ($F_{1, 116} = 4.550$, $p < 0.05$) and the interaction between the treatment and the enclosure ($F_{1, 116} = 4.829$, $p < 0.05$), meaning that behavior for the same treatment was different between enclosures (see Figure 1.3. in Appendix D for more details on each variable).

In nocturnal mammal species, the dominant behavior was Not visible, presumed resting (> 50 % of activity budget) (Fig. 1C, 1D). In armadillos, Locomotion was significantly influenced by the treatment ($F_{2, 275} = 8.711$, $p < 0.001$), the time of the day ($F_{2, 275} = 5.082$, $p < 0.01$), and the interaction between these variables ($F_{4, 275} = 3.766$, $p < 0.01$) meaning that locomotion rates for a same treatment differed whether they were happening during the day, the evening, or the night (see Figure 1.4. in Appendix E for more details on each variable). Exploration behavior was not significantly influenced by treatment ($F_{2, 275} = 2.704$, $p = 0.069$), but by its interaction with the time of the day ($F4_{, 275} = 2.404$, $p = 0.05$). In opossums, the treatment did not affect behavior. Variations in Exploration ($F_{1, 354} = 8.940$, $p < 0.01$), Locomotion ($F_{1, 354} = 20.587$, $p < 0.001$), Resting ($F_{1, 352} = 9.126$, $p < 0.01$), Not visible, presumed resting ($F_{1, 349} = 5.167$, $p < 0.05$) and Other active behaviors ($F_{1, 352} = 5.013$, $p < 0.05$) were influenced by the time of the day (see Figure 1.5. in Appendix F for more details on each variable).

In reptile species, the dominant behaviors were Resting and Not visible, presumed resting (Fig. 1E, 1F). In skinks, Resting was influenced by treatment ($F_{2, 294} = 15.358$, $p < 0.001$) and time of the day ($F_{2, 294} = 6.106$, $p < 0.01$). Not visible, presumed resting behavior was influenced significantly by treatment ($F_{2, 298} = 16.635$, $p < 0.001$), time of the day ($F_{2, 298} = 11.947$, $p < 0.001$), and the presence of environmental enrichment ($F_{1, 298} = 4.201$, $p < 0.05$) (see Figure 1.6. in

Appendix G for more details on each variable). In pythons, Resting was influenced both by treatment ($F_{2, 839} = 3.417$, $p < 0.05$) and time of the day ($F_{2, 839} = 9.273$, $p < 0.001$) (see Figure 1.7. in Appendix H for more details on each variable). The latter variable had a significant effect on the performance of many behaviors such as Locomotion ($F_{2, 839} = 212.918$, $p < 0.001$), Not visible, presumed resting ($F_{2, 835} = 18.864$, $p < 0.001$), Other active behaviors ($F_{2, 839} = 6.828$, $p < 0.01$) and Exploration ($F_{2, 835} = 8.372$, $p < 0.001$). The interaction effect between the treatment and the time of the day also influenced Exploration behavior in pythons ($F_{4, 835} = 2.916$, $p < 0.05$), meaning that exploration rates for the same treatment differed whether they were happening during the day, the evening, or the night. Finally, Periscoping was not significantly influenced by treatment ($F_{2, 835} = 2.692$, $p < 0.1$), but by its interaction with the time of the day ($F_{4, 835} = 2.377$, $p = 0.05$).

In bird species, the dominant behaviors were Resting active, Resting passive and Self-directed behaviors (Fig. 1G, 1H). Both species spent over 60 % of their time Resting active and Resting passive behaviors. We found that kestrels and owls spent respectively between 13.06 ± 13.55 % and 3.83 ± 9.00 % of their time for Self-directed behaviors. In kestrels, Exploration was not significantly influenced by treatment ($F_{2, 310} = 2.746$, $p < 0.1$) but by its interaction with the enclosure ($F_{2, 310} = 3.253$, $p < 0.05$) or by the interaction of the enclosure and the time of the day ($F_{1, 310} = 9.511$, $p < 0.01$) (see Figure 1.8. in Appendix I for more details on each variable). Treatment and enclosure significantly impacted Locomotion (treatment: $F_{2, 311} = 3.453$, $p < 0.05$; enclosure: $F_{1, 311} = 36.701$, $p < 0.001$). Resting passive was significantly influenced by treatment ($F_{2, 313} = 2.865$, $p = 0.05$), enclosure ($F_{1, 313} = 10.280$, $p < 0.01$) and time of day ($F_{1, 313} = 6.446$, $p < 0.05$). Lastly, resting behaviors were influenced by a combination of multiple variables in owls (see Figure 1.9. in Appendix J for more details on each variable). Resting active was influenced by treatment ($F_{2, 180} = 4.275$, $p < 0.05$), time of day ($F_{1, 180} = 9.825$, $p < 0.01$), enclosure ($F_{1, 180} = 3.725$,

$p = 0.05$), the interaction between treatment and enclosure ($F_{2, 180} = 4.157$, $p < 0.05$) and the interaction between enclosure and time of day ($F_{1, 180} = 11.406$, $p < 0.001$). Resting passive was influenced by treatment ($F_{2, 179} = 2.936$, $p = 0.05$), enclosure ($F_{1, 179} = 3.814$, $p = 0.05$), as well as by their interaction ($F_{2, 179} = 3.535$, $p < 0.05$).

Activity rates

None of the species exceeded the 50 % threshold variation in activity rates between treatments (Table 3). The treatment did not have a significant effect on activity rates at the animal class and among all eight ambassador species levels. Active and passive behaviors were not influenced by treatment in camels, but by a combination of time of day (Active: $F_{1, 435} = 8.788$, $p < 0.01$; Passive: $F_{1, 436} = 3.841$, $p = 0.05$) and enclosure (Active: $F_{1, 435} = 5.710$, $p < 0.05$; Passive: $F_{1, 436} = 6.840$, $p < 0.01$), as well as the interaction between time of day and absence of conspecific (Active: $F_{1, 435} = 5.205$, $p < 0.05$) and absence of conspecific (Passive: $F_{1, 436} = 11.102$, $p < 0.001$). For the ambassador dromedary, Active behaviors were influenced by treatment ($F_{1, 118} = 10.781$, $p < 0.01$) and time of day ($F_{1, 118} = 4.970$, $p < 0.05$). Passive behaviors were influenced by time of day ($F_{1, 117} = 4.093$, $p < 0.05$), its interaction with enclosure ($F_{1, 117} = 4.949$, $p < 0.05$), but not significantly by treatment ($F_{1, 117} = 3.468$, $p = 0.065$).

Nocturnal mammal and reptile species had no significant association between activity rates and treatment. Only time of day was significantly related to activity rates in opossums (Active: $F_{1, 349} = 5.314$, $p < 0.05$; Passive: $F_{1, 354} = 9.671$, $p < 0.01$), skinks (Active: $F_{2, 298} = 10.018$, $p < 0.001$; Passive: $F_{2, 298} = 8.534$, $p < 0.001$) and pythons (Active: $F_{2, 835} = 51.593$, $p < 0.001$; Passive: $F_{2, 839} = 105.643$, $p < 0.001$) at the species level. The presence of environmental enrichment also influenced Passive behaviors in skinks ($F_{1, 298} = 6.718$, $p < 0.05$). At the individual level, the treatment significantly influenced Passive behaviors in one skink ($F_{2, 141} = 3.594$, $p < 0.05$), while environment enrichment influenced the other ($F_{1, 152} = 7.954$, $p < 0.01$), but both were also related to the time of day (Ind1: $F_{2, 141} = 3.915$, $p < 0.05$; Ind2: $F_{1, 152} = 12.670$, $p < 0.001$). Treatment was also related to Passive behaviors in one python ($F_{2, 86} = 4.216$, $p < 0.05$).

In kestrels, the enclosure was the only significant predictor for Active behaviors ($F_{1, 313} = 6.389$, $p < 0.05$) at the species level. In owls, treatment ($F_{2, 180} = 3.507$, $p < 0.05$), time of day ($F_{1, 180} = 9.836$, $p < 0.01$), enclosure ($F_{1, 180} = 4.076$, $p < 0.05$), the interaction of treatment and enclosure ($F_{2, 180} = 2.941$, $p = 0.05$), as well as the interaction of enclosure and time of day ($F_{1, 180} = 8.413$, $p < 0.01$) influenced Active behaviors. At the individual level, the treatment was significant for both owls (Ind1: $F_{2, 102} = 13.424$, $p < 0.001$; Ind2: $F_{1, 77} = 6.591$, $p < 0.05$).

Undesirable behavior

Time spent performing Undesirable behavior for each species and individual in each treatment is listed in Table 4. The treatment did not have a significant effect on Undesirable behavior at the animal class and among all eight ambassador species levels. There was significant individual variation within each species ($p < 0.05$). All individuals in this study performed Undesirable behavior except one python subject and both bird species. We recorded individual time spent performing Self-directed behavior for each bird species in every treatment to compensate for the absence of Undesirable behavior and study potential excessive Self-directed behavior (Table 5). One armadillo, one camel, one skink, and three pythons exceeded the threshold of 10 % active time in performing Undesirable behavior in at least one treatment of the study. One armadillo and one python exceeded the threshold in all treatments. Finally, in camels, dromedaries, armadillos, skinks, and pythons, Undesirable behavior rates were considered potentially problematic since the mean observation rate itself did not exceed the 10 % threshold, but its sum with 1 SD exceeded it.

At the species level in camelids, Undesirable behavior was significantly influenced by the absence of conspecifics ($F_{1, 435} = 9.192$, $p < 0.01$) and the interaction between time of day and the absence of conspecifics ($F_{1, 435} = 4.217$, $p < 0.05$) meaning that behavior for a same time was different whether conspecifics were with them or gone for rides. At the individual level, there was a significant effect from the absence of conspecifics for the most used camel for rides ($F_{1, 125} = 7.371$, $p < 0.01$) and a tendency for increasing Undesirable behavior for the least used camel for rides after rides ($F_{1, 154} = 3.332$, $p < 0.1$), but it was not significant. In dromedaries, there was no significant predictor of Undesirable behaviors for the ambassador animal. However, comparing both the ambassador and the exhibited animal in the baseline treatment, the time of day ($F_{1, 183} = 4.037$, $p < 0.05$) and the interaction between time of day and the absence of conspecifics ($F_{1, 183} = 3.818$, $p = 0.05$) were related to Undesirable behavior.

In armadillos at the species level, treatment ($F_{2, 283} = 6.448$, $p < 0.01$) significantly affected the performance of Undesirable behavior which was higher after participating in a zoo workshop than a school workshop or at baseline. However, there was no difference between the treatments at the individual level. In opossums, time of day ($F_{1, 352} = 8.757$, $p < 0.01$) was the best predictor for Undesirable behavior at the species level. At the individual level, the opossum not used in activities spent more time performing Undesirable behavior at night ($F_{1, 120} = 7.751$, $p < 0.01$).

In skinks, no predictor was significantly linked to Undesirable behavior at the species level. However, at the individual level, both individuals had a different response to treatment, as one skink showed higher Undesirable behavior after participating in school workshops and the other at baseline (Ind1: $F_{2, 141} = 3.410$, $p < 0.05$; Ind2: $F_{1, 152} = 4.720$, $p < 0.05$). In pythons, time of day ($F_{2, 836} = 44.979$, $p < 0.001$) and its interaction with treatment ($F_{4, 836} = 4.372$, $p < 0.01$) influenced significantly Undesirable behaviors at the species level. At the individual level, treatment was significant for two individuals (Ind1: $F_{2, 86} = 8.734$, $p < 0.001$; Ind2: $F_{2, 64} = 7.816$, $p < 0.001$).

In birds, we did not observe Undesirable behavior during the study in any individuals. Additionally, there was no significant predictor associated with the performance of Self-directed behavior and there was an absence of variation between the treatments and the individuals in both kestrels and owls (Table 5).

Fecal sample results

The general FGM concentration trends were illustrated for each species by linear regression with 95 % confidence intervals in Figure 3. The individual FGM concentrations for each species by treatment are listed in Table 6.

The treatment had a significant impact on the FGM concentrations in camels ($F_{1, 85} = 197.010$, $p < 0.001$) (Fig. 2A). The mean concentration ± SD of corticosterone in fecal samples was 163.75 ± 102.04 ng/g after participating in rides and exceeded the baseline threshold (mean + 2 SD) of 17.75 ng/g (Fig. 3A). At the individual level, all camels exceeded their baseline threshold after participating in rides ($p < 0.001$). In dromedaries for the baseline treatment, the mean FGM concentrations of the ambassador animal were higher than the exhibited animal not used in rides (ambassador: 82.77 ± 30.62 ng/g; exhibited animal: 61.47 ± 37.22 ng/g) but not significantly different. After participating in rides, the ambassador animal's FGM concentration was 98.07 ± 76.86 ng/g and five samples exceeded the baseline threshold of 144.01 ng/g (Fig. 3B).

In armadillos, the treatment had a significant impact on FGM concentrations ($F_{2, 21} = 4.214$, $p < 0.05$), and cortisol levels were higher after school workshops than zoo workshops (Tukey test $p < 0.05$) (Fig. 2C). The FGM concentrations after school workshops were 42.02 ± 31.51 ng/g and two samples exceeded the baseline threshold of 47.05 ng/g (Fig. 3C). At the individual level, the armadillo less used in activities was the only one exceeding the baseline threshold. In opossums, the treatment also influenced FGM concentrations ($F_{2, 50} = 11.121$, $p < 0.001$) (Fig. 2D). Zoo workshop samples (71.29 ± 7.41 ng/g), as well as school workshop samples (35.6 ± 22.25 ng/g), exceeded the baseline threshold of 37.68 ng/g for two individuals used in activities (Fig. 3D). Cortisol levels were significantly different between all treatments for the animal most used in activities (F2, 22 = 4.943, $p < 0.05$).

In skinks, there was no sample collected for the school workshop treatment. The treatment did not have a significant impact on FGM concentrations ($F_{1, 9} = 0.279$, $p = 0.61$) (Fig. 2F). However, the sample size for the school workshop treatment was very small ($n = 3$) (Fig. 3F). In pythons, the treatment did not affect FGM concentrations ($F_{2, 31} = 1.949$, $p = 0.160$ (Fig. 2E), but two samples from school workshops exceeded the baseline threshold in two pythons (Fig. 3E). At the individual level, five subjects had higher FGM concentrations after school workshops which exceeded their baseline threshold. However, the individual sample size for that treatment was insufficient to make significant conclusions ($n = 1$).

In kestrels, the treatment did not have a significant impact on FGM concentrations ($F_{2, 56} = 1.447$, $p = 0.244$) (Fig. 2G), and five samples from zoo workshops exceeded the baseline threshold (329.50 ng/g) for two individuals (Fig. 3G). For one subject, there was a significant difference in corticosterone levels between the zoo workshop and the school workshop treatments ($F_{2, 17} = 4.585$, $p < 0.05$). In owls, the treatment did not significantly impact FGM concentrations ($F_{3, 27} = 1.192$, $p = 0.332$) (Fig. 2H). Two samples from zoo workshops and two from school workshops exceeded the baseline threshold (234.00 ng/g) for the individual used the most in activities (Fig. 3H).

For all species, individuals were not used equally for activities, and the total hours out or the number of rides in the last 30 days had a significant impact on FGM concentrations ($p < 0.001$) (Table 7). In camels, armadillos, opossums, skinks, and kestrels, the individuals most used in activities were the last to reach a hormone peak, whereas the individuals less used reached a peak earlier (Fig. 4). In camels, armadillos, and kestrels, the individuals less used in activities had the highest baseline FGM levels. In contrast, in opossums, skinks, and owls it was the opposite, but the difference between the individuals was not significant (Table 7). The number of samples for each individual in pythons was insufficient to perform a GAM. However, it is worth mentioning that the individuals whose FGM concentrations exceeded the baseline threshold at the species level were the first and fourth most used animals in activities.

Discussion

Behavior results

Our predictions that ambassadors' participation in programmed activities would show higher frequencies of undesirable behaviors and change the activity rate between the baseline period and the post-activity treatments were not validated. However, it is worth mentioning that undesirable behaviors were potentially problematic in a few animals and exceeded the 10 % threshold in some treatments. This reinforces the importance of studying variations both at the species and the individual levels, using individuals as their own control for optimal welfare management.

Diurnal mammal species

Camelid species like the Bactrian camel and the dromedary are known to be social animals living in hierarchical herds and spending most of their time walking to pasture (Gauthier-Pilters & Dagg, 1981; Morgan & Tromborg, 2007). They are often used as working animals because of their extreme resistance to heat, pain, or fatigue, and many are habituated to working until exhaustion without showing signs of suffering (Padalino et al., 2021; Padalino & Menchetti, 2020; Previti et al., 2016). Given the ecology and history of camelids alongside humans, life in captivity with restricted movement, reduced retreat space, forced proximity to humans, reduced feeding opportunities, and maintenance in abnormal social groups could lead to the development of undesirable behavior (Padalino et al., 2014). Despite camels' passive appearance, changes in behavioral patterns are recognizable and can be indicators of welfare for these animals, so identifying those changes early is crucial for their management (Fowler, 2000). According to a study by Padalino et al. (2014), it was shown that camels could perform locomotor stereotypies (e.g., pacing) but preferred oral stereotypies such as self-biting and bar-mouthing. The amount of time performing either stereotypic behavior was approximately 10 % of the observation period when male dromedary camels were housed in a single box for 24 hours (Padalino et al., 2014). Compared with Padalino et al.'s (2014) study, ours combined pacing, circling, and bar-mouthing in the Undesirable behavior category. Nonetheless, these behaviors were observed at similar rates to Padalino et al. (2014) in all our individuals (camels and dromedaries). Unfortunately, the literature on the potential behavioral effect of rides on captive animals in zoos is lacking. Although animal rides can increase human knowledge and awareness about the animals of concern, little is known about the welfare implications of those rides (Rahayu et al., 2022).

In a study on the daily rhythms of behavioral patterns in dromedary camels, the authors found that camels spent 42 % of their time lying down, 14 % feeding, 13 % standing, 13 % ruminating, 2 % walking and 15 % for stereotypic behaviors (Aubé et al., 2017). This agrees with our findings, that Resting up, Resting down, Ruminating, and Feeding were the most performed behaviors. After participating in rides, camels spent less time Resting up and had fewer Social interactions, while the ambassador dromedary spent more time Resting down and less time for Locomotion. Although it is unlikely, this could result from physical fatigue felt after carrying visitors for rides during the day and a preference for less active behaviors. Hence, the absence of rides could explain the higher locomotion rates in the exhibited dromedary not used for activities. The treatment of rides also decreased Social interactions in Bactrian camels when conspecifics left the enclosure for rides. Still, in Bactrian camels, even if Undesirable behaviors were slightly higher after participating in rides, the variable influencing Undesirable behavior the most was the absence of conspecifics. Knowing that camels form hierarchic groups when one animal is removed from the herd, or a new group has formed, it may increase the stress levels in the herd until the hierarchical status is re-established, so one or multiple individuals leaving for a ride day could have caused an increase of undesirable behaviors (Fowler, 2000). This has been observed in the Bactrian camels, where the individual most used in rides exceeded the 10 % threshold in our study post-activity treatment, probably because of the stress generated by the absence of conspecifics.

In dromedaries, the exhibited animal performed more Undesirable behavior than the ambassador animal, and the latter did not perform Undesirable behavior at all in the baseline treatment. The use of dromedaries for rides in zoos has only been studied once with salivary cortisol samples by Majchrzak et al. (2015). The authors concluded that salivary cortisol collected during the riding season was significantly lower than those collected during the off-ride season, which could suggest that rides provided enrichment to dromedaries (Majchrzak et al., 2015). Although the results of our study could coincide with those findings, it must be repeated that multiple variables were associated with Undesirable behavior. In our study, the treatment was not significant for the ambassador animal, and given our low sample size, our results must be interpreted with caution.

Most animal welfare studies will combine multiple physiological, behavioral, health, and other indicators to have a holistic understanding of the animal's wellbeing. Looking into the assessment of stress in riding horses, which are the closest ungulates used for rides by humans related to camelids, studies have shown that salivary cortisol concentrations were unchanged between treatments of recreational low-impact therapeutic riding but higher after horse-riding lesson programs compared to the baseline (Kang & Yun, 2016; McKinney et al., 2015). Notably, horses from riding clubs became more stressed (e.g., higher neutrophil: lymphocyte ratio, higher heart rate, and higher lactic acid levels) after multiple riding sessions a day than only one or two times a day, and some horses would perform undesirable behaviors indicative of resistance to the rider when they were in pain, fearful or anxious (Hall et al., 2013; Jung et al., 2019). Thus, the prevalence of behavioral problems in horses was proven to be affected by the riding style and other management factors, and it could be the same in camelids participating in rides across different zoos (Normando et al., 2011). This amplifies the importance of conducting more studies on camelids to assess other variables such as the amount of rides, emotional reactivity, housing conditions, or learning abilities to work in ride programs (Lesimple et al., 2011). Based on our behavior results, we cannot confirm that rides are stressful for camelids at the species level. However, the absence of conspecifics caused by the departure of individuals for rides could be a potential stressor at the individual level in Bactrian camels.

Nocturnal mammal species

Armadillos are mainly nocturnal species and show crepuscular activity peaks between 16:00 and 22:00, but they can also be active during a short period in the morning and after midnight (Attias et al., 2020; Duarte & Superina, 2015). The three-banded armadillos specifically can show increased activity during the day at low temperatures (Attias et al., 2018). They are awake on average 5.5 ± 2.8 h/day and spend between 80-99 % of their time resting hidden underground since they are fossorial animals and have the habit of excavating soil to build their burrows (Ancona & Loughry, 2010; Attias et al., 2020; Desbiez et al., 2018; Maccarini et al., 2015). Given the ecology of this species, it is not a surprise that armadillos in our study spent over 50 % of their time Not visible, presumed resting in their shelter. A study published in 2016 by Baird et al., interested in the behavioral impacts of programmed activities in ambassador animals, also found that armadillos from education programs spent approximately 50-60 % of their time resting. Contrary to our prediction, we did not find a significant effect of treatment on time spent performing active behaviors in armadillos. However, we found a significant effect of the treatment on the performance of Undesirable behavior in one individual. The main undesirable behaviors observed were pacing, circling, and interaction with the enclosure barriers, including scratching, digging, pushing, and rubbing the body against barriers (Duarte & Superina, 2015). This individual exceeded the threshold of 10 % established in all treatments and had more undesirable behaviors at the baseline treatment and after participating in school workshops. In comparison, the second individual spent less than 1 % of its time performing undesirable behaviors in all treatments. Comparing our results to those of Baird et al.'s study, they demonstrated that the amount of undesirable behavior observed was not different between education, exhibited, and off-exhibit armadillos. However, they found that the number of handling events per week was positively correlated with increasing undesirable behaviors, while substrate depth was negatively correlated with undesirable behaviors (Baird et al., 2016). In our study, the baseline treatment and the school workshop had no difference for the individual performing the most undesirable behaviors, which reduces the possibility that the treatment alone had a significant impact. It is unclear what could be the underlying cause of undesirable behaviors for that individual, but we found that they increased with time of day and were more frequent in the evening and most observed at night. Given that information, it would take more investigation to assess the other potential sources of stress in armadillos.

In opossums, Undesirable behaviors were not a concern, as they counted for less than 3 % of their active time. The potential stressors of handling and transport associated with workshops did not affect behavior compared to normal behavior observed during the baseline. The variations found for activity rates mainly were due to the time of the day, which coincides with the ecology of that species being nocturnal and mainly active during the first 3 hours after dusk and with the periodic activity of short duration during the night (Klejbor et al., 2013; Macrini, 2004). During the day, wild opossums primarily rest in their shelter, probably to avoid predators (Klejbor et al., 2013). Not much is known about their behavior post-stress. However, a study on behavioral observations of single-housed grey short-tailed opossums found that stereotypic behaviors such as bar-chewing, tail-chasing, rolling on their back, and head spinning were present in standard cage settings, whereas those behaviors were not observed in enriched environments (Wilkinson et al., 2010). Moreover, it was found that the absence of abnormal behaviors in the enriched environments allowed increased natural behaviors of running and jumping (Wilkinson et al., 2010). The fact that the opossums' enclosure in our study had multiple structures like branches and small hiding structures as permanent objects in their enclosure in addition to their regular environmental enrichment schedule could explain that this species had the lowest performance rates of undesirable behavior.

Reptile species

Contrary to common perceptions, reptiles manifest a wide range of behaviors, and their behavioral diversity can sometimes surpass that seen in birds and mammals (Warwick et al., 2001, 2013). They are ectothermic species with low metabolic rates and prioritize the energy spent on essential behaviors like hunting and basking (resting on a warm surface), which tends to reduce the behavioral welfare research for them compared to mammals (Benn et al., 2019). Given the lack of research on behavioral indicators of negative welfare in reptiles, it is crucial to identify subtle changes in behavior to recognize stress-related behaviors. For example, interaction with transparent boundaries is one of the behavioral signs of captivity stress identified by Warwick et al. (2013), along with hyper-activity (or hypo-activity) and hyper-alertness. This behavior was described as persistent attempts to push against, crawl up, dig under, or round the transparent barriers of the enclosure (Warwick et al., 2013). Warwick et al. (2013) reported interaction with transparent boundaries as an exploratory and escape behavior where individuals fail to recognize abstract, invisible barriers, which can lead to chronic friction lesions, rostral lesions, or other physical injuries. In the biological context of reptiles described earlier, excessive locomotion could be considered an undesirable behavior by causing a tremendous energetic cost to the animal and indicating discomfort or stress (Augustine et al., 2022; Conant, 2007). In our study, interaction with transparent boundaries was considered undesirable behavior for skinks and pythons since it was observed during the *ad libitum* observation period at the beginning of the data collection. As for hyper-activity – which is often associated with interaction with transparent barriers – it can also relate to abnormal high-level physical activity due to restrictive, deficient, and inappropriate environments, whereas hypoactivity can result from hypothermia, injury, pain, or transport trauma (Warwick et al., 2013). Thus, sleep patterns or time spent resting in reptile activity budgets is important to monitor since a disturbance can indicate a negative affective state (Benn et al., 2019; Clegg et al., 2015). Finally, hyper-alertness can result from nervousness or fear and act as a defense or escape behavior from exposed, inappropriate environments (Warwick et al., 2013). Specifically in pythons, hyper-alertness was associated with Periscoping behavior in which individuals made vertical movements or froze their head upwards with a 45° angle minimum while the rest of the body was tense, not in an s-curve striking position (Zdenek et al., 2023).

In skinks, time spent Resting was significantly lower in the baseline treatment than after participating in either zoo or school workshops. From the two skinks in our study, only one individual was provided with a closed hiding box in its enclosure, so the Not visible, presumed resting observations applied to that individual only. Our results showed that this individual spent less time resting in its hiding box after participating in school workshops than during the baseline. Ultimately, this means that following an activity, the individual was observed resting in open, visible areas of the enclosure instead of in its hiding place. We did not find a significant difference in activity rates between treatments in skinks. However, there was a trend to spend slightly more time in active behaviors after participating in activities than during the baseline. Both individuals in our study had different responses to activity treatments. The one provided with a hiding box (and less used for activities) had higher rates of Undesirable behavior after participating in school workshops, while the other without a hiding box (and most used for activities) had elevated rates above the 10 % threshold of Undesirable behavior during the baseline treatment. Both individuals were susceptible to exceeding the 10 % threshold of Undesirable behavior after participating in activities considering the mean observation rate. In sum, we found no evidence that the treatment influenced undesirable behavior or activity rates in skinks, but the individual most used for activities performed higher undesirable behavior rates than the other skink.

In pythons, the treatment had a significant effect, and individuals spent less time resting in open, visible areas of the enclosure after participating in activities than during baseline. As a trend in parallel, they spent more time being Not visible, presumed resting in their hiding box, which is the opposite of what was observed in skinks. Exploration and Periscoping were behaviors influenced by the interaction of the treatment and the time of the day, mostly because pythons were more active in the evening than during the day or the afternoon. Undesirable behaviors were observed in nine of the ten individuals, from which three exceeded the 10 % threshold after school workshops, and one exceeded the threshold in all treatments. The treatment significantly affected the performance of Undesirable behavior in two pythons. Although the undesirable behavior rates at the species level did not exceed the 10 % threshold, we suggest monitoring the individuals for which the activity treatments generated significant behavior changes. Finally, we found that the interaction of the treatment with the time of day influenced undesirable behavior in all individuals and increased as the day advanced, so it was highest at night. Thus, this could explain why zookeepers did not observe undesirable behaviors during the zoo's opening hours in the daytime so management should consider this new finding.

For both reptile species, it remains unclear whether the potential stressors of handling and transport for activities could alone explain changes in behavior and generate stress for the animals. A study researching the effects of handling and space constraints on reptiles found no differences in total activity after undergoing gentle handling or container restraint treatments (Kreger & Mench, 1993). However, the duration for each treatment was only 10 minutes, while workshops in our study could vary between 30 minutes and 8 hours depending on the type of workshop, the location of the activity, and the number of groups that have booked an activity in a day. Other studies found a stress effect of handling in other reptile species using indicators of locomotion/activity time, latency to novel environment/object test, tongue flicking behavior, and heart rate measures (Borgmans et al., 2021; Cabanac & Cabanac, 2000; Stockley et al., 2020). Nonetheless, to our knowledge, our study is the first incorporating interaction with transparent barriers as a behavioral criterion since it was published by Warwick et al. in 2013. In a future study, signs of acute stress and fear described by the same authors, like inflation of the body, hissing, flattened body posture, human-directed aggression, clutching, panting, or vocalizations, should be observed during the activities (Warwick et al., 2013). Although we did not find evidence that activity treatments significantly changed behavior at the species level for reptiles, we discovered that a few individuals exceeded the 10 % threshold of undesirable behavior no matter the treatment.

Bird species

Measuring stress-related behaviors differs in bird species, especially in raptor species that rarely show undesirable behaviors (Park, 2003; Smith & Forbes, 2009). For captive raptor species, stressors can include being on exhibit for displays, enclosures with a limited view, the sight of other predators, unfamiliar environment, training, and deprivation of social interaction or mental stimulation for extended periods (Jones, 2001; Smith & Forbes, 2009). All those stressors can lead to undesirable behavior in birds, and it is already known that handling and human contact can be stressful for raptors (Park, 2003). This explains why a permit is mandatory to possess raptors in many countries, and only skilled specialists should handle them to minimize stress (Huckabee, 2000). Based on our literature review, we identified feather-damaging behavior as undesirable behaviors for our bird species (Smith & Forbes, 2009). Feather-damaging behavior provokes birds to pluck, chew, bite, and fray all feathers accessible to the bird's beak and results in loss or damage of them (van Zeeland et al., 2009). Although feather-damaging behavior is rarely observed in raptors, they have been demonstrated in Harris' hawks and red-tailed hawks (Baird et al., 2016; Harris, 2005; Jones, 2001; Park, 2003; Smith & Forbes, 2009). To our knowledge, this is the first study looking into potential undesirable behaviors in American kestrels and great-horned owls. In our study, we differentiated feather-damaging behavior into two categories: 1) Self-directed behavior: feather plucking as part of grooming, and 2) Undesirable behavior: feather damaging due to locomotor stereotypic movements such as hitting cage walls, escape behavior, or self-harming behavior against cage surfaces or perches (Park, 2003). Contrary to our predictions, none of the ambassador bird species performed Undesirable behavior, but the results for Self-directed behavior will be further discussed.

Based on a study also assessing the welfare of ambassador animals, it was found in red-tailed hawks that handling frequency and duration were negatively correlated to Self-directed behavior, and the latter behavior was used as an equivalent of undesirable behaviors in birds (Baird et al., 2016). The authors did not mention the observation rates of self-directed behaviors from their study, but according to the literature, birds spend on average between 5-10 % of their time preening

(Bush & Clayton, 2023; Cotgreave & Clayton, 1994). Feather-damaging behavior may be considered undesirable behavior when the normal behavior of preening increases in time, duration and/or intensity as a coping mechanism to stress (van Zeeland et al., 2009). However, the distinction between preening, and feather-damaging behavior is hard to establish and feather-damaging behavior often follows the same pattern as grooming in self-directed behavior (Lefebvre, 1982; Van Hoek & King, 1997; van Zeeland et al., 2009). Looking into our results, the mean observation rate of self-directed behavior after participating in programmed activities was not significantly different between treatments for either species. Notably, we found that kestrels and owls spent respectively 13 % and 4 % of their time on Self-directed behaviors, and there was no significant difference between individuals. These rates agree with those found in the literature on owls but exceed the 10 % rate in kestrels. Whether feather-damaging behavior occurred more than normal preening behavior remains unknown, so we suggest monitoring self-directed behavior in kestrels, investigating the number of feathers found in the enclosure, and potentially analyzing cortisol rates in them.

Time spent perching and actively hunting or foraging varies greatly between raptor species according to their preferred hunting modes, so activity budgets can be very species-specific (Baladrón et al., 2016). In the context of programmed activities, the study from Baird et al. (2016) also found that red-tailed hawks exhibited less Resting active behavior after participating in activities than at baseline or during no handling periods, so that behavior was positively correlated with handling frequency. Our results are similar since Resting passive in kestrels was significantly influenced by treatment, among other variables. For example, kestrels spent more time Resting passive after school workshops than at baseline or after zoo workshops. This means that after school workshops, kestrels spent less time being vigilant and had fewer head and eye movements than during the baseline. American kestrels are diurnal hunting falcons and the allocation of time spent for various activities, including Resting active to hunt prey, can vary due to extrinsic factors such as time of day and human-related disturbance (Palmer et al., 2001). Hence, a disturbance such as school workshops could reduce the time spent Resting active or vigilant and change the activity budget resulting in more Resting passive behavior. Similarly, in owls, both resting behaviors were significantly different between treatments, with more time spent Resting active during the baseline treatment than after participating in a workshop and more time spent Resting passive after participating in a workshop than during the baseline. Knowing that great-horned owls are nocturnal birds, light, noise, and anthropogenic disturbances for workshops during the daytime - when they are supposed to rest in a restorative way - could lead to impairments in normal time dedicated to rest, including the total sleep duration and the quality of sleep (Caorsi et al., 2019; Rudolph, 1978). Hence, this could explain the increased time spent Resting passive during the night when they are supposed to be active. Thus, we can confirm that treatments impacted resting behaviors in both bird species, but further investigation is needed to determine if that impacts their welfare negatively.

Fecal sample results

The baseline and activity treatments did not have a significant impact on FGM levels in all species. Specifically, camels and opossums had significantly high FGM means exceeding the baseline threshold after rides and workshops, respectively. In dromedaries and opossums, the baseline FGM levels of the ambassador animals were noticeable but not significantly higher than the exhibited animals. All ambassador species, except skinks, had a few samples with potentially problematic high FGM levels exceeding the baseline threshold after participating in activities. Nonetheless, the activity treatments were not always significantly associated with higher FGM levels in comparison to the baseline. For example, zoo workshops had lower FGM concentrations than the baseline in armadillos and pythons, while school workshops had the lowest FGM concentrations of all treatments in kestrels. We can confirm our prediction in camels and opossums based on our results. However, these results must be interpreted cautiously since many variables can influence FGM levels, such as environmental challenges and intrinsic or ecological factors.

Environmental challenges from biotic or abiotic factors and anthropogenic disturbances can create a physiological stress response and modify FGM concentrations (Dantzer et al., 2014). In our study, most ambassador species had a strict husbandry routine and were kept off-exhibit: individuals were housed alone, their diet and frequency of feeding were similar every week, the ambient temperature was regulated to always stay the same, the lights automatically turned on and shut off every day at the same hour and the zookeepers cleaned the enclosures according to a specific schedule. However, that was not the case for camels and dromedaries who were kept outside in the exhibited part of the zoo open to visitors. Exposure to familiar and unfamiliar people represents a significant part of zoo animals' life; consequently, the animals will perceive humans negatively, neutrally, or positively (Hosey, 2013; Sherwen & Hemsworth, 2019). The way zoo animals will change their behavior and physiology in response to the presence of visitors is called the visitor effect (Hosey, 2013; Sherwen & Hemsworth, 2019). In our study, fecal samples for the baseline treatment were collected before the zoo's opening for the summer season and many factors could not be controlled such as the weather and the visitor effect. Since there were no visitors before the zoo's opening and rides occurred every day once it opened, it was arduous to collect more baseline samples afterward. The latter – if they were significantly higher – would have been eliminated through the iterative process described in the methods section. However, it has been shown that exposure to humans through tourism could be associated with increased FGM levels

(Barja et al., 2007; Zwijacz-Kozica et al., 2013). Thus, our results confirm that the treatment of rides significantly affected FGM levels in camels, but that could have been amplified by the visitor effect when the activity fecal samples were collected after the zoo's opening. To our knowledge, this study is the first to measure FGM concentrations in Bactrian camels, so it would be interesting for further studies to investigate their welfare.

In opossums, individuals were housed in controlled settings without environmental challenges, so the FGM concentration results interpretation had fewer variables to consider. In marsupial species, only a few studies focused on stress responses to capture and handling, such as in koalas, numbats, and wombats (Hajduk et al., 1992; Hing et al., 2014; Hogan et al., 2011, 2012). The effects of those stressors were increasing FGM levels after regular handling and no behavioral reaction (Hogan et al., 2011). The latter suggested that learned helplessness occurred because the stressor was continuous, and the physiological stress did not diminish with time (Hogan et al., 2011). This corresponds to our elevated FGM results, which significantly exceeded the baseline threshold for opossums after participating in activities requiring handling compared to the baseline treatment. Comparing our FGM results to recent research on wild-caught brushtail possums (Cope et al., 2022), the mean baseline FGM concentrations were similar but slightly higher in wild brushtail possums (62.75 ± 24.49 ng/g) compared to ambassador opossums (23.51 ± 7.08 ng/g). The mean peak FGM concentrations in wild brushtail possums after trapping and brought into captivity, which includes handling and transport stressors, were reached after 19 ± 13 h and approximately measured 178,67 ± 67,83 ng/g (Cope et al., 2022). This magnitude of change from baseline FGM at a peak of over 100 % corresponds to the significant difference found in our results between the mean baseline FGM concentrations and the multiple samples that we identified exceeding the baseline threshold that also had a magnitude of change over 100 %. However, it remains unclear why zoo workshops were associated with higher FGM concentrations than school workshops. During the summer, the opossums participated in very few zoo workshops, all for day camp presentations rather than programmed shows with regular zoo visitors. It would be interesting for future studies to measure the impact of the number of guests during programmed activities, the potential effects of the visitors' age range, and the number of times the animal was touched along with other relevant stimuli to deepen our knowledge on potential stressors related to handling and transport in those two different workshops.

We did not find a significant difference between the activity and the baseline treatments in armadillos. Only two studies have measured adrenocortical activity in zoo-captive armadillos. The first published in 2012 from Howell-Stephens et al. characterized FGM concentrations in different treatments after ACTH challenge (injection of a synthetic adrenocorticotropic hormone (ACTH) analog), natural and routine events (pairing/breeding individuals), and veterinary procedures to validate the use of fecal hormone analysis. They found elevated FGM concentrations in male armadillos of 3805.5 ± 1809.1 ng/g above the baseline concentration (1080.2 ± 240.5 ng/g) with peak FGM concentrations reached within 30-94 h and a return to baseline between 1-7 days according to the treatment (Howell-Stephens et al., 2012). The FGM concentrations in their study were significantly higher than those measured in ours. The second study published in 2016 by Baird et al. was interested in the physiological impacts of programmed activities in ambassador animals. It was found in that study that the FGM concentrations did not differ between groups of education, exhibit, and off-exhibit armadillos, with baseline concentrations in male armadillos ranging between 12.33 ng/g and 42.95 ng/g and over 100 % increases in FGM concentrations post ACTH challenge for both males. The peak FGM concentrations were reached within 19-46 h and returned to baseline between 3-5 days. In our study, ambassador armadillos had programmed activities at least twice a week from April to August 2022, and that period coincided with the collection period of most of the fecal samples. Therefore, it was more accurate to the armadillos' schedule to keep the activity treatment duration at three consecutive days following the participation in an activity and collect baseline fecal samples minimally 72 h after coming back from the last activity. Realistically, it would not have been feasible otherwise to collect baseline samples during the summer season without compromising the reservations already booked and the work schedule of educators.

Furthermore, armadillos had significantly different FGM concentrations among the activity treatments, whereas the school workshops had higher FGM concentrations than the zoo workshops. Going back to Baird et al. (2016) study, they found that the effect of handling was not significant, however, the overall amount of handling that animals experienced was positively correlated to FGM in armadillos. This agrees with our findings since the zoo workshop treatment with handling was not significantly different from the baseline treatment. School workshops with the additional transport stressor could be potentially more stressful for armadillos because of three variables: 1) the substrate type in transport boxes, 2) the transport box size, and 3) the daily activity pattern in armadillos. Baird et al. (2016) found that the type and depth of substrate provided in armadillo enclosures were associated with differences in FGM levels, meaning that armadillos had higher FGM concentrations when no substrate was provided. The transport boxes used for school workshops cannot meet the optimal needs regarding substrate type and depth, so changing the environment to a container less suited to the burrowing lifestyle of armadillos could generate stress. It was also found that the enclosure size was negatively correlated with FGM, meaning that armadillos with smaller enclosures had higher FGM concentrations than individuals housed in bigger enclosures (Baird et al., 2016). Transport boxes are usually smaller than the animal's usual enclosure size for transport purposes, which could lead to stress due to space constraints. Finally, as mentioned before, armadillos are animals mainly active during the night and spend between 80-99 % of the time in their burrows, so the potential stressors of handling and transport occurring during the daytime for workshops conflict with these habits (Attias et al., 2020; Desbiez et al., 2018; Maccarini et al., 2015).

To continue, intrinsic and ecological factors can affect physiological stress responses and therefore, increase or decrease FGM levels (Dantzer et al., 2014). Intrinsic factors, including sex, age, reproductive status, and developmental and past experiences, can either sensitize or desensitize the individual to stressful challenges. Among the ambassadors used in this project, only pythons and bird species had both male and female subjects. Although FGM levels in reptiles can differ between males and females, we did not collect sufficient samples per individual in pythons to make conclusive statements about sex, but we can comment on the results for bird species (Martínez Silvestre, 2014). A study found that male free-living northern spotted owls (*Strix occidentalis caurina*) exhibit a more pronounced rise in FGM levels in response to logging roads and traffic noise compared with females (Hayward et al., 2010; Wasser et al., 1997). The opposite is true in American kestrels (*Falco sparverius*), where females were found to exhibit higher baseline plasma FGM levels in response to anthropogenic disturbances of road traffic and human development (Strasser & Heath, 2013). This information could partially explain the individual baseline and post-activity FGM levels in both bird species studied. For instance, if the results of the northern spotted owls can be generalized to the great-horned owls, then the male ambassador had higher but non-significant baseline and zoo workshops FGM concentrations than the female owl ambassador. The FGM concentrations found in our study were similar to the ones found in the literature for the great-horned owls, with peak values reached 2 h and 12 h post-ACTH challenge and concentrations between approximately 250-350 ng/g (Wasser et al., 2000; Wasser & Hunt, 2005). As for the kestrel ambassadors, the results were inconclusive; one female had higher FGM baseline levels than the male, while the other female had higher FGM school workshop levels than the male, but ultimately the male had higher FGM zoo workshop levels than both females. Our baseline FGM concentrations were similar to the ones found in the literature for wild American kestrels induced to ACTH challenge (114.27 ± 15.23 ng/g) with peak values of 446.10 ± 60.73 ng/g reached within 4 h and returning to normal values within 4 days (Pereira et al., 2009). Overall, it is essential to consider sex in the interpretation of FGM concentrations, but it was not conclusive in our study.

Age is another factor that can influence physiological stress responses (Dantzer et al., 2014). This factor was not included in our analyses because of the small sample size of individuals per species. However, it is interesting to know that in mammalian species, young individuals' HPA axis is hyporesponsive, whereas when they grow older, it becomes hyperresponsive to challenges, meaning that individuals will exhibit more prolonged and exaggerated stress responses to disturbances (Sapolsky et al., 1986; Sapolsky & Altmann, 1991; Wada, 2008). Looking into the FGM concentrations of our mammal species following activity treatments, there was no significant variation in age between the individuals in opossums. On the other hand, the oldest individual reached the highest FGM levels in camels, dromedaries, and armadillos. In both camelid species, the oldest individual was the most used in rides and the last one to reach an FGM peak in comparison to younger less used animals. In armadillos, the oldest individual was the least used animal for workshops and the first to reach an FGM peak when looking at the total activity hours. However, it has been showed that FGM levels were not influenced by sex or age in armadillos (Howell-Stephens et al., 2012). It would be interesting for a future study to compare the interaction effect between the age of ambassadors and the participation rate for activities.

The diurnal rhythm is another intrinsic and species-specific variable that influences the excretion of FGM levels (Dantzer et al., 2014; Touma & Palme, 2005). Daily rhythmicity of cortisol secretion has been studied in many species, such as bulls and horses (Bohák et al., 2013; Hemmann et al., 2012; Thun et al., 1981). Results show that cortisol levels in ungulates are higher around midday than in the evening, so Aubé et al. (2017) suggest sampling at the same time of day to compare cortisol levels. Even if the hour of the day was a random factor in our FGM analysis, it is still important to explain the literature behind the method used for data collection. In research studying daily rhythms of hormonal patterns in captive male dromedaries housed in boxes, the mean cortisol blood levels peaked at 14:00 and returned to normal levels after 18:00, with the lowest concentrations at 20:00 before increasing again at noon the following day (Aubé et al., 2017). Plasma cortisol and feces cortisol are positively correlated and adequately reflect the HPA axis activity in dromedaries, but most studies found on camelid species used plasma samples rather than fecal samples (Bargaâ et al., 2016). Thus, almost all samples collected for camelids in our study were collected at the beginning of the day between 8:00 and 10:00, so daily pattern variations were not a concern. The only study on male dromedaries using fecal samples was a study from Sid-Ahmed et al. (2013) in which the maximal peak values were reached 24 h after the ACTH challenge and declined after 36 h (Sid-Ahmed et al., 2013).

Moreover, one of the most critical variables to consider in FGM analysis is the species-specific intestinal passage time and the digestive rate allowing the excretion of hormones in feces samples (Palme, 2005). Although the animals' diet did not change during the study period, the feeding frequency was not the same among species due to different metabolic needs. For example, pythons were fed only once every week or two, meaning they rarely defecated, and the sample size obtained per individual was small. That is a common practical problem in reptiles, particularly in the Python taxa, which has a passage time from 5 to 17 days and explains why most of the existing literature used blood samples (Bedford & Christian, 2000; Benn et al., 2019). Unfortunately, non-invasive sampling methods remain understudied in comparison to blood sampling in reptiles (Benn et al., 2019). To our knowledge, this is the first study measuring FGM concentrations in ball pythons and blue-tongued skinks, and based on our results, we did not find a significant effect of the treatment on FGM concentrations. However, it has been demonstrated that after taking into account gut passage time, general patterns of FGM concentrations were similar to those in blood samples, so it is still relevant to compare our results with other welfare studies in captive reptiles (Palme et al., 2005; Schwarzenberger et al., 1996). For instance, research on the impacts of animal care on snake welfare in zoological institutions found that increased paper changes for the enclosure's ground covering generated an increase in FGM concentrations in Wagner's vipers (Augustine et al., 2022). Another past research interested in the effects of handling and space restraint in the ball python and the blue-tongued skink found that container-restrained pythons had higher plasma corticosterone levels than those of controls, while container-restrained skinks had higher but not significant levels than those of controls (Kreger & Mench, 1993). This agrees with our results, where FGM concentrations associated with the handling and transport stressors after a school workshop were higher but not significantly different from the baseline treatment for both ball pythons and blue-tongued skinks.

Finally, our study's last variable of interest was the effect of activities experienced by the ambassador animals. Contrary to what was predicted, the activity treatment, including handling only in zoo workshops, was not significantly different from the baseline treatment in all species, except opossums. However, this could be explained by the fact that the ambassador used in our study went through several years in the education program and became familiar with handling procedures, so FGM concentrations were unchanged (Palme, 2019). Thus, we were interested to know if the total use of animals for activities had an impact on FGM concentrations over several activities in time. We found a significant effect of the activity outings rate on FGM concentrations in all species. This relationship was also found in Baird et al.'s study (2016), where the overall amount of handling was positively correlated with FGM concentrations. Interestingly, we also found that the individuals that were the most used in all species, except pythons, were the last to reach an FGM peak, whereas those less used for activities reached an FGM peak earlier. This could indicate that less used animals experience acute stress sooner after participating in fewer activities than most used animals. These results emphasize the importance of monitoring the outings of each ambassador animal since parameters like frequency and duration of activities can lead to different stress responses between animals less and more used over time. To conclude, multiple variables can influence FGM concentrations, and for this reason, they must be analyzed in parallel with other welfare indicators such as behavior.

Implications on welfare

In the hope of understanding the impacts of education programs on ambassador animal welfare, we were mainly interested in the behavioral and physiological stress consequences generated by handling and transport after participating in programmed activities and whether the participation in animal rides, zoo workshops or school workshops would: 1) increase FGM levels; 2) increase the rate of undesirable behavior; or 3) change the activity rate post-stress in comparison to a baseline treatment. Assessing whether animal welfare is good or poor is complex, as it requires a comprehensive and holistic point of view, and an animal's current wellbeing status can vary with time. Thus, to provide an insightful understanding of our results to the reader, we summarized the outcome of our predictions for each species separately.

Combining the behavior and physiological measure results, our initial predictions were not unanimous across species. In camels, FGM levels were more elevated than baseline levels and undesirable behaviors were potentially problematic after participating in rides. In dromedaries, the ambassador animal did not have elevated FGM levels nor problematic undesirable behavior rates after participating in rides. Additionally, the ambassador animal had no significant but higher FGM levels than the exhibited animal and performed less undesirable behavior. In armadillos, a difference was found between the FGM levels associated with the zoo workshops and the school workshops; undesirable behavior was only observed in one individual at rates exceeding the established threshold. In opossums, the FGM levels were higher after participating in activity treatments than at baseline, but the undesirable behavior rates were not a concern given that they were the lowest rates observed of all species. In skinks, FGM levels respected the thresholds and only one individual had elevated undesirable behavior during the baseline treatment. In pythons, FGM levels did not exceed the thresholds, but a few targeted individuals performed elevated undesirable behavior rates. Finally, in both kestrels and owls, FGM levels were under thresholds, and no undesirable behavior was observed, but we recommend monitoring self-directed behavior, particularly in kestrels, to prevent feather-damaging behavior. For all species, our third prediction on activity rates was not validated and was not different between activity and baseline treatments. However, we found a positive association between FGM levels and the frequency of outings of ambassador animals. Looking into our results, we do not have enough evidence to suggest that the ambassador animals in the Zoo de Granby and the Ecomuseum Zoo experienced significant signs of stress threatening their welfare due to programmed activities.

Improving ambassador animal welfare

The main difference between ambassador animals and exhibited animals is the increased handling and manipulations that ambassadors undergo for activities where visitors can touch animals (Powell et al., 2020). Multiple studies have been conducted on the effects of being exposed to handling or hand-reared by humans in early life stages to reduce adults stress responses (Baird et al., 2016; Bryan Jones, 1994; Costa et al., 2016). Otherwise, when adult animals have negative past experiences with handling, they can develop immediate responses to certain stimuli preceding handling and become fearful or stressed at the sight of these stimuli (Baskir et al., 2020; Meagher et al., 2011). In the case of ambassador animals frequently participating in programmed activities, the lack of significant changes in behavioral responses between the treatments could be explained by the phenomenon of habituation, according to which the magnitude of the responses to a specific stimulus decreases with repeated exposure to that stimulus (Grissom & Bhatnagar, 2009; Thompson & Spencer, 1966). Habituation protocols are commonly used by zookeepers in the present context to habituate and introduce new ambassador animals to handling and transport procedures which are particularly useful in decreasing stress-related behavior associated with handling and enhance the safety of both humans and animals (Ujita et al., 2021). The issue with interpreting reduced stress responses is the probability of overlooking that ambassador animals might suffer from learned helplessness (Hogan et al., 2011). Thus, when contacts with visitors become frequent and unavoidable if emotional reactivity does not prevent animals from being handled or transported, they may stop avoidance or defensive behaviors altogether and become non-responsive (Hogan et al., 2011). In that case, learned helplessness would indicate poor welfare and be reflected by constantly elevated glucocorticoid hormone concentrations (Hogan et al., 2011). Therefore, establishing individual baseline thresholds is imperative to monitor stress-related hormone concentrations in the long term, especially for ambassador animals. Thus, we highly recommend education programs using ambassador animals and zookeepers training new ambassadors to implement behavioral and physiological records if they are not presently used to improve welfare and prevent learned helplessness.

Aside from the direct contact with the public that ambassador animals undergo, they live in different enclosures from those designed for exhibited animals (Powell et al., 2020). For example, the living spaces can have different sizes, environmental structures for decoration purposes, or even be in different buildings that are not accessible to zoo visitors. Notably, that was the case for all the species studied in our project, except camelids, who were housed on exhibit next to the camel rides route for visitors. Since the other ambassador animals were smaller in size and weight, they were housed in a remote building open to zoo staff. A common trend observed was that those ambassador animals had smaller enclosures than exhibited animals while respecting the minimal space regulations. It would be interesting to compare the enclosure size differences between the same species of ambassador animals and exhibited animals to explore the potential impacts on the welfare of the enclosure sizes provided to ambassador animals across multiple zoological institutions. Another difference is the absence of decorative structures in the enclosures of ambassadors. Since the enclosures are not visible to the public, there is no need to provide unnecessary decorations if they add more responsibilities to zookeepers, including cleaning and/or must be frequently replaced or repaired because of their usage by animals. On the other hand, bigger enclosures with structural complexity provide a wider range of natural behaviors such as hiding, climbing, digging, etc. and it was proven that space use could be used as an indicator of enclosure appropriateness and welfare (Ross et al., 2009). A first concern for the ambassador reptile species' enclosures was that they did not provide opportunities to move up or down and one skink did not have a closed hiding space. Another example of exhibited pythons at the zoo is that they had a tree-like structure with branches and suspended baskets or platforms to allow the animals to climb at different heights, rest in different spots, and, more importantly, stretch their whole body. It was recommended by multiple studies that snakes should be able to adopt rectilinear behavior and live in enclosures that are at least the length of the animal, not only for comfort to stretch out fully but also to avoid potential clinical illnesses (Cargill et al., 2022; D'Cruze et al., 2020; Warwick et al., 2021). Thus, it emphasizes the bias regarding reptiles, whose welfare is more often overlooked compared to mammals, and findings from space use studies for exhibited animals should also be transferred to improve ambassador animals' enclosures.

Using non-invasive methods to measure stress should always be favored over other invasive methods, so we collected fecal samples instead of blood samples. However, it can be challenging to collect many fecal samples in the case of species that defecate rarely. That is why we recommend using multiple glucocorticoid sampling techniques in future studies in addition to fecal samples. For example, it would have been interesting to analyze keratinized tissues, including hair, feathers, and shed skin. The benefit of combining techniques is that they estimate different time frames and response latencies after a stressful event, so more variables can be analyzed for stress duration, peaks, return to normal, and cyclic patterns (Gormally & Romero, 2020). Furthermore, it would have been interesting to measure immediate metabolic responses with those of the nervous system by measuring variations in heart rates, breathing rates, and body temperature. In the context of programmed activities, measuring heart rate would be relevant since it can be affected by acute stress, chronic stress, and repeated stressors (Cyr & Romero, 2008; Fischer et al., 2018). Moreover, it was found that breathing rates could change quickly within minutes in birds in response to handling (Carere & van Oers, 2004). Since our raptor bird species' behavior changes were inconclusive, measuring breathing rates could have been helpful. Finally, measuring fluctuations in body temperature could be applied to reptiles since they are ectothermic species, and changes in ambient temperatures were found to be potentially harmful to them (Mancera et al., 2014). In our context, especially during school workshops, the temperature can vary greatly from moving between the initial animal's enclosure, the transport box, inside and outside the vehicle, and between different rooms, so the exposure and adaptation time to return to normal temperature could be a stressor for reptiles participating to activities.

It is commonly known in zoos that environmental enrichment provides multiple benefits to captive animals, the first of which is reducing undesirable behavior (Podturkin, 2021; Swaisgood & Shepherdson, 2005). Although the presence or absence of environmental enrichment was noted during this study, the detailed results will not be discussed here since they were not the focal point of the research. However, we were surprised to find that the presence of environmental enrichment in the animals' enclosure did not significantly change the time spent performing exploration behavior, undesirable behavior, or active behaviors overall. It has already been suggested that human-animal interactions could be rewarding for some species and provide a form of enrichment (Clegg et al., 2018). Although it is still debated whether programmed activities are stressful for ambassador animals or not, another important question that has arisen would be whether the presence of enrichment programs has a significant effect on their welfare and determine if enrichment can remediate the performance of undesirable behavior in ambassador animals.

We believe that a few criteria should be considered when choosing ambassador animals. For example, a good ambassador would be one who can express affiliative behavior or be more socially engaged towards humans. Acaralp-Rehnberg et al. (2020) observed that hand-reared cheetahs were more suitable for interactive activities during presentations with visitors possibly because of their higher tolerance for close contact with human caretakers but it could also be due to reduced fear responses after extensive training (Kozlowski et al., 2021). The authors also reported that further investigation should be done on how positive zookeeper-animal relationships can affect the response of zoo animals to visitors since one of the keepers was responsible for rearing cheetahs when they were kittens and closely bonded with them (Acaralp-Rehnberg et al., 2020). Moreover, a good ambassador should have a high tolerance to human disturbance to reduce the potential impacts related to anthropogenic stressors, including unfamiliar environments, noise, frequency and duration of contacts, handling, and transport, experienced during programmed activities (Bardo & Bird, 2009; Morgan & Tromborg, 2007). For example, American kestrels are well documented among raptor species and are known for their high tolerance for human disturbances in agricultural and urban habitats (Bardo & Bird, 2009; Dantzer et al., 2014; Heath & Dufty, 1998). The numerous studies on blood and fecal glucocorticoid levels, reproductive success, normal behaviors, and handling stress can be applied to captive settings to insure optimal conditions (Bardo & Bird, 2009; Bush & Clayton, 2023; Pereira et al., 2009; Sockman & Schwabl, 2001; Strasser & Heath, 2013). Thus, considering the literature available on that species, kestrels seem to be a good model for education programs.

Other important aspects in the choice of ambassador species are the animals' physical characteristics and ecology. For example, smaller size and weight of the animal are variables that can facilitate housing, handling, and transport (Bardo & Bird, 2009; Fuhrman & Ladewig, 2008). Thus, smaller animals are preferable to use in workshops outside of the zoo while bigger ones are more suited for interactions on zoo grounds, including animal petting, feeding, and rides. In addition to size, the species' activity rates and light cycles must be considered, since more active animals tend to increase visitor engagement but programmed activities during the day can conflict with normal sleeping schedules of nocturnal species (Fuhrman & Ladewig, 2008; Kozlowski et al., 2021). For example, Kozlowski et al. (2021) found that nocturnal ambassador foxes had higher activity rates during the day than off-exhibit foxes, but they could not determine whether the greater time spent active was related to stress and the potential impacts on welfare. Similarly, in our study owls spent more time resting passive during the night while they should have been active. Therefore, the potential impact of programmed activities on diurnal species compared to nocturnal or crepuscular species on their welfare is unknown. Lastly, the habituation process to desensitize future ambassador animals to handling and transport takes time and must be done carefully following specific guidelines to ensure that the animal is adequately trained to participate in programmed activities. This process can take up to several months until the animal is considered ready to become an active ambassador. For that reason, we believe that species with short lifespan, like grey short-tailed opossums who live in average between one to three years, are less suited for education programs because the proportion of time spent on training compared with the time spent participating in activities is not practical.

Finally, the choice of ambassadors should not rely on generalization of a species' behavior and should take into account individual variations. For example, personality traits like aggressivity, fearfulness, dominance, curiousness, friendliness, or nervousness, can determine whether or not an individual can be a successful ambassador compared to another one (Kozlowski et al., 2021). From the educator's perspective, the behavior of the ambassador animal must be under control at all times to ensure the safety of visitors and employees. Specifically, in raptor birds, Caudell and Riddleberger (2001) describe a few popular beliefs that most hawks are viewed as a "beginner's bird" for falconers, while ospreys are considered very difficult to maintain in captivity, and eagles should only be handled by experienced handlers. Although these recommendations may be accurate, it is crucial to evaluate each bird individually to assess its potential as an ambassador

(Caudell & Riddleberger, 2001). Furthermore, the favorable personality traits could potentially have an effect on the selected individual ambassadors’ behavioral or physiological response and, ultimately, its welfare.

As mentioned above, following specific guidelines for behavioral training, handling and transport desensitization, and environmental enrichment programs, are important considerations for improving ambassador animals’ welfare. It is worth mentioning that all those practices were used both at the Zoo de Granby and the Ecomuseum Zoo. This demonstrates the importance given to animal welfare in these zoological institutions and can be reflected by the results of this study. Overall, although the activity treatments of animal rides, zoo workshops or school workshops influenced some individuals who exceeded established thresholds for both behavioral and physiological indicators, we did not find evidence at the species level that ambassador animals used for programmed activities experienced poor welfare. Therefore, future studies should assess animal welfare both at the species and the individual level to ensure that all animals benefit from optimal welfare management practices and are a good fit to become ambassadors in education programs.

Conclusion

Overall, we found potential sources of stress across ambassadors by comparing different activity treatments associated with handling and/or transport to a baseline treatment with no stressor experienced. Notably, the latter treatments had a physiological effect on camels, opossums, and armadillos' fecal samples. We characterized the variation of behavioral stress indicators in activity budgets, such as undesirable behavior and activity rates for each species. A few individuals exceeded the 10 % threshold of undesirable behavior for specific treatments in camels, armadillos, skinks, and pythons, but the activity rates between treatments was unchanged. We also found significant FGM concentration variations depending on the use of ambassador animals for programmed activities. As a result, most used ambassador animals experienced acute stress FGM peaks delayed later than less used animals after participating in activities. Finally, we studied intra and inter-variations of stress responses between all treatments within mammals, reptiles, and bird species. Our results do not suggest that ambassador animals from the Zoo de Granby and the Ecomuseum Zoo are stressed by education programs on both a behavioral and physiological level concurrently. The average effect for each ambassador species did not significantly exceed the established thresholds of wellbeing for both behavior and hormone analyses together. However, our findings also suggest that potentially problematic values for either behavioral or physiological measures separately could exceed our thresholds in a few of the studied species, so it is crucial to continue monitoring ambassador animal welfare, especially for targeted individuals. To optimize management methods, we recommend inspecting welfare indicators at the individual level and using each animal as its own control in addition to general species assessments. Given individual variations, we found that a few individuals exceeded the established behavioral and physiological thresholds of wellbeing, indicating potentially poor welfare, but their results did not affect means at the species level. As part of the principal commitments of zoos to enhance animal welfare and provide optimal animal care, zookeepers and managers should identify the individuals having more difficulty coping with stressful situations first and implement regular animal welfare inspection protocols following species-specific guidelines. We hope our findings will benefit the zoological community and inspire other research project ideas to improve ambassador animal welfare.

Tables and Figures

Table 1. Ambassador species demography and husbandry in each zoological facility.

	Granby Zoo species						Ecomuseum Zoo species	
Details	Three-banded armadillo (*Tolypeutes matacus*)	Grey short-tailed opossum (*Monodelphis domestica*)	Bactrian camel (*Camelus bactrianus*)	Dromedary (*Camelus dromedarius*)	Ball python (*Python regius*)	Blue-tongued skink (*Tiliqua scincoides*)	American kestrel (*Falco sparverius*)	Great-horned owl (*Bubo virginianus*)
Sex								
Male	2	3	3	2	6	2	1	1
Female	-	-	-	-	4	-	2	1
Total	2	3	3	2	10	2	3	2
Age (years range)	6 - 12	2 - 3	8 - 10	15 - 18	5 - 33	7 - 21	1 - 12	12
Light cycle	Crepuscular	Nocturnal	Diurnal	Diurnal	Crepuscular	Diurnal	Diurnal	Nocturnal
Observation time	9:00 – 0:00	16:00 – 0:00	8:00 – 17:00	8:00 – 17:00	8:00 – 22:00	8:00 – 22:00	8:00 – 19:00	15:00 – 5:00
Status								
Ambassador	2	2	3	1	10	2	3	2
Exhibited animal	-	1	-	1	-	-	-	-
Occupation	Workshops	Workshops	Rides	Rides	Workshops	Workshops	Workshops	Workshops
Enclosure								
Location	Inside	Inside	Outside	Outside	Inside	Inside	Both	Both
Shared with conspecifics	No	No	Yes	Yes	No	No	No	No
Contact with visitors	No	No	Yes	Yes	No	No	No	No
Environment enrichment program	Yes	Yes	Yes	Yes	Yes	Yes	No	No

Table 2. Ethogram used for all ambassadors and species-specific behaviors.

Behavior	Key	Category	Description
Exploration	EXP	Active	Interaction with a non-food item in the animal's enclosure. Includes environmental enrichment objects, shelter, or enclosure substrate.
Feeding and drinking	FEED	Active	Interaction with provided food or water by consuming it.
Locomotion	LOCO	Active	Movement of the animal from one point to another of at least the body length in distance. Includes walking, running, climbing, and crawling.
Not visible	NOTV		The behavior performed by the animal is not visible to the observer.
Not visible, presumed resting	NVPR	Passive	The animal is not visible to the observer but is known to be in a resting space. There is no visual or auditory sign from the animal.
Resting	REST	Passive	The animal is not engaged in any active behavior.
Other active	OTHER	Active	The animal is performing other active behaviors not defined in this study. Includes defecating, vigilance, and vocalizing.
Self-directed	SELF	Active	Behavior targeted to the animal itself. Includes grooming, scratching, or rubbing itself on an object in an unharmful way.
Undesirable	UND	Active	Abnormal stereotypic behavior observed at least three times (pacing or circling back and forth the same path), escape behavior (pushing, climbing, crawling alongside enclosure bars or invisible barriers), or potentially damaging behaviors that may cause injury (licking or mouthing enclosure bars).
Species-specific			
Resting up	RUP	Passive	In camelids, the animal is resting standing up on its four legs.
Resting down	RDOWN	Passive	In camelids, the animal is resting lying down on the ground on its abdomen or its side.
Ruminating	RUMI	Active	In camelids, the animal regurgitates partially digested food and chews it without consuming additional food.
Social interaction	SOCIAL	Active	In camelids, interaction with conspecifics including physical contact and vocalizing.
Resting active	RACT	Active	In birds, the animal is staring or is in a vigilant state with its surroundings with head movement.
Resting passive	RPASS	Passive	In birds, the animal is not engaged with its surroundings and does not have head movements. Eyes can be open or closed.
Running wheel	WHEEL	Active	In opossums, interaction with a running wheel for exercise running.
Preparing shelter	SHELT	Active	In armadillos, digging and excavating the enclosure's substrate to bury itself in its shelter box.
Periscoping	PERIS	Active	In pythons, standing upright position with head straight immobile to scope surroundings.

Table 3. Proportion of time spent performing active behaviors in mammals and birds and passive behaviors in reptiles (% mean ± SD) for each treatment in all species.

Species	Individual	Baseline	Zoo Activities (Rides or Workshops)	School Workshops
Camel	Clyde	42.70 ± 35.30	34.27 ± 33.85	NA
	Denzel	41.55 ± 32.57	34.69 ± 36.06	NA
	Junior	30.32 ± 30.28	30.63 ± 31.41	NA
Dromedary	Shawn	63.91 ± 30.96	37.53 ± 31.99	NA
	Solomon	49.59 ± 34.72	NA	NA
Armadillo	Antonio	43.62 ± 47.42	39.50 ± 54.09	42.71 ± 44.84
	Esteban	30.19 ± 45.94	35.90 ± 42.07	31.42 ± 42.17
Opossum	Pablo	28.16 ± 38.78	10.42 ± 25.52	28.33 ± 39.31
	Pedro	20.60 ± 24.72	NA	NA
	Sancho	14.80 ± 22.46	13.18 ± 17.92	21.22 ± 28.60
Skink	Agent 005	90.48 ± 16.75	NA	95.65 ± 9.53
	Slush	77.52 ± 28.28	84.14 ± 27.42	88.16 ± 14.89
Python	Congo	97.78 ± 5.35	90.17 ± 24.88	74.64 ± 34.51
	Kenya	99.91 ± 0.46	99.94 ± 0.28	NA
	Libye	99.34 ± 1.47	87.57 ± 25.91	96.83 ± 8.40
	Malawi	68.27 ± 40.31	76.42 ± 36.15	46.88 ± 36.29
	Mali	97.57 ± 7.37	99.22 ± 3.10	99.94 ± 0.28
	Ouganda	88.36 ± 20.81	99.90 ± 0.35	97.50 ± 10.63
	Sénégal	86.99 ± 28.29	84.06 ± 29.68	79.86 ± 39.22
	Tanzanie	96.13 ± 10.56	90.37 ± 24.26	96.09 ± 12.14
	Tchad	92.88 ± 22.01	99.97 ± 0.21	100.00 ± 0.00
	Zimbabwe	94.50 ± 17.55	90.93 ± 19.80	93.10 ± 20.67
Kestrel	Artémis	97.50 ± 6.11	95.12 ± 13.12	NA
	Saphira	88.16 ± 22.98	90.02 ± 21.24	91.14 ± 10.88
	Zéphyr	98.72 ± 3.49	98.84 ± 2.61	97.59 ± 4.51
Owl	Cléopâtre	87.02 ± 22.00	62.89 ± 37.39	NA
	Spartakus	94.83 ± 8.41	85.49 ± 29.71	58.83 ± 38.76

Table 4. Proportion of time spent performing undesirable behaviors (% mean ± SD) for each treatment in all species.

Species	Individual	Baseline	Zoo Activities (Rides or Workshops)	School Workshops
Camel	Clyde	0.88 ± 4.00	3.09 ± 9.79	NA
	Denzel	4.10 ± 14.57	12.44 ± 26.22	NA
	Junior	3.20 ± 9.86	3.78 ± 10.48	NA
Dromedary	Shawn	0 ± 0	0.89 ± 8.57	NA
	Solomon	4.29 ± 14.33	NA	NA
Armadillo	Antonio	20.22 ± 26.44	12.91 ± 23.62	19.63 ± 25.13
	Esteban	0.19 ± 0.70	0.09 ± 0.36	0.17 ± 0.88
Opossum	Pablo	0.02 ± 0.17	0 ± 0	0.14 ± 1.08
	Pedro	0.50 ± 1.44	NA	NA
	Sancho	0.47 ± 1.62	0.58 ± 1.88	1.15 ± 2.48
Skink	Agent 005	0.69 ± 3.49	NA	2.75 ± 8.35
	Slush	12.15 ± 20.88	5.97 ± 15.63	4.25 ± 14.58
Python	Congo	0 ± 0	2.57 ± 11.17	19.93 ± 34.05
	Kenya	0 ± 0	0 ± 0	0 ± 0
	Libye	0 ± 0	5.54 ± 14.79	0 ± 0
	Malawi	20.90 ± 32.03	17.07 ± 27.90	73.10 ± 26.80
	Mali	0.23 ± 1.34	0 ± 0	0 ± 0
	Ouganda	7.91 ± 21.10	0 ± 0	0 ± 0
	Sénégal	8.35 ± 23.87	9.19 ± 24.13	13.37 ± 28.26
	Tanzanie	0.37 ± 1.17	3.49 ± 13.40	1.77 ± 9.00
	Tchad	5.47 ± 19.67	0 ± 0	0 ± 0
	Zimbabwe	0.75 ± 3.20	1.83 ± 6.88	3.10 ± 14.85

Table 5. Proportion of time spent performing self-directed behaviors (% mean ± SD) for each treatment in bird species.

Species	Individual	Baseline	Zoo Activities (Workshops)	School Workshops
Kestrel	Saphira	14.92 ± 14.03	12.97 ± 12.05	16.66 ± 14.20
	Zéphyr	14.70 ± 11.17	12.47 ± 16.79	5.00 ± 10.56
	Artémis	14.00 ± 13.80	12.34 ± 13.44	NA
Owl	Spartakus	1.70 ± 4.96	4.54 ± 12.31	3.45 ± 7.63
	Cléopâtre	3.02 ± 5.98	4.75 ± 9.47	NA

Table 6. Individual FGM concentrations (mean ng/g ± SD) and number of fecal samples (N) collected for each treatment in Bactrian camels, dromedaries, three-banded armadillos, grey short-tailed opossums, ball pythons, blue-tongued skinks, American kestrels, and great-horned owls.

Species	Individual	Baseline		Zoo Activities (Rides or Workshops)		School Workshops	
		Mean ± SD	N	Mean ± SD	N	Mean ± SD	N
Camel	Clyde	20.03 ± 26.71	6	130.71 ± 121.31	17	NA	NA
	Denzel	11.07 ± 3.43	5	194.12 ± 103.86	30	NA	NA
	Junior	22.42 ± 18.36	6	149.77 ± 76.34	25	NA	NA
Dromedary	Shawn	82.77 ± 30.62	4	98.07 ± 76.86	28	NA	NA
	Solomon	61.47 ± 37.22	8	NA	NA	NA	NA
Armadillo	Esteban	26.29 ± 7.12	7	12.64 ± 6.37	3	22.79 ± 10.33	3
	Antonio	42.99 ± 27.70	7	19.87 ± 5.87	2	70.88 ± 31.37	2
Opossum	Pablo	23.93 ± 11.07	10	64.74	1	35.03 ± 29.07	12
	Pedro	22.39 ± 10.51	6	NA	NA	29.06	1
	Sancho	26.17 ± 4.32	9	74.57 ± 6.75	2	36.73 ± 15.15	12
Skink	Agent 005	117.66 ± 33.90	5	NA	NA	128.14	1
	Slush	154.65 ± 51.72	3	NA	NA	145.29 ± 9.55	2
Python	Congo	9.50 ± 5.10	2	15.27	1	NA	NA
	Kenya	23.47	1	19.19	1	86.31	1
	Libye	33.04	1	NA	NA	21.4	1
	Malawi	8.83 ± 5.08	3	6.37	1	24.55	1
	Mali	22.11 ± 0.77	2	NA	NA	46.58	1
	Ouganda	34.19 ± 13.09	3	NA	NA	17.11 ± 7.24	2
	Sénégal	71.84 ± 12.98	2	13.22 ± 10.35	2	38.95	1
	Tanzanie	39.82	1	10.81	1	40.97	1
	Tchad	31.66	1	NA	NA	57.86	1
	Zimbabwe	32.26 ± 7.68	2	29.28	1	9.15 ± 4.59	2
Kestrel	Saphira	114.99 ± 105.16	6	177.11 ± 158.00	6	105.37 ± 17.88	6
	Zéphir	127.69 ± 76.42	6	243.19 ± 193.65	12	38.55 ± 11.29	2
	Artémis	164.32 ± 106.50	8	142.37 ± 78.33	13	NA	NA
Owl	Spartakus	174.90 ± 246.11	4	151.08 ± 137.51	5	176.81 ± 195.25	6
	Cléopâtre	171.19 ± 144.03	6	74.19 ± 57.11	10	NA	NA

Table 7. Activity outings information for all species and individual baseline hormone concentration (ng/g). In camels and dromedaries, the number of activities, the total hours out, and the mean hours correspond to the number of ride days, the total number of rides, and the mean number of rides per day respectively during the summer 2022. The statistics for the other mammals and reptile species are from August 2021 to August 2022 while those of birds are also from July to September 2022.

Species	Individual	Number of activities	Total hours out for activities	Mean hours by activity	Rank	Baseline concentration (mean ± SD)	N
Camel	Denzel	42	4865	115.83	1	11.07 ± 3.43	5
	Junior	24	988	41.17	2	22.42 ± 18.36	6
	Clyde	17	1018	59.88	3	20.03 ± 26.71	6
Dromedary	Shawn	47	5858	124.64	1	82.77 ± 30.62	4
	Solomon	0	0	0	2	61.47 ± 37.22	8
Armadillo	Esteban	85	584.74	7.13	1	26.29 ± 7.12	7
	Antonio	70	445.67	6.65	2	42.99 ± 27.70	10
Opossum	Sancho	46	355.16	7.72	1	26.17 ± 4.32	9
	Pablo	41	297.42	7.43	2	23.93 ± 11.07	12
	Pedro	16	136.00	8.50	3	22.39 ± 10.51	7
Skink	Slush	58	394.26	7.04	1	154.65 ± 51.72	3
	Agent 005	43	289.00	6.88	2	117.66 ± 33.90	5
Python	Tchad	76	314.01	4.24	1	31.66	1
	Zimbabwe	72	276.54	4.00	2	32.26 ± 7.68	2
	Libye	69	271.89	4.18	3	33.04	1
	Kenya	69	264.87	4.01	4	23.47	1
	Congo	65	209.84	3.38	5	9.50 ± 5.10	2
	Ouganda	60	239.96	4.20	6	34.19 ± 13.09	3
	Mali	60	210.26	3.56	7	22.11 ± 0.77	2
	Sénégal	59	188.19	3.36	8	71.84 ± 12.98	2
	Tanzanie	48	151.79	3.22	9	39.82	1
	Malawi	41	152.48	3.81	10	8.83 ± 2.93	3
Kestrel	Saphira	7	8.5	1.21	1	114.99 ± 105.16	6
	Zéphyr	7	6	0.86	2	127.69 ± 76.42	6
	Artémis	6	3	0.50	3	164.32 ± 106.50	8
Owl	Spartakus	5	7.5	1.5	1	174.90 ± 246.11	4
	Cléopâtre	4	2	0.5	2	171.19 ± 144.03	6

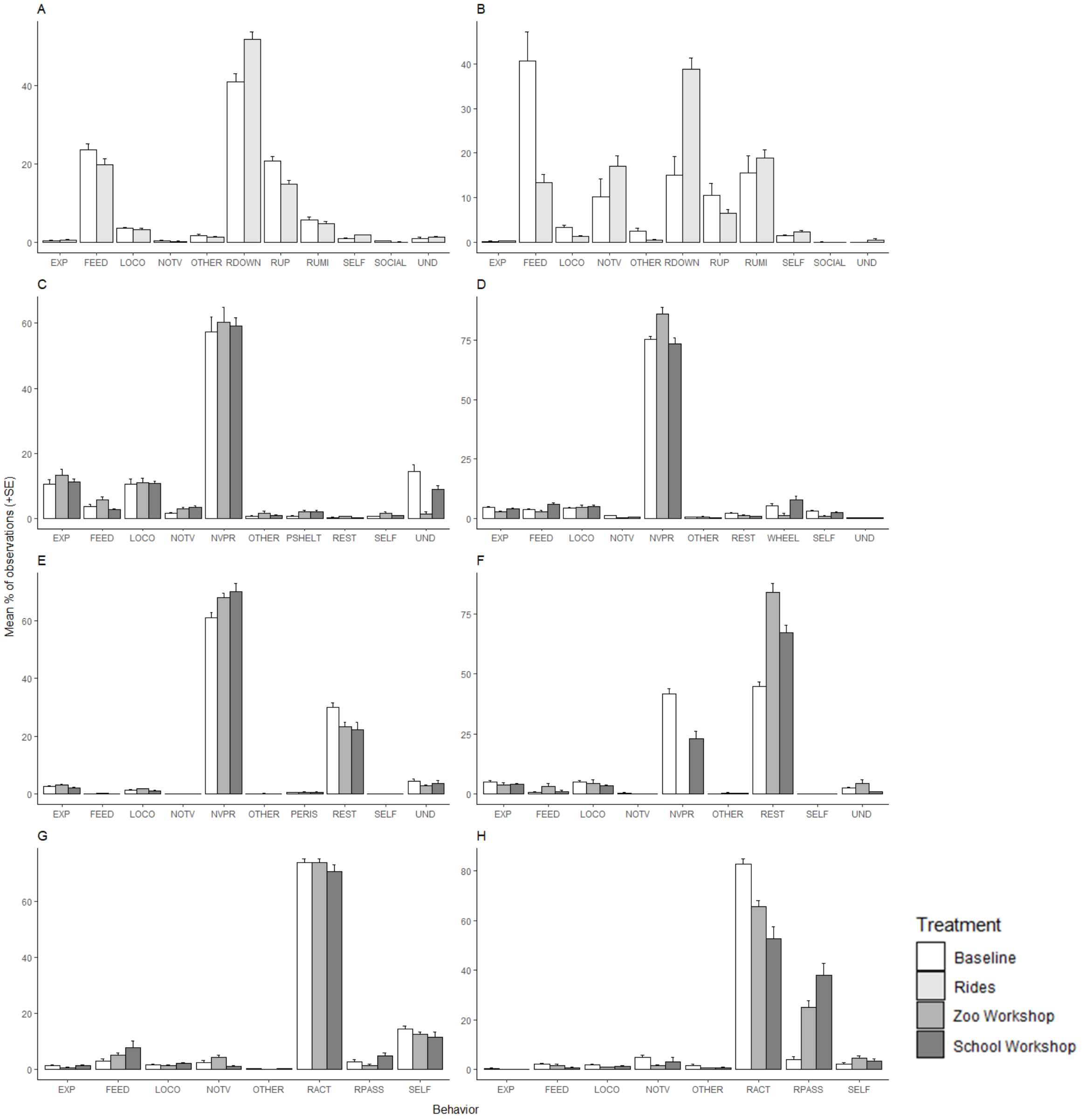

Figure 1. Activity budgets of all species in response to each treatment in (A) Bactrian camels, (B) dromedaries, (C) three-banded armadillos, (D) grey short-tailed opossums, (E) ball pythons, (F) blue-tongued skinks, (G) American kestrels, and (H) great-horned owls. EXP: Exploration, FEED: Feeding and drinking, LOCO: Locomotion, NOTV: Not visible, NVPR: Not visible, presumed resting, OTHER: Other active, REST: Resting, SELF: Self-directed, UND: Undesirable, RUP: Resting up, RDOWN: Resting down, RUMI: Ruminating, SOCIAL: Social interaction, RACT: Resting active, RPASS: Resting passive, WHEEL: Running wheel, SHELT: Preparing shelter, PERIS: Periscoping.

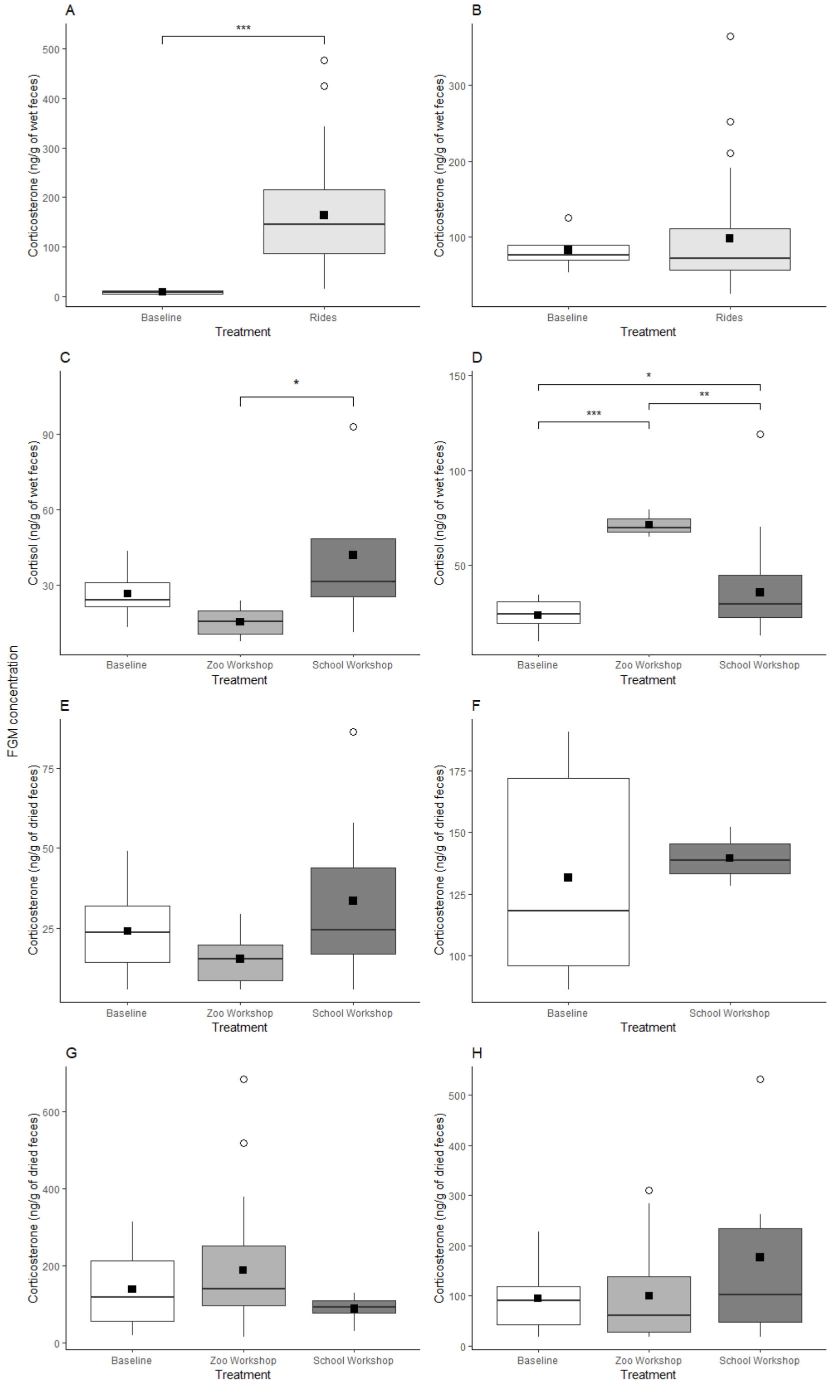

Figure 2. FGM concentrations (ng/g) for each treatment in (A) Bactrian camels, (B) dromedaries, (C) three-banded armadillos, (D) grey short-tailed opossums, (E) ball pythons, (F) blue-tongued skinks, (G) American kestrels, and (H) great-horned owls. The black squares represent the mean hormone concentration.

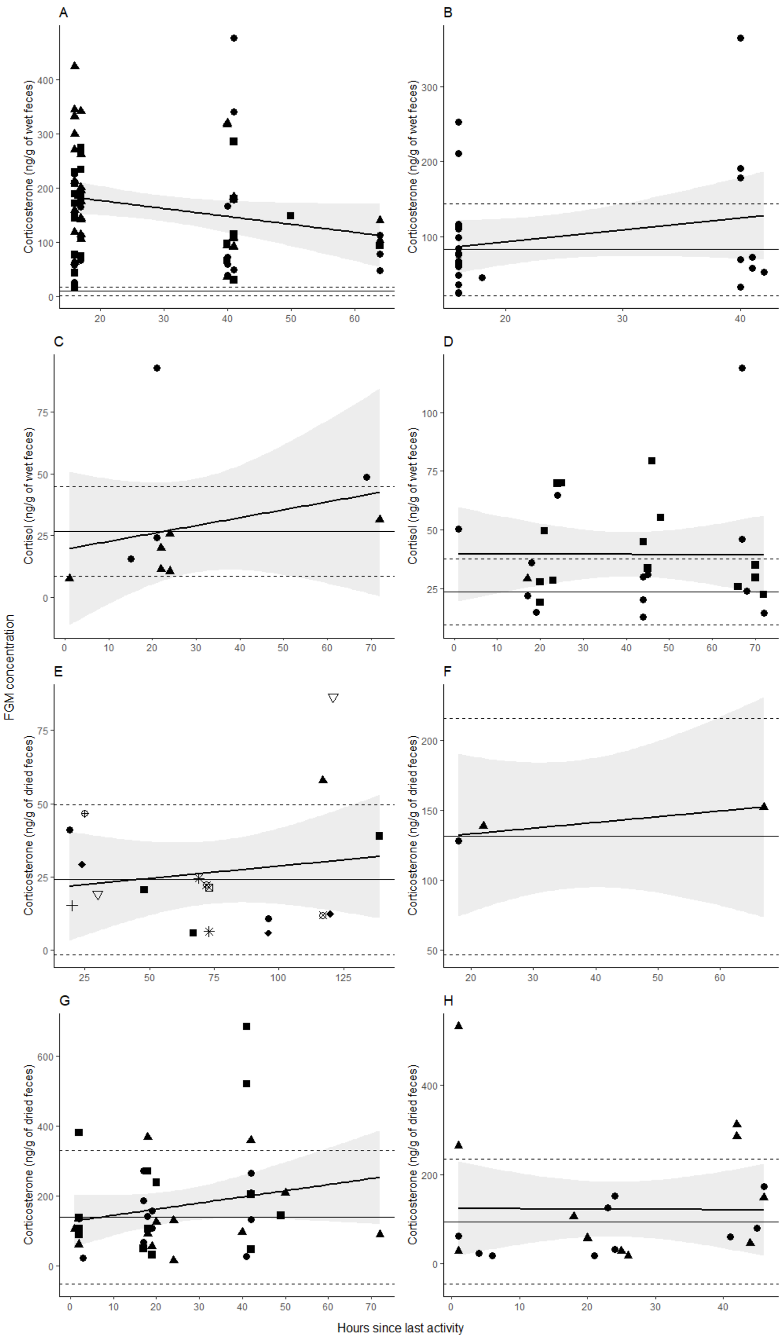

Figure 3. FGM concentrations (ng/g) in (A) Bactrian camels, (B) dromedaries, (C) three-banded armadillos, (D) grey short-tailed opossums, (E) ball pythons, (F) blue-tongued skinks, (G) American kestrels, and (H) great-horned owls. Shapes correspond to samples from different individuals. The mean + 2 SD baseline threshold is represented by the straight horizontal line with two dashed lines. The mean FGM concentration for a given species is represented by linear regression with 95 % shaded confidence intervals.

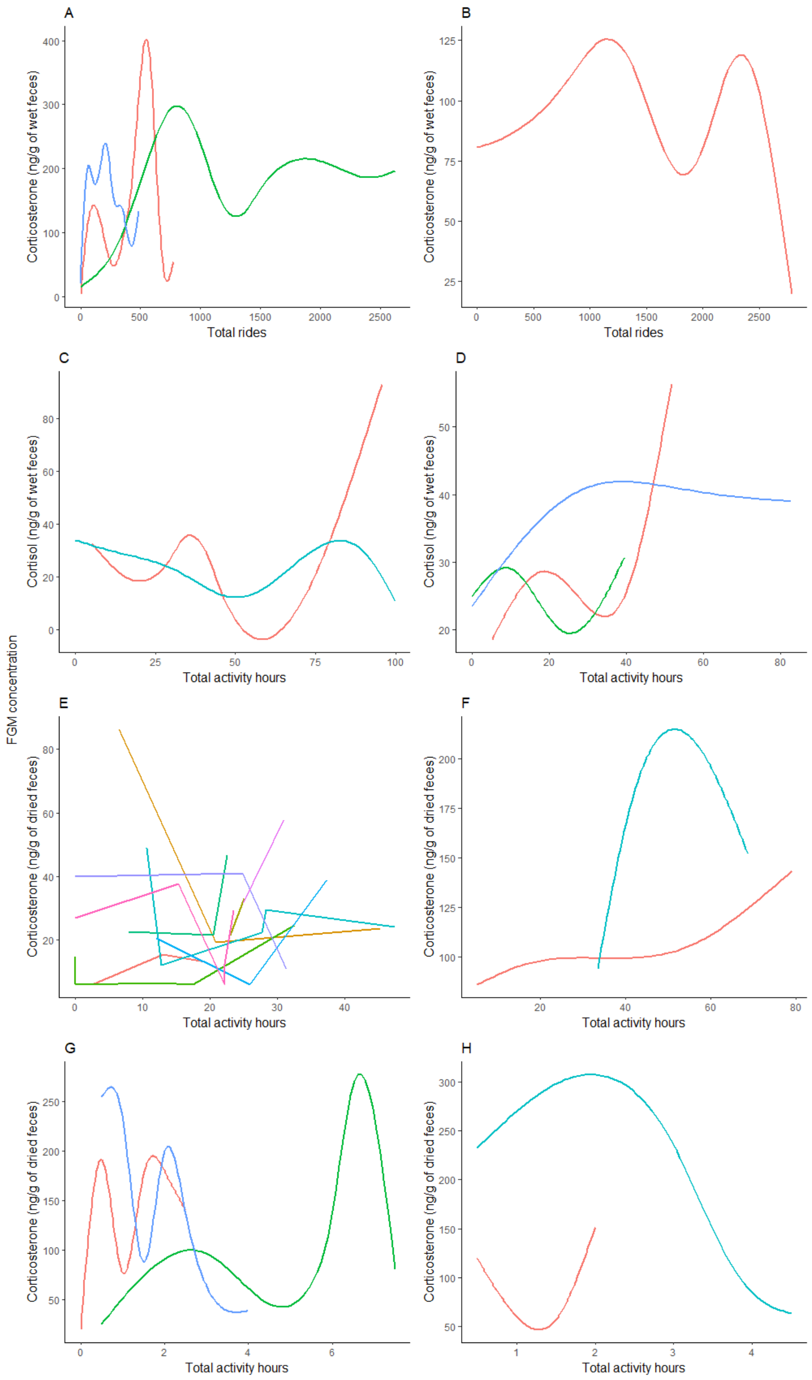

Figure 4. FGM concentrations (ng/g) after the total activity hours or the number of rides in the last 30 days for (A) Bactrian camels, (B) dromedaries, (C) three-banded armadillos, (D) grey short-tailed opossums, (E) ball pythons, (F) blue-tongued skinks, (G) American kestrels, and (H) great-horned owls. The line colors represent different individuals.

References

Acaralp-Rehnberg, L. K., Coleman, G. J., Magrath, M. J. L., Melfi, V., Fanson, K. V., & Bland, I. M. (2020). The effect of behind-the-scenes encounters and interactive presentations on the welfare of captive servals (*Leptailurus serval*). *Animals*, *10*(4), 743. https://doi.org/10.3390/ani10040743

Ancona, K., & Loughry, W. (2010). Sources of variation in the time budgets of wild nine-banded armadillos. *Mammalia*, *74*, 127-134. https://doi.org/10.1515/MAMM.2010.022

Association of Zoos and Aquariums. (2015). *Ambassador animal policy*. Retrieved August 16, 2023 from https://assets.speakcdn.com/assets/2332/ambassador_animal_policy_2015.pdf

Attias, N., Gurarie, E., Fagan, W. F., & Mourão, G. (2020). Ecology and social biology of the southern three-banded armadillo (*Tolypeutes matacus; Cingulata : Chlamyphoridae*). *Journal of Mammalogy*, *101*(6), 1692-1705. https://doi.org/10.1093/jmammal/gyaa117

Attias, N., Oliveira-Santos, L. G. R., Fagan, W. F., & Mourão, G. (2018). Effects of air temperature on habitat selection and activity patterns of two tropical imperfect homeotherms. *Animal Behaviour*, *140*, 129-140. https://doi.org/10.1016/j.anbehav.2018.04.011

Aubé, L., Fatnassi, M., Monaco, D., Khorchani, T., Lacalandra, G. M., Hammadi, M., & Padalino, B. (2017). Daily rhythms of behavioral and hormonal patterns in male dromedary camels housed in boxes. *PeerJ*, *5*, e3074. https://doi.org/10.7717/peerj.3074

Augustine, L., Baskir, E., Kozlowski, C. P., Hammack, S., Elden, J., Wanner, M. D., Franklin, A. D., & Powell, D. M. (2022). Investigating welfare metrics for snakes at the Saint Louis Zoo. *Animals*, *12*(3), 373. https://doi.org/10.3390/ani12030373

Baird, B. A., Kuhar, C. W., Lukas, K. E., Amendolagine, L. A., Fuller, G. A., Nemet, J., Willis, M. A., & Schook, M. W. (2016). Program animal welfare : Using behavioral and physiological measures to assess the well-being of animals used for education programs in zoos. *Applied Animal Behaviour Science*, *176*, 150-162. https://doi.org/10.1016/j.applanim.2015.12.004

Baladrón, A. V., Cavalli, M., Pretelli, M. G., & Bó, M. S. (2016). Time-activity budgets and hunting behavior of the roadside hawk (*Rupornis magnirostris*) and the long-winged harrier (*Circus buffoni*). *Revista Brasileira de Ornitologia*, *24*(3), 197-203. https://doi.org/10.1007/BF03544346

Bargaâ, R., Lektib, I., Chakir, Y., Abbadi, N. E., Belhouari, A., Hammoumi, A., Farh, M., Tahri, E.-H., Riad, F., Mzibri, M. E., & Khasmi, M. E. (2016). Corrélation des taux de cortisol dans le sérum, les poils et les fèces chez le dromadaire (*Camelus dromedarius*). *Revue d'élevage et de médecine vétérinaire des pays tropicaux*, *69*(2), 87-91. https://doi.org/10.19182/remvt.31184

Barja, I., Silván, G., Rosellini, S., Piñeiro, A., González-Gil, A., Camacho, L., & Illera, J. C. (2007). Stress physiological responses to tourist pressure in a wild population of European pine marten. *The Journal of Steroid Biochemistry and Molecular Biology*, *104*(3-5), 136-142. https://doi.org/10.1016/j.jsbmb.2007.03.008

Bartolomucci, A., Pederzani, T., Sacerdote, P., Panerai, A. E., Parmigiani, S., & Palanza, P. (2004). Behavioral and physiological characterization of male mice under chronic psychosocial stress. *Psychoneuroendocrinology*, *29*(7), 899-910. https://doi.org/10.1016/j.psyneuen.2003.08.003

Bashaw, M. J., Gibson, M. D., Schowe, D. M., & Kucher, A. S. (2016). Does enrichment improve reptile welfare? Leopard geckos (*Eublepharis macularius*) respond to five types of environmental enrichment. *Applied Animal Behaviour Science*, *184*, 150-160. https://doi.org/10.1016/j.applanim.2016.08.003

Baskir, E. A., Kucharski, S., & Powell, D. M. (2020). Chinchilla (*Chinchilla lanigera*) behavioral responses to a visual signal preceding handling. *Zoo Biology*, *39*(6), 391-396. https://doi.org/10.1002/zoo.21564

Bedford, G. S., & Christian, K. A. (2000). Digestive efficiency in some Australian pythons. *Copeia*, *2000*(3), 829-834. http://doi.org/10.1643/0045-8511(2000)000%5B0829:DEISAP%5D2.0.CO;2

Benn, A. L., McLelland, D. J., & Whittaker, A. L. (2019). A review of welfare assessment methods in reptiles, and preliminary application of the welfare quality® protocol to the pygmy blue-tongue skink, *Tiliqua adelaidensis*, using animal-based measures. *Animals*, *9*(1), 27. https://doi.org/10.3390/ani9010027

Binding, S., Farmer, H., Krusin, L., & Cronin, K. (2020). Status of animal welfare research in zoos and aquariums : Where are we, where to next? *Journal of Zoo and Aquarium Research*, *8*(3), 166-174. https://doi.org/10.19227/jzar.v8i3.505

Bohák, Z., Szabó, F., Beckers, J.-F., Melo de Sousa, N., Kutasi, O., Nagy, K., & Szenci, O. (2013). Monitoring the circadian rhythm of serum and salivary cortisol concentrations in the horse. *Domestic Animal Endocrinology*, *45*(1), 38-42. https://doi.org/10.1016/j.domaniend.2013.04.001

Boissy, A., Veissier, I., & Roussel, S. (2001). Behavioural reactivity affected by chronic stress : An experimental approach in calves submitted to environmental instability. *Animal Welfare*, *10*(S1), S175-S185. https://doi.org/10.1017/S0962728600023605

Borgmans, G., Palme, R., Sannen, A., Vervaecke, H., & Van Damme, R. (2021). The effect of long term captivity on stress levels in *Anolis carolinensis* lizards. *Journal of Applied Animal Welfare Science*, *24*(3), 321-330. https://doi.org/10.1080/10888705.2021.1916937

Bracke, M. B. M., & Hopster, H. (2006). Assessing the importance of natural behavior for animal welfare. *Journal of Agricultural and Environmental Ethics*, *19*(1), 77-89. https://doi.org/10.1007/s10806-005-4493-7

Brando, S., & Buchanan-Smith, H. M. (2018). The 24/7 approach to promoting optimal welfare for captive wild animals. *Behavioural Processes*, *156*, 83-95. https://doi.org/10.1016/j.beproc.2017.09.010

Broom, D. M. (2001). Coping, stress and welfare. In *Coping with Challenge: Welfare in Animals including Humans, Proceedings of Dahlem Conference*, ed. D. M. Broom, p. 1-9. Berlin: Dahlem University Press.

Broom, D. M. (1991). Animal welfare : Concepts and measurement. *Journal of Animal Science*, *69*(10), 4167-4175. https://doi.org/10.2527/1991.69104167x

Brown, J. L., Schmitt, D. L., Bellem, A., Graham, L. H., & Lehnhardt, J. (1999). Hormone secretion in the Asian elephant (*Elephas maximus*): Characterization of ovulatory and anovulatory luteinizing hormone surges. *Biology of reproduction*, *61*(5), 1294–1299. http://doi.org/10.1095/biolreprod61.5.1294

Bryan Jones, R. (1994). Regular handling and the domestic chick's fear of human beings : Generalisation of response. *Applied Animal Behaviour Science*, *42*(2), 129-143. https://doi.org/10.1016/0168-1591(94)90153-8

Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: a practical information-theoretic approach* (Second edition). New York: Springer.

Bush, S. E., & Clayton, D. H. (2023). Grooming time predicts survival : American kestrels, *Falco sparverius*, on a subtropical island. *The American Naturalist*, *201*(4), 603-609. https://doi.org/10.1086/723412

Cabanac, A., & Cabanac, M. (2000). Heart rate response to gentle handling of frog and lizard. *Behavioural Processes*, *52*(2), 89-95. https://doi.org/10.1016/S0376-6357(00)00108-X

Caorsi, V., Sprau, P., Zollinger, S. A., & Brumm, H. (2019). Nocturnal resting behaviour in urban great tits and its relation to anthropogenic disturbance and microclimate. *Behavioral Ecology and Sociobiology*, *73*(19). https://doi.org/ 10.1007/s00265-018-2624-1

Carere, C., & van Oers, K. (2004). Shy and bold great tits (*Parus major*) : Body temperature and breath rate in response to handling stress. *Physiology & Behavior*, *82*(5), 905-912. https://doi.org/10.1016/j.physbeh.2004.07.009

Cargill, B. M., Benato, L., & Rooney, N. J. (2022). A survey exploring the impact of housing and husbandry on pet snake welfare. *Animal Welfare*, *31*(2), 193-208. https://doi.org/10.7120/09627286.31.2.004

Carlstead, K., & Brown, J. L. (2005). Relationships between patterns of Fecal corticoid excretion and behavior, reproduction, and environmental factors in captive black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceros. *Zoo Biology*, *24*(3), 215-232. https://doi.org/10.1002/zoo.20050

Carlstead, K., Brown, J. L., & Strawn, W. (1993). Behavioral and physiological correlates of stress in laboratory cats. *Applied Animal Behaviour Science*, *38*(2), 143-158. https://doi.org/10.1016/0168-1591(93)90062-T

Caudell, N., & Riddleberger, A. (2001). Management of nonreleasable raptors for conservation education programs. *Journal of Raptor Research*, *35*(1), 49-57. https://sora.unm.edu/sites/default/files/journals/jrr/v035n01/p00049-p00057.pdf

Clark, F. E., & Melfi, V. A. (2012). Environmental enrichment for a mixed-species nocturnal mammal exhibit. *Zoo Biology*, *31*(4), 397-413. https://doi.org/10.1002/zoo.20380

Clegg, I. L. K., Borger-Turner, J. L., & Eskelinen, H. C. (2015). C-Well : The development of a welfare assessment index for captive bottlenose dolphins (*Tursiops truncatus*). *Animal Welfare*, *24*(3), 267-282. https://doi.org/10.7120/09627286.24.3.267

Clegg, I. L. K., Rödel, H. G., Boivin, X., & Delfour, F. (2018). Looking forward to interacting with their caretakers : Dolphins' anticipatory behaviour indicates motivation to participate in specific events. *Applied Animal Behaviour Science*, *202*, 85-93. https://doi.org/10.1016/j.applanim.2018.01.015

Clifford-Clarke, M. M., Whitehouse-Tedd, K., & Ellis, C. F. (2022). Conservation education impacts of animal ambassadors in zoos. *Journal of Zoological and Botanical Gardens*, *3*(1), 1-18. https://doi.org/10.3390/jzbg3010001

Conant, R. (2007). Reptile and amphibian management practices at Philadelphia Zoo. *International Zoo Yearbook*, *11*, 224-230. https://doi.org/10.1111/j.1748-1090.1971.tb01912.x

Cope, H. R., Keeley, T., Keong, J., Smith, D., Silva, F. R. O., McArthur, C., Webster, K. N., Mella, V. S. A., & Herbert, C. A. (2022). Validation of an enzyme immunoassay to measure faecal glucocorticoid metabolites in common brushtail possums (*Trichosurus vulpecula*) to evaluate responses to rehabilitation. *Animals*, *12*(13), 1627. https://doi.org/10.3390/ani12131627

Costa, P., Macchi, E., Valle, E., De Marco, M., Nucera, D. M., Gasco, L., & Schiavone, A. (2016). An association between feather damaging behavior and corticosterone metabolite excretion in captive African grey parrots (*Psittacus erithacus*). *PeerJ*, *4*, e2462. https://doi.org/10.7717/peerj.2462

Cotgreave, P., & Clayton, D. H. (1994). Comparative analysis of time spent grooming by birds in relation to parasite load. *Behaviour*, *131*(3/4), 171-187. https://www.jstor.org/stable/4535237

Cyr, N. E., & Romero, L. M. (2008). Fecal glucocorticoid metabolites of experimentally stressed captive and free-living starlings : Implications for conservation research. *General and Comparative Endocrinology*, *158*(1), 20-28. https://doi.org/10.1016/j.ygcen.2008.05.001

Dantzer, B., Fletcher, Q. E., Boonstra, R., & Sheriff, M. J. (2014). Measures of physiological stress : A transparent or opaque window into the status, management and conservation of species? *Conservation Physiology*, *2*(1), cou023. https://doi.org/10.1093/conphys/cou023

Davey, G. (2007). Visitors' effects on the welfare of animals in the zoo : A review. *Journal of applied animal welfare science*, *10*, 169-183. https://doi.org/10.1080/10888700701313595

D'Cruze, N., Khan, S., Carder, G., Megson, D., Coulthard, E., Norrey, J., & Groves, G. (2019). A global review of animal–visitor interactions in modern zoos and aquariums and their implications for wild animal welfare. *Animals*, *9*(6), 332. https://doi.org/10.3390/ani9060332

D'Cruze, N., Paterson, S., Green, J., Megson, D., Warwick, C., Coulthard, E., Norrey, J. D., Auliya, M., & Carder, G. (2020). Dropping the ball? The welfare of ball pythons traded in the EU and North America. *Animals*, *10*, 413. https://doi.org/10.3390/ani10030413

Dembiec, D. P., Snider, R. J., & Zanella, A. J. (2004). The effects of transport stress on tiger physiology and behavior. *Zoo Biology*, *23*(4), 335-346. https://doi.org/10.1002/zoo.20012

Desbiez, A. L. J., Massocato, G. F., Kluyber, D., & Santos, R. C. F. (2018). Unraveling the cryptic life of the southern naked-tailed armadillo, *Cabassous unicinctus squamicaudis* (Lund, 1845), in a Neotropical wetland : Home range, activity pattern, burrow use and reproductive behaviour. *Mammalian Biology*, *91*, 95-103. https://doi.org/10.1016/j.mambio.2018.02.006

Duarte, A. C., & Superina, M. (2015). Etograma para tres especies de armadillos (*Dasypus sabanicola*, *D. novemcinctus* y *Cabassous unicinctus*) mantenidas en condiciones controladas en Villavicencio, Colombia. *Edentata*, *16*, 1-10. https://ri.conicet.gov.ar/bitstream/handle/11336/49695/CONICET_Digital_Nro.c1e0abd4-b287-4e93-a1cf-f0e210fa7d32_A.pdf?sequence=2&isAllowed=y

Fischer, C. P., & Romero, L. M. (2018). Chronic captivity stress in wild animals is highly species-specific. *Conservation Physiology*, *7*, 38. http://doi.org/10.1093/conphys/coz093

Fischer, C. P., Wright-Lichter, J., & Romero, L. M. (2018). Chronic stress and the introduction to captivity : How wild house sparrows (*Passer domesticus*) adjust to laboratory conditions. *General and Comparative Endocrinology*, *259*, 85-92. https://doi.org/10.1016/j.ygcen.2017.11.007

Fowler, M. E. (2000). The influence of behaviour on the health and well-being of camels and their handlers. *Journal of Camel Practice and Research*, *7*, 129-142. https://www.researchgate.net/publication/289896999_The_influence_of_behaviour_on_the_health_and_well-being_of_camels_and_their_handlers

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

Grissom, N., & Bhatnagar, S. (2009). Habituation to repeated stress : Get used to it. *Neurobiology of Learning and Memory*, *92*(2), 215-224. https://doi.org/10.1016/j.nlm.2008.07.001

Hajduk, P., Copland, M. D., & Schultz, D. A. (1992). Effects of capture on hematological values and plasma cortisol levels of free-range koalas (*Phascolarctos cinereus*). *Journal of Wildlife Diseases*, *28*(3), 502-506. https://doi.org/10.7589/0090-3558-28.3.502

Hall, C., Huws, N., White, C., Taylor, E., Owen, H., & McGreevy, P. (2013). Assessment of ridden horse behavior. *Journal of Veterinary Behavior*, *8*(2), 62-73. https://doi.org/10.1016/j.jveb.2012.05.005

Harris, L. W. (2005). *The Harris Hawk : Management, Training and Hunting*. Swan Hill Press.

Hayward, L. S., Booth, R. K., & Wasser, S. K. (2010). Eliminating the artificial effect of sample mass on avian fecal hormone metabolite concentration. *General and Comparative Endocrinology*, *169*(2), 117-122. https://doi.org/10.1016/j.ygcen.2010.08.004

Hemmann, K., Raekallio, M., Kanerva, K., Hänninen, L., Pastell, M., Palviainen, M., & Vainio, O. (2012). Circadian variation in ghrelin and certain stress hormones in crib-biting horses. *The Veterinary Journal*, *193*(1), 97-102. https://doi.org/10.1016/j.tvjl.2011.09.027

Hill, S., & Broom, D. (2009). Measuring zoo animal welfare : Theory and practice. *Zoo Biology*, *28*, 531-544. https://doi.org/10.1002/zoo.20276

Hing, S., Narayan, E., Thompson, R. C. A., & Godfrey, S. (2014). A review of factors influencing the stress response in Australian marsupials. *Conservation Physiology*, *2*(1), cou027. https://doi.org/10.1093/conphys/cou027

Hogan, L. A., Johnston, S. D., Lisle, A. T., Keeley, T., Wong, P., Nicolson, V., Horsup, A. B., Janssen, T., & Phillips, C. J. C. (2011). Behavioural and physiological responses of captive wombats (*Lasiorhinus latifrons*) to regular handling by humans. *Applied Animal Behaviour Science*, *134*(3), 217-228. https://doi.org/10.1016/j.applanim.2011.07.010

Hogan, L. A., Lisle, A. T., Johnston, S. D., & Robertson, H. (2012). Non-invasive assessment of stress in captive numbats, *Myrmecobius fasciatus* (*Mammalia : Marsupialia*), using faecal cortisol measurement. *General and Comparative Endocrinology*, *179*(3), 376-383. https://doi.org/10.1016/j.ygcen.2012.09.020

Hosey, G. (2013). Hediger revisited : How do zoo animals see us? *Journal of Applied Animal Welfare Science*, *16*(4), 338-359. https://doi.org/10.1080/10888705.2013.827916

Howell-Stephens, J. A., Brown, J. S., Bernier, D., Mulkerin, D., & Santymire, R. M. (2012). Characterizing adrenocortical activity in zoo-housed southern three-banded armadillos (*Tolypeutes matacus*). *General and Comparative Endocrinology*, *178*(1), 64-74. https://doi.org/10.1016/j.ygcen.2012.04.003

Huckabee, J. R. (2000). Raptor therapeutics. *Veterinary Clinics of North America: Exotic Animal Practice*, *3*(1), 91-116. https://doi.org/10.1016/S1094-9194(17)30096-8

Iossa, G., Soulsbury, C., & Harris, S. (2009). Are wild animals suited to a travelling circus life? *Animal Welfare*, *18*(2), 129-140. http://doi.org/10.1017/S0962728600000270

Jones, M. P. (2001). Behavioral aspects of captive birds of prey. *Veterinary Clinics of North America: Exotic Animal Practice*, *4*(3), 613-632. https://doi.org/10.1016/S1094-9194(17)30026-9

Jung, A., Jung, H., Choi, Y., Colee, J., Wickens, C., Lee, J.-W., & Yoon, M. (2019). Frequent riding sessions daily elevate stress, blood lactic acid, and heart rate of thoroughbred riding horses. *Journal of Veterinary Behavior*, *32*, 1-5. https://doi.org/10.1016/j.jveb.2019.03.012

Kang, O.-D., & Yun, Y.-M. (2016). Influence of horse and rider on stress during horse-riding lesson program. *Asian-Australasian Journal of Animal Sciences*, *29*(6), 895-900. https://doi.org/10.5713/ajas.15.1068

Kleiman, D. G. (1992). Behavior research in zoos : Past, present, and future. *Zoo Biology*, *11*(5), 301-312. https://doi.org/10.1002/zoo.1430110502

Klejbor, I., Ludkiewicz, B., & Turlejski, K. (2013). Effect of light-dark changes on the locomotor activity in open field in adult rats and opossums. *Folia Morphologica*, *72*(4), 300-305. https://doi.org/10.5603/fm.2013.0050

Kozlowski, C. P., Bauman, K. L., Franklin, A. D., Sahrmann, J. M., Gartner, M., Baskir, E., Hanna, S., LaMattina, K., Seyfried, A., & Powell, D. M. (2021). Glucocorticoid production, activity levels, and personality traits of fennec foxes (*Vulpes zerda*) managed for different roles in zoos. *Journal of Applied Animal Welfare Science*, *26*(3), 1-18. https://doi.org/10.1080/10888705.2021.1980725

Kreger, M. D., & Mench, J. A. (1993). Physiological and behavioral effects of handling and restraint in the ball python (*Python regius*) and the blue-tongued skink (*Tiliqua scincoides*). *Applied Animal Behaviour Science*, *38*(3-4), 323-336. https://doi.org/10.1016/0168-1591(93)90030-S

Lefebvre, L. (1982). The organization of grooming in budgerigars. *Behavioural Processes*, *7*(2), 93-106. https://doi.org/10.1016/0376-6357(82)90019-5

Lesimple, C., Fureix, C., LeScolan, N., Richard-Yris, M.-A., & Hausberger, M. (2011). Housing conditions and breed are associated with emotionality and cognitive abilities in riding school horses. *Applied Animal Behaviour Science*, *129*(2), 92-99. https://doi.org/10.1016/j.applanim.2010.11.005

Luebke, J. F., Watters, J. V., Packer, J., Miller, L. J., & Powell, D. M. (2016). Zoo visitors' affective responses to observing animal behaviors. *Visitor Studies*, *19*(1), 60-76. https://doi.org/10.1080/10645578.2016.1144028

Maccarini, T. B., Attias, N., Medri, Í. M., Marinho-Filho, J., & Mourão, G. (2015). Temperature influences the activity patterns of armadillo species in a large neotropical wetland. *Mammal Research*, *60*(4), 403-409. https://doi.org/10.1007/s13364-015-0232-2

Macrini, T. E. (2004). Monodelphis domestica. *Mammalian Species*, *760*, 1-8. https://doi.org/10.1644/760

Majchrzak, Y. N., Mastromonaco, G. F., Korver, W., & Burness, G. (2015). Use of salivary cortisol to evaluate the influence of rides in dromedary camels. *General and Comparative Endocrinology*, *211*, 123-130. https://doi.org/10.1016/j.ygcen.2014.11.007

Mancera, K., Murray, P. J., Gao, Y. N., Lisle, A., & Phillips, C. J. C. (2014). The effects of simulated transport on the behaviour of eastern blue tongued lizards (*Tiliqua scincoides*). *Animal Welfare*, *23*(3), 239. http://doi.org/10.7120/09627286.23.3.239

Martínez Silvestre, A. (2014). How to assess stress in reptiles. *Journal of Exotic Pet Medicine*, *23*(3), 240-243. https://doi.org/10.1053/j.jepm.2014.06.004

Mason, G. J. (2010). Species differences in responses to captivity : Stress, welfare and the comparative method. *Trends in Ecology & Evolution*, *25*(12), 713-721. https://doi.org/10.1016/j.tree.2010.08.011

Mason, G., & Latham, N. R. (2004). Can't stop, won't stop : Is stereotypy a reliable animal welfare indicator? *Animal Welfare*, *13*, 57-69. http://doi.org/10.1017/S096272860001438X

Mason, G., & Mendl, M. (1993). Why is there no simple way of measuring animal welfare? *Animal Welfare*, *2*(4), 301-319. http://doi.org/10.1017/S0962728600016092

McKinney, C., Mueller, M. K., & Frank, N. (2015). Effects of therapeutic riding on measures of stress in horses. *Journal of Equine Veterinary Science*, *35*(11), 922-928. https://doi.org/10.1016/j.jevs.2015.08.015

Meagher, R. K., Duncan, I., Bechard, A., & Mason, G. J. (2011). Who's afraid of the big bad glove? Testing for fear and its correlates in mink. *Applied Animal Behaviour Science*, *133*(3), 254-264. https://doi.org/10.1016/j.applanim.2011.05.009

Melfi, V. (2009). There are big gaps in our knowledge, and thus approach, to zoo animal welfare : A case for evidence-based zoo animal management. *Zoo Biology*, *28*, 574-588. https://doi.org/10.1002/zoo.20288

Menchetti, L., Faye, B., & Padalino, B. (2021). New animal-based measures to assess welfare in dromedary camels. *Tropical Animal Health and Production*, *53*(6), 533. https://doi.org/10.1007/s11250-021-02978-8

Mineur, Y. S., Prasol, D. J., Belzung, C., & Crusio, W. E. (2003). Agonistic behavior and unpredictable chronic mild stress in mice. *Behavior Genetics*, *33*(5), 513-519. https://doi.org/10.1023/a:1025770616068

Morgan, K. N., & Tromborg, C. T. (2007). Sources of stress in captivity. *Applied Animal Behaviour Science*, *102*(3), 262-302. https://doi.org/10.1016/j.applanim.2006.05.032

Mormède, P., Andanson, S., Aupérin, B., Beerda, B., Guémené, D., Malmkvist, J., Manteca, X., Manteuffel, G., Prunet, P., van Reenen, C. G., Richard, S., & Veissier, I. (2007). Exploration of the hypothalamic–pituitary–adrenal function as a tool to evaluate animal welfare. *Physiology & Behavior*, *92*(3), 317-339. https://doi.org/10.1016/j.physbeh.2006.12.003

Möstl, E. (2014). Glucocorticoids, their metabolites and their measurement in various animal species. *Medycyna Weterynaryjna*, *70*, 524-529.

https://www.researchgate.net/publication/287245901_Glucocorticoids_their_metabolites_and_their_measurement_in_various_animal_species

Möstl, E., & Palme, R. (2002). Hormones as indicators of stress. *Domestic Animal Endocrinology*, *23*(1-2), 67-74. https://doi.org/10.1016/S0739-7240(02)00146-7

Nagel, A. H., Beshel, M., DeChant, C. J., Huskisson, S. M., Campbell, M. K., & Stoops, M. A. (2019). Non-invasive methods to measure inter-renal function in aquatic salamanders—Correlating fecal corticosterone to the environmental and physiologic conditions of captive *Necturus*. *Conservation Physiology*, *7*(1), coz074. https://doi.org/10.1093/conphys/coz074

Normando, S., Meers, L., Samuels, W. E., Faustini, M., & Ödberg, F. O. (2011). Variables affecting the prevalence of behavioural problems in horses. Can riding style and other management factors be significant? *Applied Animal Behaviour Science*, *133*(3), 186-198. https://doi.org/10.1016/j.applanim.2011.06.012

Padalino, B., Abdelali, Z., Monaco, D., Freccero, F., Menchetti, L., & Padalino, B. (2021). Dromedary camel health care practices reported by caretakers working at a permanent market. *Emirates Journal of Food and Agriculture*, 348-361. https://doi.org/10.9755/ejfa.2021.v33.i4.2688

Padalino, B., Aubé, L., Fatnassi, M., Monaco, D., Khorchani, T., Hammadi, M., & Lacalandra, G. M. (2014). Could dromedary camels develop stereotypy? The first description of stereotypical behaviour in housed male dromedary camels and how it is affected by different management systems. *PLOS ONE*, *9*(2), e89093. https://doi.org/10.1371/journal.pone.0089093

Padalino, B., & Menchetti, L. (2020). The first protocol for assessing welfare of camels. *Frontiers in Veterinary Science*, *7*, 631876. https://doi.org/10.3389/fvets.2020.631876

Palme, R. (2005). Measuring fecal steroids : Guidelines for practical application. *Annals of the New York Academy of Sciences*, *1046*, 75-80. https://doi.org/10.1196/annals.1343.007

Palme, R. (2019). Non-invasive measurement of glucocorticoids : Advances and problems. *Physiology & Behavior*, *199*, 229-243. https://doi.org/10.1016/j.physbeh.2018.11.021

Palme, R., Rettenbacher, S., Touma, C., El-Bahr, S. M., & Möstl, E. (2005). Stress hormones in mammals and birds : comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. *Annals of the New York Academy of Sciences*, *1040*(1), 162-171. https://doi.org/10.1196/annals.1327.021

Palmer, A. G., Nordmeyer, D. L., & Roby, D. D. (2001). Factors influencing nest attendance and time-activity budgets of peregrine falcons in interior Alaska. *ARCTIC*, *54*(2), 105-206. https://doi.org/10.14430/arctic769

Park, F. (2003). Behavior and behavioral problems of Australian raptors in captivity. *Seminars in Avian and Exotic Pet Medicine*, *12*(4), 232-241. https://doi.org/10.1053/S1055-937X(03)00038-0

Pereira, R. J. G., Granzinolli, M. A. M., De Barros, F. M., & Duarte, J. M. B. (2009). Influence of radiotransmitters on fecal glucocorticoid levels of free-ranging male American kestrels. *The Journal of Wildlife Management*, *73*(5), 772-778. http://doi.org/10.2193/2008-184

Podturkin, A. A. (2021). In search of the optimal enrichment program for zoo-housed animals. *Zoo Biology*, *40*(6), 527-540. https://doi.org/10.1002/zoo.21642

Pohlin, F., Hooijberg, E. H., & Meyer, L. C. R. (2021). Challenges to animal welfare during transportation of wild mammals : a review (1990–2020). *Journal of Zoo and Wildlife Medicine*, *52*(1), 1-13. https://doi.org/10.1638/2019-0029

Povey, K. (2002). Close encounters : The benefits of using education program animals. *Annual Proceedings of the Association of Zoos and Aquariums*, 117-121. https://scholar.google.com/scholar_lookup?title=Close%20encounters%3A%20the%20benefits%20of%20using%20education%20program%20animals&publication_year=2002&author=K.%20Povey

Povey, K. D., & Rios, J. (2002). Using interpretive animals to deliver affective messages in zoos. *Journal of Interpretation Research*, *7*(2), 19-28. https://doi.org/10.1177/109258720200700203

Powell, D. M., Kozlowski, C. P., Clark, J., Seyfried, A., Baskir, E., & Franklin, A. D. (2020). Physical and physiological indicators of welfare in guinea pigs (*Cavia porcellus*) serving as ambassador animals. *Animals*, *10*(5), 815. https://doi.org/10.3390/ani10050815

Previti, A., Guercio, B., & Passantino, A. (2016). Protection of farmed camels (*Camelus Dromedarius*) : Welfare problems and legislative perspective. *Animal Science Journal*, *87*(2), 183-189. https://doi.org/10.1111/asj.12446

Rahayu, S. Y. S., Priatna, D., Rosadi, R., & Suryanto, S. (2022). Management strategy of elephant riding at the zoo. *Indonesian Journal of Forestry Research*, *9*(1), 29-47. https://doi.org/10.20886/ijfr.2022.9.1.29-47

Ralph, C. R., & Tilbrook, A. J. (2016). Invited review : The usefulness of measuring glucocorticoids for assessing animal welfare. *Journal of Animal Science*, *94*(2), 457-470. https://doi.org/10.2527/jas.2015-9645

Rank, S. J., Roberts, S.-J., & Manion, K. (2021). The impact of ambassador animal facilitated programs on visitor curiosity and connections : A mixed-methods study. *Animal Behavior and Cognition*, *8*(4), 558-575. https://doi.org/10.26451/abc.08.04.08.2021

R Core Team. (2022). R: A language and environment for statistical computing. (R. D. C. Team, Ed.), *R Foundation for Statistical Computting*. R Foundation for Statistical Computing. Retrieved August 16, 2023 from http://www.r-project.org

Rommel, R. E., Crump, P. S., & Packard, J. M. (2016). Leaping from awareness to action : Impacts of an amphibian educator workshop. *Journal of Herpetology*, *50*(1), 12-16. http://doi.org/10.1670/14-025

Rose, P. E., Nash, S. M., & Riley, L. M. (2017). To pace or not to pace? A review of what abnormal repetitive behavior tells us about zoo animal management. *Journal of Veterinary Behavior*, *20*, 11-21. https://doi.org/10.1016/j.jveb.2017.02.007

Ross, S. R., Schapiro, S. J., Hau, J., & Lukas, K. E. (2009). Space use as an indicator of enclosure appropriateness : A novel measure of captive animal welfare. *Applied Animal Behaviour Science*, *121*(1), 42-50. https://doi.org/10.1016/j.applanim.2009.08.007

Rudolph, S. G. (1978). Predation ecology of coexistng great horned and barn owls. *The Wilson Bulletin*, *90*(1), 134-137. https://sora.unm.edu/sites/default/files/journals/wilson/v090n01/p0134-p0137.pdf

Rutherford, K., Haskell, M., Glasbey, C., Jones, B., & Lawrence, A. (2004). Fractal analysis of animal behaviour as an indicator of animal welfare. *Animal Welfare*, *13*, 99-103. https://doi.org/10.1017/S0962728600014433

Saiyed, S. T., Hopper, L. M., & Cronin, K. A. (2019). Evaluating the behavior and temperament of African penguins in a non-contact animal encounter program. *Animals*, *9*(6), 326. https://doi.org/10.3390/ani9060326

Sapolsky, R. M., & Altmann, J. (1991). Incidence of hypercortisolism and dexamethasone resistance increases with age among wild baboons. *Biological Psychiatry*, *30*(10), 1008-1016. https://doi.org/10.1016/0006-3223(91)90121-2

Sapolsky, R. M., Krey, L. C., & McEwen, B. S. (1986). The neuroendocrinology of stress and aging : The glucocorticoid cascade hypothesis. *Endocrine Reviews*, *7*(3), 284-301. https://doi.org/10.1210/edrv-7-3-284

Schouten, W. G. P., & Wiegant, V. M. (1997). Individual responses to acute and chronic stress in pigs. *Acta Physiologica Scandinavica Supplementum*, *640*, 88-91. https://pubmed.ncbi.nlm.nih.gov/9401614/

Schwarzenberger, F., Möstl, E., Palme, R., & Bamberg, E. (1996). Faecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals. *Animal Reproduction Science*, *42*(1), 515-526. https://doi.org/10.1016/0378-4320(96)01561-8

Sejian, V., Lakritz, J., Ezeji, T., & Lal, R. (2011). Assessment methods and indicators of animal welfare. *Asian Journal of Animal and Veterinary Advances*, *6*, 301-315. https://doi.org/10.3923/ajava.2011.301.315

Shepherdson, D., & Carlstead, K. (2001). New approaches to the evaluation of zoo animal well-being using multiple institutions, assessment of behavior and temperament, and non-invasive physiological measures. *AZA Annual Conference Proceedings.*, 131-136. https://www.researchgate.net/publication/258495213_New_Approaches_to_the_Evaluation_of_Zoo_Animal_Well-Being_Using_Multiple_Institutions_Assessment_of_Behavior_and_Temperament_and_Non-Invasive_Physiological_Measures

Sheriff, M. J., Krebs, C. J., & Boonstra, R. (2010). Assessing stress in animal populations : Do fecal and plasma glucocorticoids tell the same story? *General and Comparative Endocrinology*, *166*(3), 614-619. https://doi.org/10.1016/j.ygcen.2009.12.017

Sherwen, S. L., & Hemsworth, P. H. (2019). The visitor effect on zoo animals : Implications and opportunities for zoo animal welfare. *Animals*, *9*(6), 366. https://doi.org/10.3390/ani9060366

Sid-Ahmed, O.-E., Sanhouri, A., Elwaseela, B.-E., Fadllalah, I., Mohammed, G.-E. E., & Möstl, E. (2013). Assessment of adrenocortical activity by non-invasive measurement of faecal cortisol metabolites in dromedary camels (*Camelus dromedarius*). *Tropical Animal Health and Production*, *45*(6), 1453-1458. https://doi.org/10.1007/s11250-013-0374-7

Smith, S. P., & Forbes, N. A. (2009). A novel technique for prevention of self-mutilation in three Harris' hawks (*Parabuteo unicinctus*). *Journal of Avian Medicine and Surgery*, *23*(1), 49-52. http://doi.org/10.1647/2005-046R1.1

Spooner, S., Farnworth, M., Ward, S., & Whitehouse-Tedd, K. (2021). Conservation education : are zoo animals effective ambassadors and is there any cost to their welfare ? *Journal of Zoological and Botanical Gardens*, *2*(1), 41-65. https://doi.org/10.3390/jzbg2010004

Stockley, V. R., Wilkinson, A., & Burman, O. H. P. (2020). How to handle your dragon : Does handling duration affect the behaviour of bearded dragons (*Pogona Vitticeps*)? *Animals*, *10*(11), 2116. https://doi.org/10.3390/ani10112116

Strasser, E. H., & Heath, J. A. (2013). Reproductive failure of a human-tolerant species, the American kestrel, is associated with stress and human disturbance. *Journal of Applied Ecology*, *50*(4), 912-919. https://doi.org/10.1111/1365-2664.12103

Suchy, P., Bedanova, I., Vecerek, V., Voslarova, E., Pistekova, V., Chloupek, P., & Vitula, F. (2007). Effects of transport stress and floor space reduction on selected biochemical indices in common pheasant (*Phasianus colchicus*). *Archiv Fur Geflugelkunde*, *71*, 56-61. https://www.european-poultry-science.com/effects-of-transport-stress-and-floor-space-reduction-on-selected-biochemical-indices-in-common-pheasant-span-classws-name-phasianus-colchicusspan,QUlEPTQyMTc2NjcmTUlEPTE2MTAxNA.html

Swaisgood, R. R., & Shepherdson, D. J. (2005). Scientific approaches to enrichment and stereotypies in zoo animals : What's been done and where should we go next? *Zoo Biology*, *24*(6), 499-518. https://doi.org/10.1002/zoo.20066

Swanagan, J. S. (2000). Factors influencing zoo visitors' conservation attitudes and behavior. *The Journal of Environmental Education*, *31*(4), 26-31. https://doi.org/10.1080/00958960009598648

Thiel, D., Jenni-Eiermann, S., & Palme, R. (2005). Measuring corticosterone metabolites in droppings of capercaillies (*Tetrao urogallus*). *Annals of the New York Academy of Sciences*, *1046*(1), 96-108. https://doi.org/10.1196/annals.1343.009

Thompson, R. F., & Spencer, W. A. (1966). Habituation : A model phenomenon for the study of neuronal substrates of behavior. *Psychological Review*, *73*(1), 16-43. https://doi.org/10.1037/h0022681

Thun, R., Eggenberger, E., Zerobin, K., Lüscher, T., & Vetter, W. (1981). Twenty-four-hour secretory pattern of cortisol in the bull : Evidence of episodic secretion and circadian rhythm. *Endocrinology*, *109*(6), 2208-2212. https://doi.org/10.1210/endo-109-6-2208

Touma, C., & Palme, R. (2005). Measuring fecal glucocorticoid metabolites in mammals and birds : The importance of validation. *Annals of the New York Academy of Sciences*, *1046*(1), 54-74. https://doi.org/10.1196/annals.1343.006

Ujita, A., Seekford, Z., Kott, M., Goncherenko, G., Dias, N. W., Feuerbacher, E., Bergamasco, L., Jacobs, L., Eversole, D. E., Negrão, J. A., Mercadante, V. R. G. (2021). Habituation protocols improve behavioral and physiological responses of beef cattle exposed to students in an animal handling class. *Animals*, *11*(8), 2159. https://doi.org/10.3390/ani11082159

Van Hoek, C. S., & King, C. E. (1997). Causation and influence of environmental enrichment on feather picking of the crimson-bellied conure (*Pyrrhura perlata perlata*). *Zoo Biology*, *16*(2), 161-172. https://doi.org/10.1002/(SICI)1098-2361(1997)16:2<161::AID-ZOO6>3.0.CO;2-8

van Heerbeek, R. J. A., Brox, B. W., & Macaskill, A. C. (2021). Using a novel ethogram of tuatara behaviour to evaluate the impact of interactions with zoo visitors. *New Zealand Journal of Zoology*, *49*(3), 1-11. https://doi.org/10.1080/03014223.2021.1967415 van Zeeland, Y. R. A., Spruit, B. M., Rodenburg, T. B., Riedstra, B., van Hierden, Y. M., Buitenhuis, B., Korte, S. M., & Lumeij, J. T. (2009). Feather damaging behaviour in parrots : A review with consideration of comparative aspects. *Applied Animal Behaviour Science*, *121*(2), 75-95. https://doi.org/10.1016/j.applanim.2009.09.006

van Zeeland, Y., Spruit, B., Rodenburg, B., Riedstra, B., Hierden, Y. M., Buitenhuis, A. J., Korte, S., & Lumeij, J. (2009). Feather damaging behaviour in parrots : A review with consideration of comparative aspects. *Applied Animal Behaviour Science*, *121*, 75-95. http://doi.org/10.1016/j.applanim.2009.09.006

Wada, H. (2008). Glucocorticoids : Mediators of vertebrate ontogenetic transitions. *General and Comparative Endocrinology*, *156*(3), 441-453. https://doi.org/10.1016/j.ygcen.2008.02.004

Wark, J., Cronin, K., Niemann, T., Shender, M., Horrigan, A., Kao, A., & Ross, M. (2019). Monitoring the behavior and habitat use of animals to enhance welfare using the Zoomonitor app. *Animal Behavior and Cognition*, *6*, 158-167. https://doi.org/10.26451/abc.06.03.01.2019

Warwick, C., Arena, P., Lindley, S., Jessop, M., & Steedman, C. (2013). Assessing reptile welfare using behavioural criteria. *In Practice*, *35*(3), 123-131. https://doi.org/10.1136/inp.f1197

Warwick, C., Frye, F. L., Murphy, J. B., & Murphy, J. B. (2001). *Health and Welfare of Captive Reptiles*. Springer Science & Business Media.

Warwick, C., Grant, R., Steedman, C., Howell, T. J., Arena, P. C., Lambiris, A. J. L., Nash, A.-E., Jessop, M., Pilny, A., Amarello, M., Gorzula, S., Spain, M., Walton, A., Nicholas, E., Mancera, K., Whitehead, M., Martínez-Silvestre, A., Cadenas, V., Whittaker, A., & Wilson,

A. (2021). Getting it straight : Accommodating rectilinear behavior in captive snakes — A review of recommendations and their evidence base. *Animals*, *11*(5), 1459. https://doi.org/10.3390/ani11051459

Washburn, B. E., Millspaugh, J. J., Schulz, J. H., Jones, S. B., & Mong, T. (2003). Using fecal glucocorticoids for stress assessment in mourning doves. *Condor*, *105*(4), 696-706. http://doi.org/10.1093/condor/105.4.696

Wasser, S. K., Bevis, K., King, G., & Hanson, E. (1997). Noninvasive physiological measures of disturbance in the northern spotted owl. *Conservation Biology*, *11*(4), 1019-1022. http://doi.org/10.1046/j.1523-1739.1997.96240.x

Wasser, S. K., & Hunt, K. E. (2005). Noninvasive measures of reproductive function and disturbance in the barred owl, great horned owl, and northern spotted owl. *Annals of the New York Academy of Sciences*, *1046*(1), 109-137. https://doi.org/10.1196/annals.1343.010

Wasser, S. K., Hunt, K. E., Brown, J. L., Cooper, K., Crockett, C. M., Bechert, U., Millspaugh, J. J., Larson, S., & Monfort, S. L. (2000). A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. *General and Comparative Endocrinology*, *120*(3), 260-275. https://doi.org/10.1006/gcen.2000.7557

Watters, J. V., Margulis, S. W., & Atsalis, S. (2009). Behavioral monitoring in zoos and aquariums : A tool for guiding husbandry and directing research. *Zoo Biology*, *28*(1), 35-48. https://doi.org/10.1002/zoo.20207

Wemmer, C. (1981). Desert domesticates: The camel. Its evolution, ecology, behavior, and relationship to man. *Science*, *214*, 653-654. https://doi.org/10.1126/science.214.4521.653

Whitehouse-Tedd, K. M., Lozano-Martinez, J., Reeves, J., Page, M., Martin, J. H., & Prozesky, H. (2021). Assessing the visitor and animal outcomes of a zoo encounter and guided tour program with ambassador cheetahs. *Anthrozoös*, *35*(2), 1-16. https://doi.org/10.1080/08927936.2021.1986263

Wilkinson, M., Stirton, C., & McConnachie, A. (2010). Behavioural observations of singly-housed grey short-tailed opossums (*Monodelphis domestica*) in standard and enriched environments. *Laboratory Animals*, *44*(4), 364-369. https://doi.org/10.1258/la.2010.010040

Yerke, R., & Burns, A. (1991). Measuring the impact of animal shows on visitor attitudes. *Annual Proceedings of the American Association of Zoological Parks and Aquariums*, 532-534. https://scholar.google.com/scholar_lookup?title=Measuring+the+impact+of+animal+shows+on+visitor+attitudes.&publication_year=1991&journal=Annual+Proceedings+of+the+American+Association+of+Zoological+Parks+and+Aquariums&pages=532-534

Zdenek, C. N., Staples, T., Hay, C., Bourke, L. N., & Candusso, D. (2023). Sound garden : How snakes respond to airborne and groundborne sounds. *PLOS ONE*, *18*(2), e0281285. https://doi.org/10.1371/journal.pone.0281285

Zwijacz-Kozica, T., Selva, N., Barja, I., Silvan, G., Martínez-Fernández, L., Illera, J., & Jodłowski, M. (2013). Concentration of fecal cortisol metabolites in chamois in relation to tourist pressure in Tatra National Park (South Poland). *Acta theriologica*, *58*, 227-235. https://doi.org/10.1007/s13364-012-0108-7

Appendix A

Table 1.1. Information chart on the studied individuals housed at Zoo de Granby and Ecomuseum Zoo, Québec, Canada. The age is calculated as in the end of September 2022.

Zoological institution	Species	Individual	Status	Sex	Age
Zoo de Granby	Camel	Clyde	Ambassador	M	10
		Denzel	Ambassador	M	8
		Junior	Ambassador	M	8
	Dromedary	Shawn	Ambassador	M	18
		Solomon	Exhibited animal	M	15
	Armadillo	Antonio	Ambassador	M	12
		Esteban	Ambassador	M	6
	Opossum	Pablo	Ambassador	M	3
		Pedro	Retired ambassador	M	3
		Sancho	Ambassador	M	2
	Skink	Agent 005	Ambassador	M	7
		Slush	Ambassador	M	21
	Python	Congo	Ambassador	M	24
		Kenya	Ambassador	F	33
		Libye	Ambassador	F	20
		Malawi	Ambassador	F	5
		Mali	Ambassador	F	22
		Ouganda	Ambassador	M	6
		Sénégal	Ambassador	M	29
		Tanzanie	Ambassador	M	19
		Tchad	Ambassador	M	5
		Zimbabwe	Ambassador	M	16
Ecomuseum Zoo	Kestrel	Artémis	Ambassador	F	12
		Saphira	Ambassador	F	12
		Zéphyr	Ambassador	M	1
	Owl	Cléopâtre	Ambassador	F	12
		Spartakus	Ambassador	M	12

Appendix B

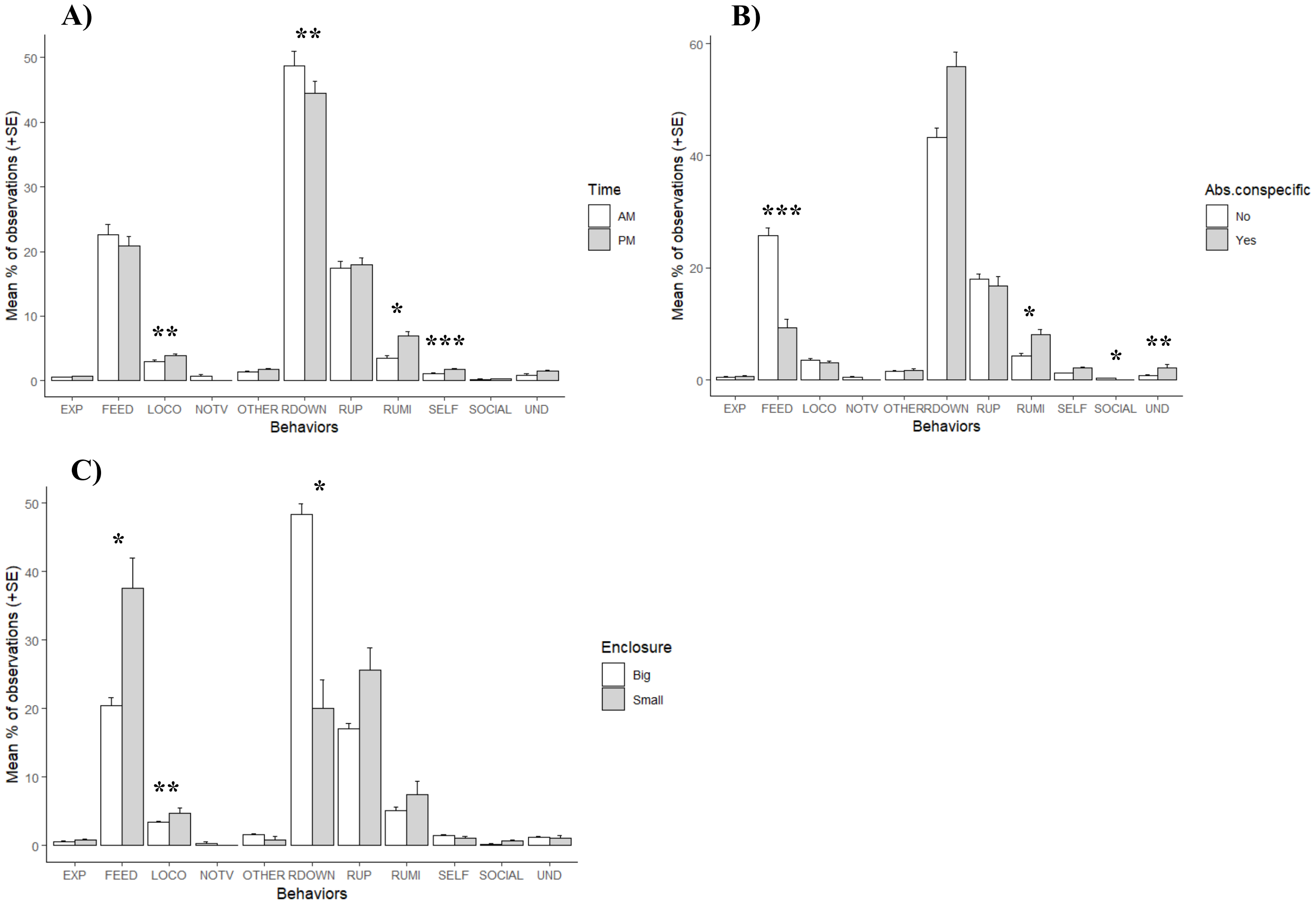

Figure 1.1. Activity budgets of Bactrian camels according to A) the time of the day, B) the absence of conspecific, and C) the enclosure. EXP: Exploration, FEED: Feeding and drinking, LOCO: Locomotion, NOTV: Not visible, OTHER: Other active, RDOWN: Resting down, RUP: Resting up, RUMI: Ruminating, SELF: Self-directed, SOCIAL: Social interaction, UND: Undesirable. Significance levels are indicated with stars.

Appendix C

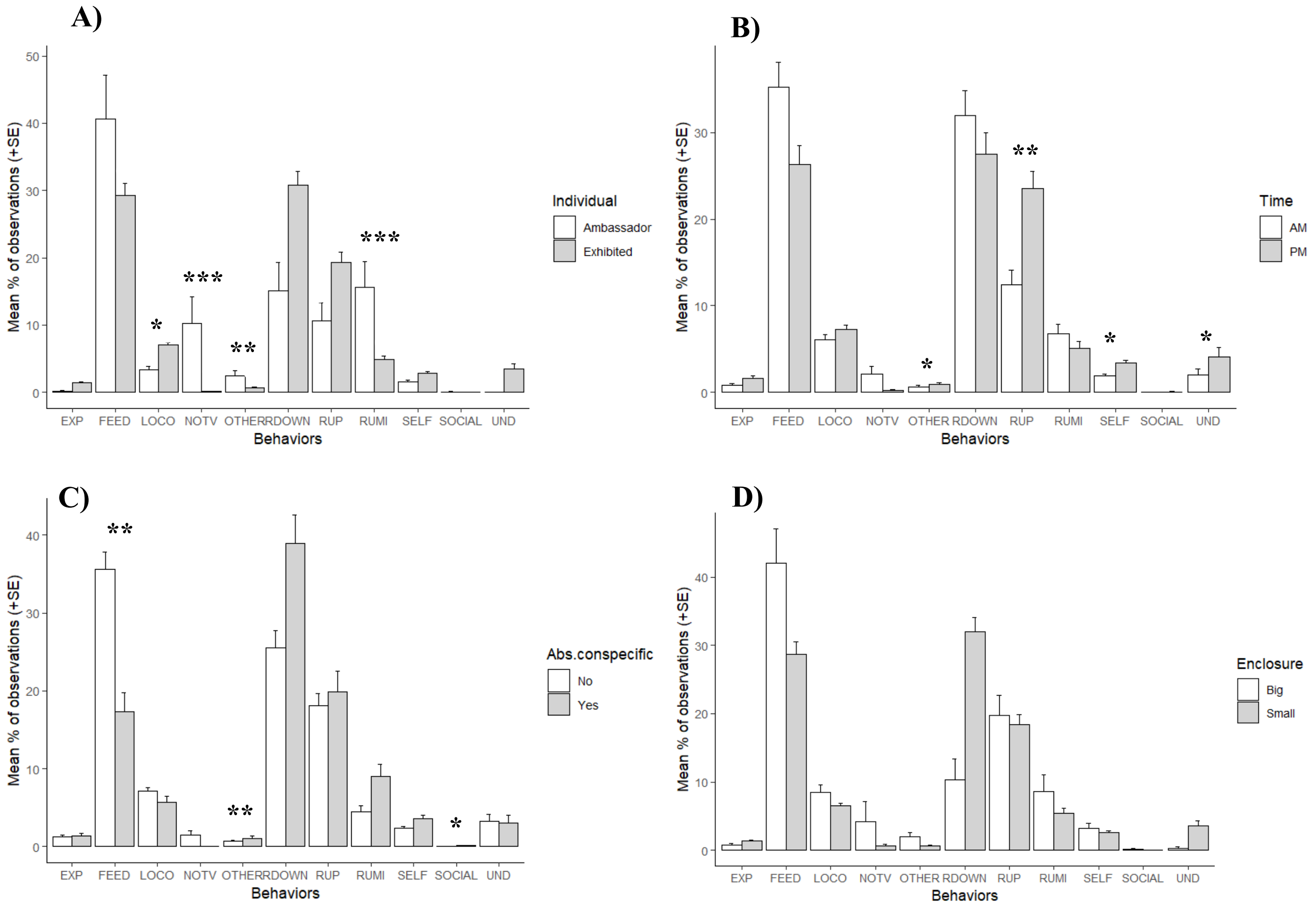

Figure 1.2. Activity budgets of dromedaries according to A) the ambassador and the exhibited animal, B) the time of the day, C) the absence of conspecific, and D) the enclosure. EXP: Exploration, FEED: Feeding and drinking, LOCO: Locomotion, NOTV: Not visible, OTHER: Other active, RDOWN: Resting down, RUP: Resting up, RUMI: Ruminating, SELF: Self-directed, SOCIAL: Social interaction, UND: Undesirable. Significance levels are indicated with stars.

Appendix D

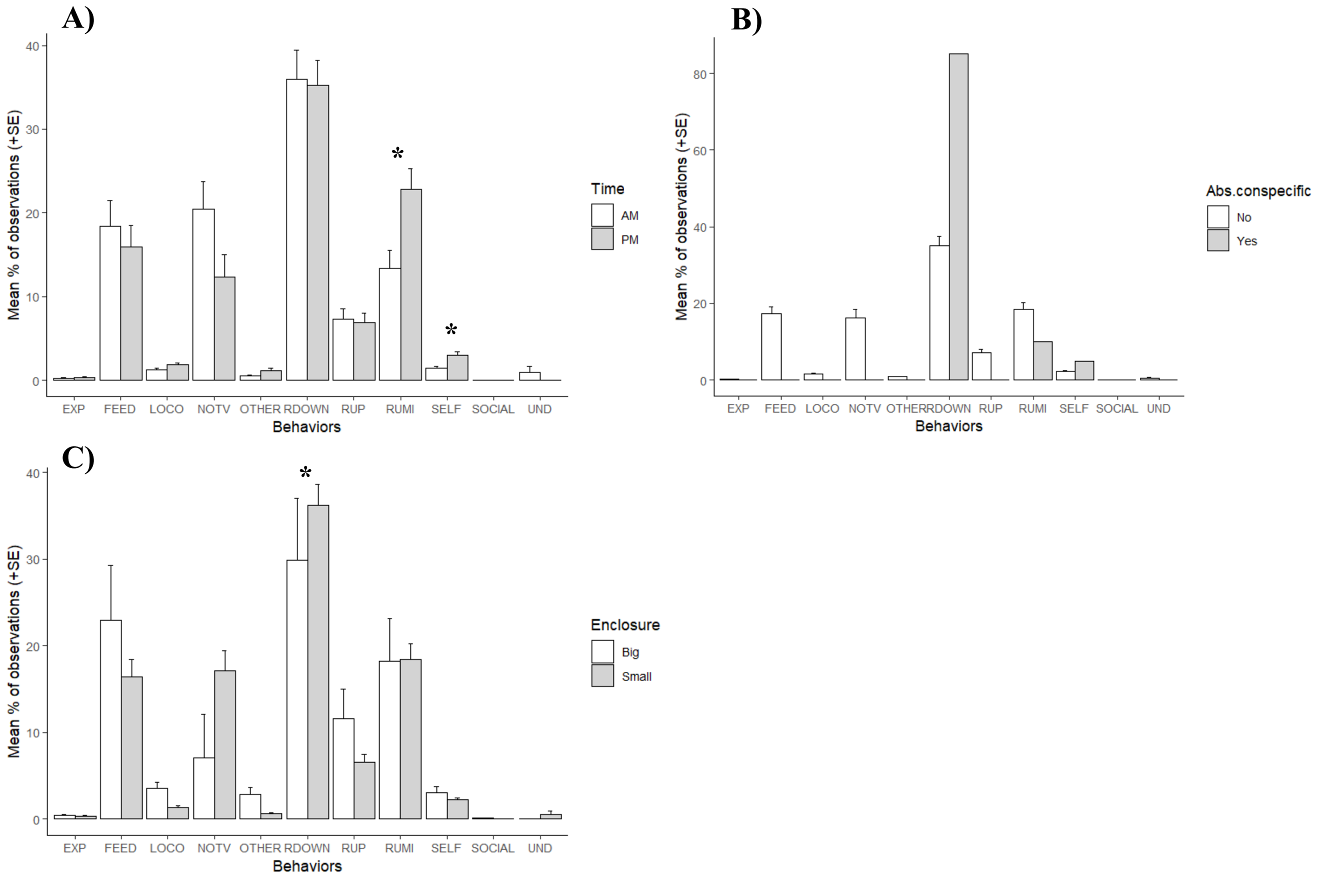

Figure 1.3. Activity budgets of the ambassador dromedary according to A) the time of the day, B) the absence of conspecific, and C) the enclosure. EXP: Exploration, FEED: Feeding and drinking, LOCO: Locomotion, NOTV: Not visible, OTHER: Other active, RDOWN: Resting down, RUP: Resting up, RUMI: Ruminating, SELF: Self-directed, SOCIAL: Social interaction, UND: Undesirable. Significance levels are indicated with stars.

Appendix E

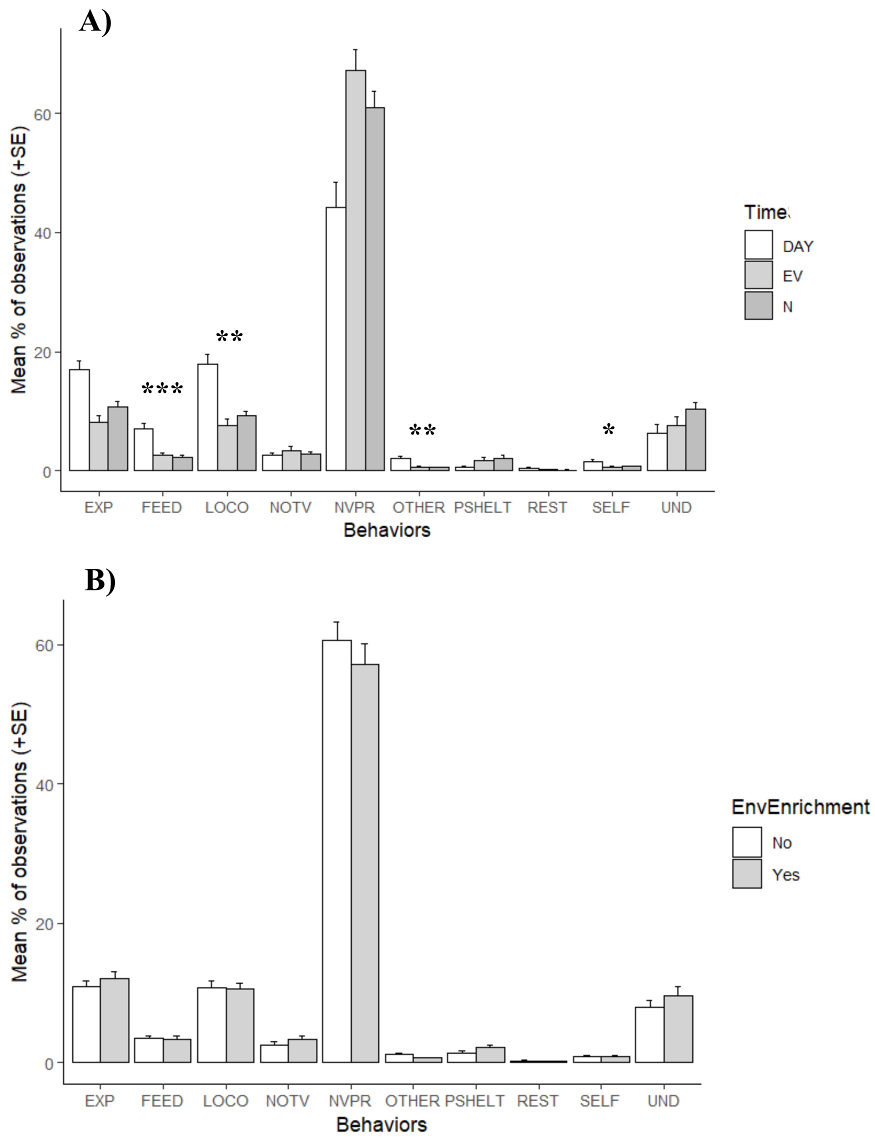

Figure 1.4. Activity budgets of three-banded armadillos according to A) the time of the day and B) the presence of environmental enrichment. EXP: Exploration, FEED: Feeding and drinking, LOCO: Locomotion, NOTV: Not visible, NVPR: Not visible, presumed resting, OTHER: Other active, PSHELT: Preparing shelter, REST: Resting, SELF: Self-directed, UND: Undesirable. Significance levels are indicated with stars.

Appendix F

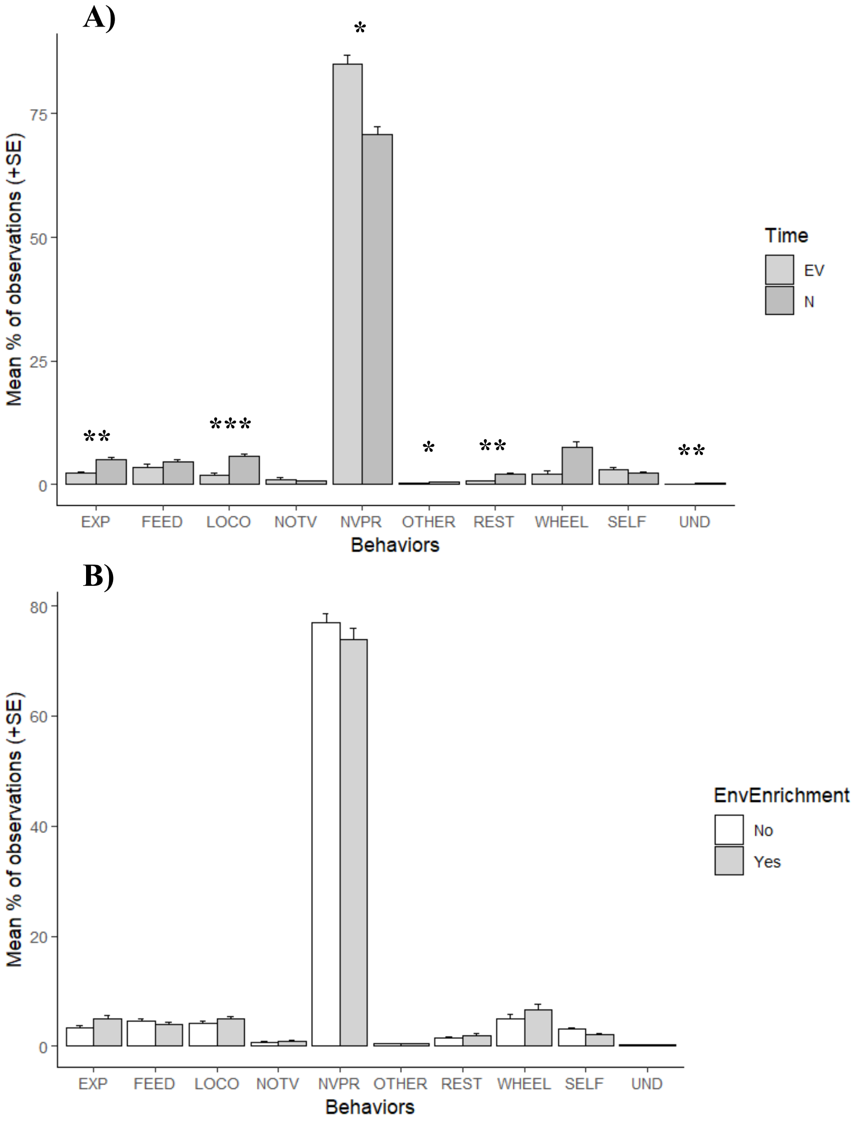

Figure 1.5. Activity budgets of grey short-tailed opossums according to A) the time of the day and B) the presence of environmental enrichment. EXP: Exploration, FEED: Feeding and drinking, LOCO: Locomotion, NOTV: Not visible, NVPR: Not visible, presumed resting, OTHER: Other active, REST: Resting, WHEEL: Running wheel, SELF: Self-directed, UND: Undesirable. Significance levels are indicated with stars.

Appendix G

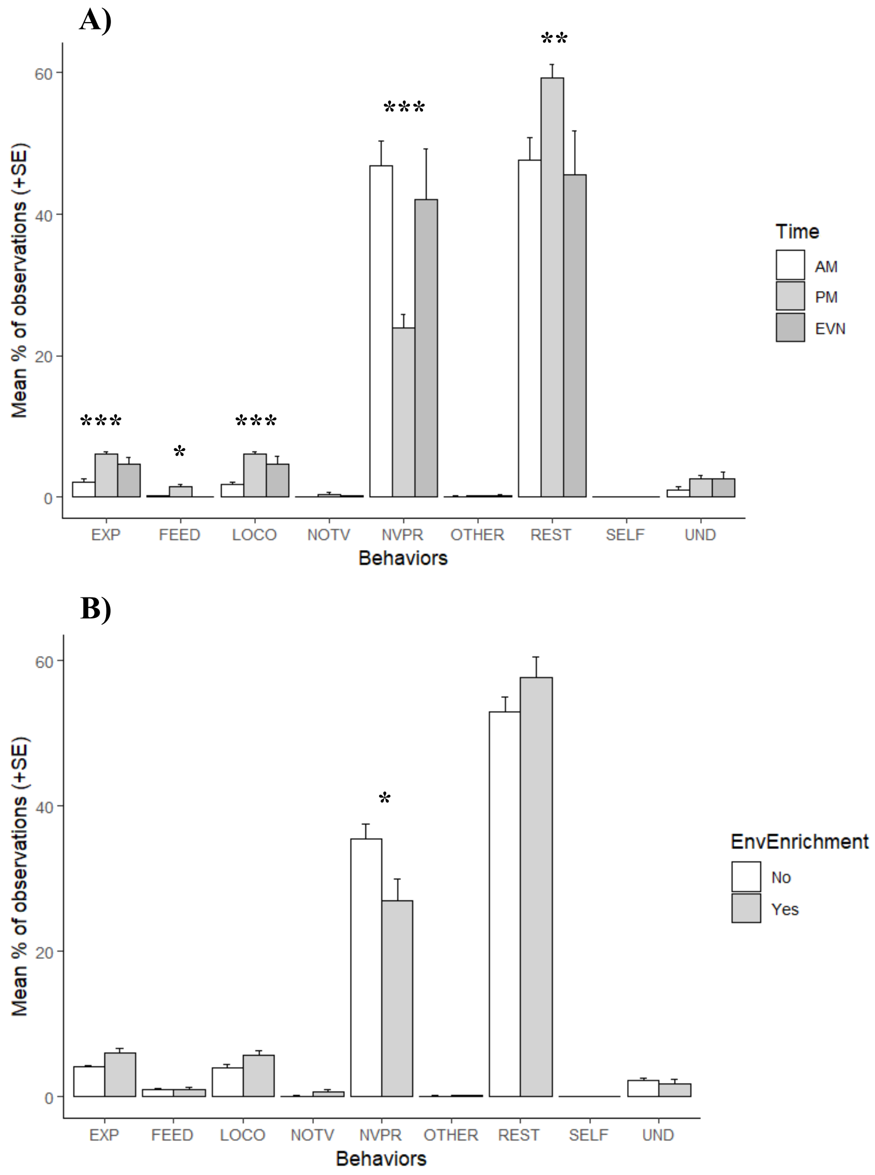

Figure 1.6. Activity budgets of blue-tongued skinks according to A) the time of the day and B) the presence of environmental enrichment. EXP: Exploration, FEED: Feeding and drinking, LOCO: Locomotion, NOTV: Not visible, NVPR: Not visible, presumed resting, OTHER: Other active, REST: Resting, SELF: Self-directed, UND: Undesirable. Significance levels are indicated with stars.

Appendix H

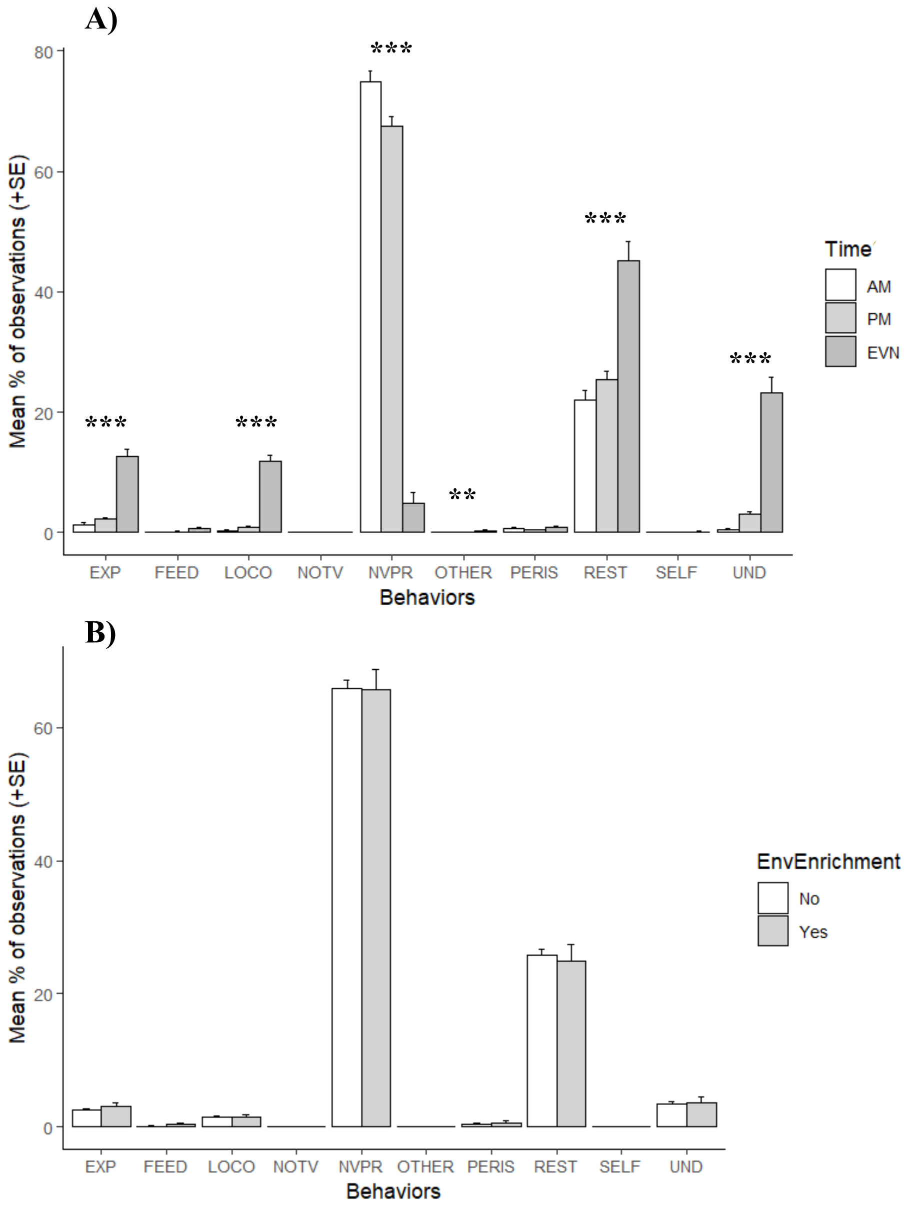

Figure 1.7. Activity budgets of ball pythons according to A) the time of the day and B) the presence of environmental enrichment. EXP: Exploration, FEED: Feeding and drinking, LOCO: Locomotion, NOTV: Not visible, NVPR: Not visible, presumed resting, OTHER: Other active, PERIS: Periscoping, REST: Resting, SELF: Self-directed, UND: Undesirable. Significance levels are indicated with stars.

Appendix I

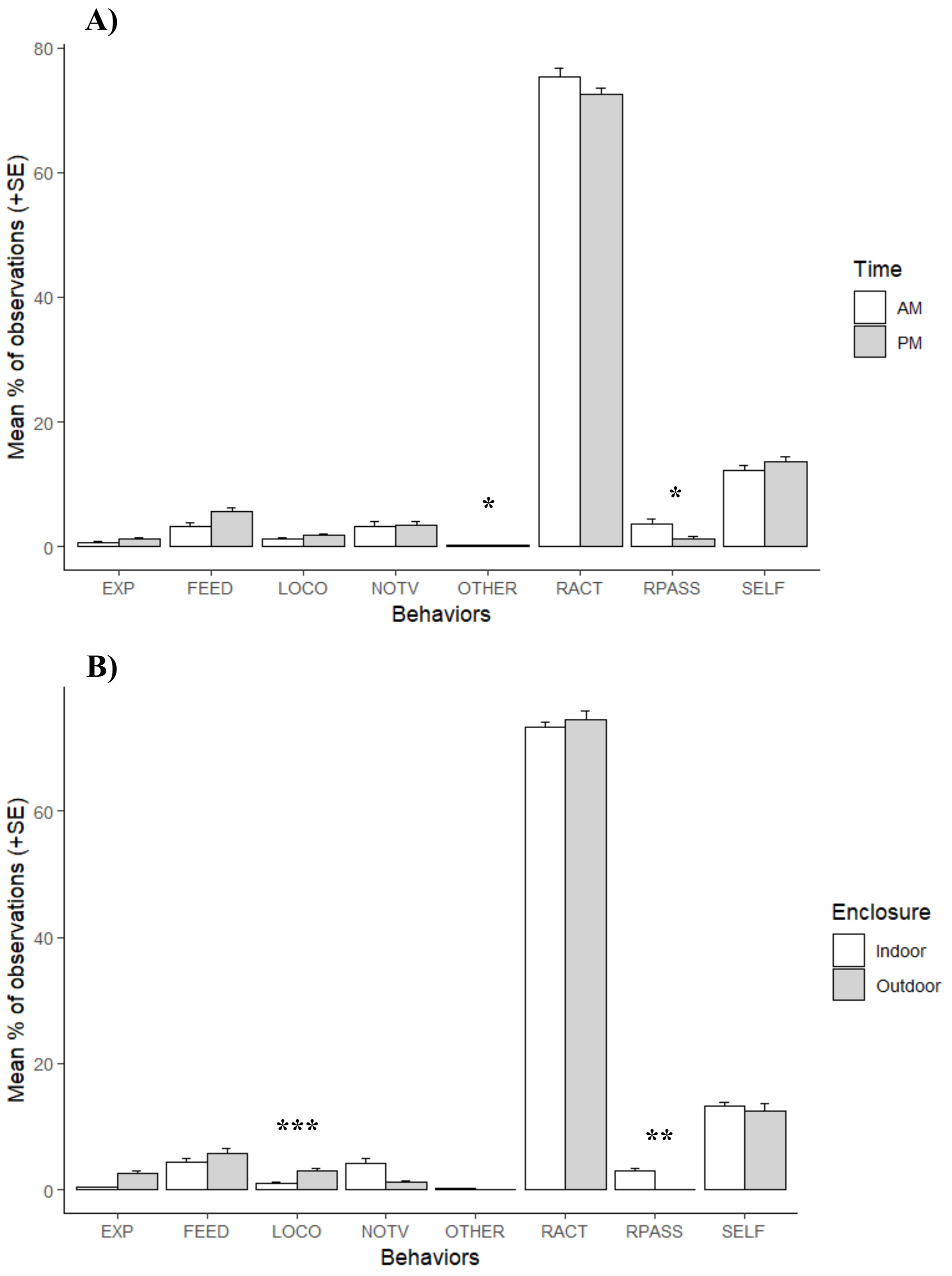

Figure 1.8. Activity budgets of American kestrels according to A) the time of the day and B) the enclosure. EXP: Exploration, FEED: Feeding and drinking, LOCO: Locomotion, NOTV: Not visible, OTHER: Other active, RACT: Resting active, RPASS: Resting passive, SELF: Self-directed. Significance levels are indicated with stars.

Appendix J

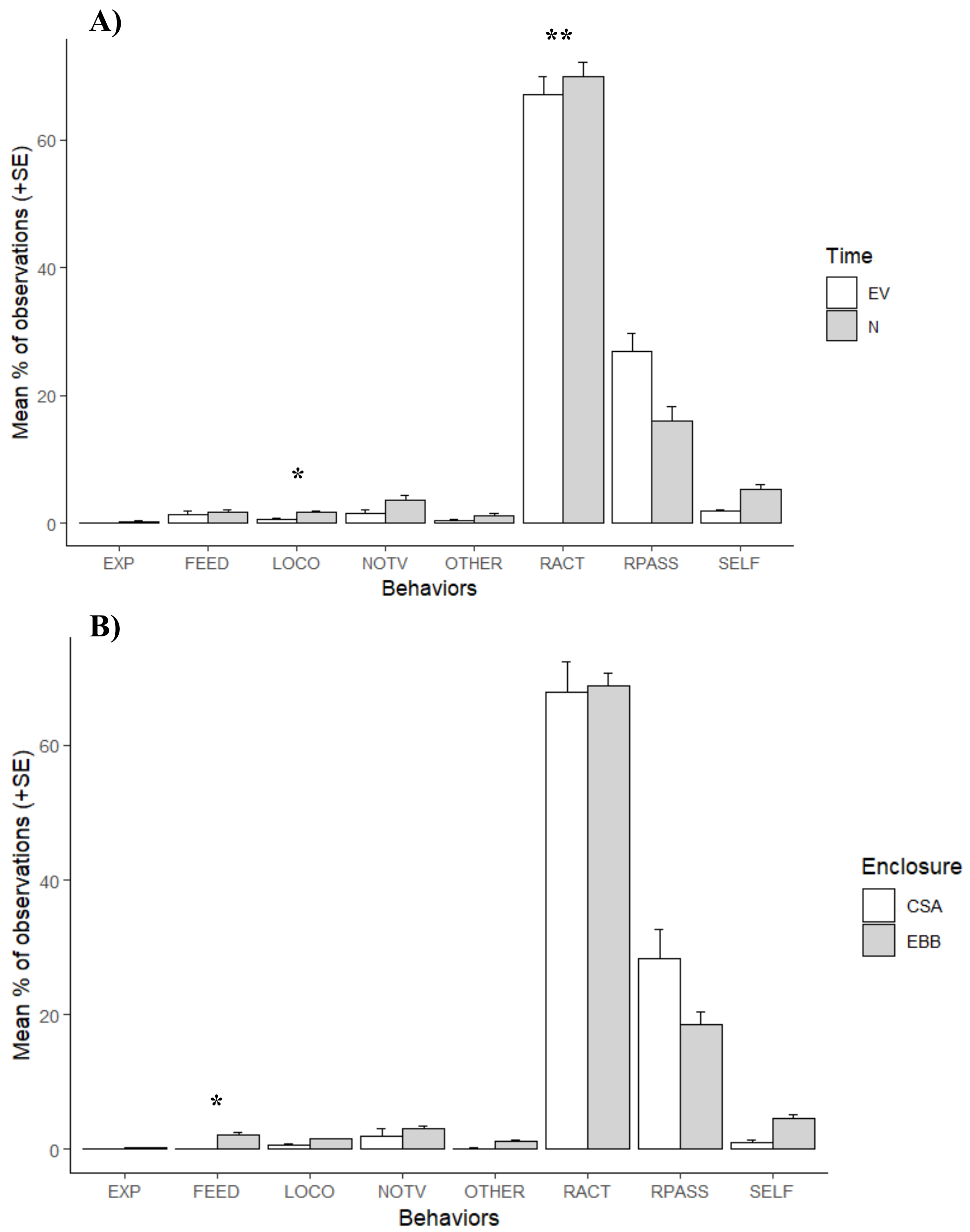

Figure 1.9. Activity budgets of great-horned owls according to A) the time of the day and B) the enclosure. EXP: Exploration, FEED: Feeding and drinking, LOCO: Locomotion, NOTV: Not visible, OTHER: Other active, RACT: Resting active, RPASS: Resting passive, SELF: Self-directed. Significance levels are indicated with stars.