RESEARCH ARTICLE

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# Characterization and correlations of behavioral and adrenocortical activities of zoo-housed lesser anteaters (*Tamandua tetradactyla*)

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We characterized behavioral and adrenocortical activities of *Tamandua tetradactyla* under human care driven by the hypothesis that they vary between males and females. We also assessed the potential association between natural or abnormal behaviors and adrenocortical activity. We kept females and males *T. tetradactyla* in individual, contiguous enclosures at Córdoba Zoo (Argentina), under natural photoperiod and temperature. During 29 consecutive days we monitored the animals' behavior by recording their activity pattern every 5 min using infrared cameras (8352 records/individual). We collected all feces and measured fecal glucocorticoid metabolites (FGM) with an 11-oxoaetiocholanolone enzyme immunoassay. We found individual differences in all behavioral variables. We detected that females exhibited lower total activity than males ($23.8 \pm 0.2\%$ and $32.3 \pm 0.3\%$, respectively; $p = .005$). Females were more active at night and males during the day ($p < .05$) and exhibited less abnormal behaviors than males ($p = .05$). Although we did not find sex-related differences for average FGM, we detected individual differences ($p < .0001$). We found that daily FGM showed negative ($-0.39$) and positive ($0.38$) correlations with natural and abnormal behaviors, respectively ($p < .0001$). Thus, we consider that individual input and sex are factors to be considered in stress responses of the species in captivity. Natural and abnormal behaviors may demand different levels of adrenocortical activity. Our findings may prove useful as normative data for ex situ management of conservation programs.

**KEYWORDS**
activity pattern, fecal cortisol metabolites, noninvasive hormone monitoring, pilosa, stereotypic behavior

## 1 | INTRODUCTION

Coping with environmental perturbations such as climatic extremes, social disruption, and/or rapid human-induced changes is one of the most essential physiological and behavioral processes (Mason et al., 2013; Wingfield, 2013). Taking into account the difficulties involved in studying stress responses of free-ranging mammals, zoo-derived data have enormous potential value for elucidating animal responses to captivity (Mason, Clubb, Latham, & Vickery, 2007; Schwarzenberger & Brown, 2013) and improving normative data to enhance the precision of studies in free-ranging wildlife. For example, different taxa have been studied in terms of how management and husbandry impact behavior and/or adrenocortical responses in captivity (Cortés Duarte, Trujillo, & Superina, 2016; Howell-Stephens, Brown, Bernier, Mulkerin, & Santymire, 2012; Wielebnowski, Fletchall, Carlstead, Busso, & Brown, 2002). Furthermore, in view of the increasing

disappearance of animal species worldwide, breeding animals in captivity is one way of stemming the tide of extinction by reintroducing individuals into the wild and assisting in the recolonization of areas where species have become locally extinct (Vignieri, 2014). However, some species apparently thrive in captivity, whereas others are prone to problems including poor health, breeding difficulties, and behavioral problems such as repetitive stereotypic behaviors (R. Clubb & Mason, 2007). According to the latter authors, some particular lifestyles in the wild confer vulnerability in captivity, for example in wide-ranging carnivores. For carnivores, the most widely cited hypothesis for the motivation underlying locomotory stereotypies is that in captivity they are restrained from developing their natural foraging behaviors (R. E. Clubb & Vickery, 2006). Understanding the reasons for abnormal behavior of animals under human care is indispensable, as it allows institutions to improve enclosure design and enrichment techniques (Polanco, Díez-León, & Mason, 2018).

The hypothalamic–pituitary–adrenocortical (HPA) axis is a key player associated with behavioral responses to environmental factors. Recent advances in the study of HPA activity suggest that the degree of HPA activation is a direct reflection of the amount of physical activity and the accompanying metabolic demands (Koolhaas et al., 2011; Spencer & Deak, 2016). Additionally, recent studies in physiology and behavior have begun to explore stress responses in free-ranging mammals. These studies benefit from the advent of new methods for quantifying stress, including techniques of noninvasive hormonal monitoring (Reeder & Kramer, 2005; Sheriff, Dantzer, Delehanty, Palme, & Boonstra, 2011). For example, Rizo-Aguilar, Guerrero, Montoya-Lara, and Valdespino (2014) postulate fecal glucocorticoid metabolite measurements as potential indicators of physiological health in volcano rabbits *Romerolagus diazi*. Hormone–behavior interactions are now widely used to investigate and address questions in the fields of ecology, conservation biology and welfare (Palme, 2012, 2019; Sheriff et al., 2011). For example, to learn more about mating patterns in the aardwolf *Proteles cristata*, a small insectivorous hyaenid, Marneweck, Cameron, Ganswindt, and Dalerum (2015) analyzed sex differences by monitoring correlations between behavior and fecal glucocorticoid measurements. Measuring behavioral changes (e.g., sleep pattern, stereotyped behaviors, etc.) and physiological stress-related hormone levels (e.g., glucocorticoid mediators) may therefore help in understanding the implications of stress for individual life-history strategies (Buchanan, 2000).

The present study focuses on a specialized mammalian myrmecophage (ant and termite-eater) endemic to South America (Redford & Eisenberg, 1992): *Tamandua tetradactyla* (Mammalia: Xenarthra: Pilosa). This species is listed by the IUCN Red List of Threatened Species as Least Concern in view of its wide distribution, presumed large population and occurrence in a number of protected areas (Miranda et al., 2014). However, since it is affected by habitat loss and fragmentation in parts of its range, it is listed as Near Threatened

in Argentina (Superina, Abba, & Vizcaíno, 2012) and as Threatened in Uruguay (Soutullo, Clavijo, & Martínez-Lanfranco, 2013). Wild *T. tetradactyla* individuals usually spend a large proportion of their activity time foraging, which is associated with large home-ranges (100-375 ha) and mean daily movements (3,000 m; Hayssen, 2011). The literature cites both *Tamandua* species (*Tamandua mexicana* and *T. tetradactyla*) as nocturnal to diurnal–crepuscular, with activity during approximately 8 hr/day (Brown, 2011; Montgomery, 1985). According to Navarrete and Ortega (2011), temperature and the incidence of direct sunlight have a strong effect on the daily movements of *T. mexicana*. Information about *T. tetradactyla* reviewed by Hayssen (2011) indicates that captive animals may be active during the day. A previous study described several directly observed behavioral categories and fecal glucocorticoid metabolite measurements validated for captive *T. tetradactyla* individuals at Córdoba Zoo, Argentina. Analyses demonstrated that food-based environmental enrichment improved the welfare of these individuals (e.g., increased natural behaviors and reduced adrenocortical activity), although it was not useful for tackling stereotypies (Eguizábal et al., 2013). Recently, Eguizábal et al. (unpublished data) carried out a noninvasive assessment of zoo-housed *T. tetradactyla*, showing that management procedures do not compromise animal welfare (e.g., daily behavior and adrenocortical activity did not change after body weight check).

Sex differences in behavioral and HPA activities are generally well-documented (e.g., Handa, Burguess, Kerr, & O'Keefe, 1994; Kelley, 1988; Wearmouth & Sims, 2008), but to our knowledge they have not been assessed in any Xenarthra. Surmising that behavioral and adrenocortical activity varies between males and females, we therefore characterized these activities in *T. tetradactyla* under human care. We also assessed the potential association between natural or abnormal behaviors and adrenocortical activity.

## 2 | MATERIALS AND METHODS

### 2.1 | Animals, housing conditions, and management

We studied six adult *T. tetradactyla* (three females: 6.35 ± 0.43 kg and three males: 8.53 ± 0.66 kg) during 29 consecutive days in autumn 2016 (April 21–May 20). Animals were housed at Córdoba Zoo (31°12.32′S, 64°16.84′W; Córdoba, Argentina), under natural conditions of photoperiod (sunrise 07:50 hr, sunset 20:00 hr), temperature (average min.–max. = 11.8–17.8°C; an external heat source (150 W, E27, General Electric) was turned on in the shelter when the ambient temperature was lower than 10°C) and humidity (average min.–max. = 69.7–83.3%). All individuals were at least 4 years old. Housing was individual in contiguous, similar-sized (all enclosures approximately 38 m$^2$) enclosures and each animal had at least one individual of the opposite sex as a neighbor (Figure 1); this housing condition led to the application of standardized experimental design. Following Superina, Miranda, and Plese (2008), enclosures contained a
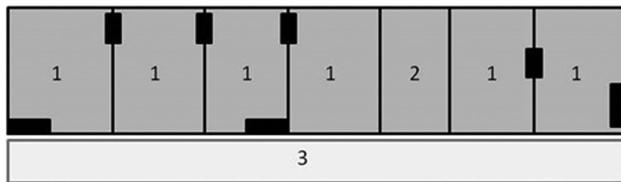
**FIGURE 1** Zoo-housing of adult (*n* = 6) *Tamandua tetradactyla* individuals, top view. Enclosures (1) were under natural conditions of photoperiod, temperature and humidity. Laboratory (2) was located between enclosures. Public view is marked as 3. Wire gates (small black rectangles) connecting enclosures and glass gates (large black rectangles) for entering enclosures are shown

wooden shelter, four plastic feeders and a watering trough, several climbing structures (i.e., logs, stairs, and wire roof), plants, soil and wood substrate, and wire gates between contiguous enclosures.

The animals' balanced diet consisted of a mixed shake containing lactose-reduced whole powdered milk (La Serenísima, 15% of total ration), baby cereal (Nestum, Nestlé, 14%), balanced dog feed for puppies (Eukanuba Small Breed, 71%), drinking water and vitamin K. The individual quantity varied (700–900 ml) depending on the energetic need of each animal, which we calculated following Dierenfeld and Graffam (1996). Food consumption was 486.8 ± 7.6 ml for females and 669.5 ± 18.4 ml for males. In accordance with Eguizábal et al. (2013), food-based environmental enrichment (e.g., ants, honey, mealworms, fruits, etc.) was additionally offered 2–4 times/week.

We subjected the animals to the following institutional management strategies: We performed daily cleaning routines between 9:00 and 11:00 hr, supplied food between 12:00 and 15:00 hr, carried out a veterinary check every 3 months (May 4) and checked body weight once per month (May 11). Finally, taking into account the possible beneficial effects of providing new exploration opportunities, we rotated animals between enclosures every 2 weeks (April 27 and May 11). According to veterinary records, animals showed good health and nutritional status (data not shown).

We carried the study out in accordance with the CONICET Ethics Committee (Resolution 1047 Annex II, 2005) and the Argentinean National Law of Animal Protection (N° 14346). It was evaluated and approved by the Bioethics and Laboratory Animal Commission, following the Annex to the Regulation of "Care and Use of Laboratory Animals" (from IIBYT/CONICET- FCEFyN-UNC). It was not possible to increase sample size (number of animals) since *T. tetradactyla* individuals in other Argentinean zoos are kept under different environmental and husbandry conditions, making it impossible to compare the results with precision. Furthermore, transferring additional animals from other institutions to Córdoba zoo to keep them under similar conditions during the study period was not permitted by local regulations.

## 2.2 | Behavioral measurements

Behavior was continuously monitored by infrared video cameras located in each enclosure (HIKVISION Turbo HD- IR Turret Camera-DS 2CE56C2T IRM) and recorded by a digital video recorder (HIKVISION Turbo HD DVR-DS 7200 Series) located in the lab next to the enclosures. Daily measurements began at dawn (6:30 hr) and ended the next day (6:29 hr). We used an adaptation of the instantaneous sampling method proposed by Altmann (1974) to register animal behavior (Martin & Bateson, 2013). Based on preliminary observations, we chose an interval of 5 min, considering 15 s before and after sampling points (e.g., 4:59:45 to 5:00:15; 5:04:45 to 5:05:15; etc.). Briefly, during preliminary observations we analyzed behavioral percentages from 12-hr video, considering 1, 2, 5, and 10 min sampling interval points. Percentages were not different for 1, 2, and 5. However, 10 min led to missing behavioral data. Thus, we chose the 5-min interval for the 29-days study to minimize sampling effort. We collected a total of 288 frequency records per individual during 24 hr. Behavioral categories are based on the ethogram developed by Eguizábal et al. (2013) as described in Table 1. All observations were made by the same researcher (G. V. E.).

We calculated *total activity* (TA) as the number of frequency records (considering all active behaviors) per day and then the percentage of activity. For example, 70 active behavior records

**TABLE 1** Ethogram of adult zoo-housed *Tamandua tetradactyla*. Behaviors are categorized as inactive, natural active, or abnormal active

| Behavior | Category | Definition |
| --- | --- | --- |
| Rest | Inactive | Lying or rolled up, belly down or up, with eyes closed or open, motionless. |
| Exploration | Natural Active | Variable position, sniffing while moving head laterally or vertically and/or using forelimbs and claws to rip apart logs, plants or soil. |
| Locomotion | | Using all four limbs and usually the tail to move, either walking or climbing. |
| Feeding | | Variable position, using tongue to introduce food into the mouth. |
| Social | | Variable position, introducing tongue, claws, tail and/or snout into the adjacent enclosure, while the neighbor is performing the same behavior. |
| Alert | | Quadrupedal or tripedal position, motionless. |
| Others | | Grooming, defecating, urinating and other behaviors not previously described. |
| Repetitive locomotion | Abnormal active | Using all four limbs to move along a given route (in front of the door, around enclosure, etc.) for two or more repetitions, either walking or running. |

*Note:* Behavioral categories were based on the ethogram developed by Eguizábal et al. (2013).

per day correspond to 24.3% of activity (calculated as 70 × 100/288). We obtained percentage of activity per hour by averaging all observations every 5 min of each hour for each animal (288 records/individual/day). We calculated the *activity pattern according to light–dark phases* (APLD) as the number of active records for each phase (dawn: 06:30–07:49 hr, day: 07:50 hr–18:39 hr, dusk: 18:40–19:59 hr, night: 20:00–06:29 hr) during the day. Finally, we calculated *natural activity* (NAT) and *abnormal activity* (ABN) as the numbers of records of each category (considering all natural active, and abnormal active behaviors, respectively) during the day.

## 2.3 | Measurement of fecal glucocorticoid metabolites

We collected fresh feces daily after deposition (during behavioral sample intervals) between 09:00 and 13:00 hr, froze them immediately at −20°C and stored them until analysis. Frequency of deposition was 4–7 times per week (total number of samples: 174). We extracted fecal glucocorticoid metabolites (FGM) by a simple method. Briefly, we added 5 ml methanol/water (80%) to a portion (0.5 g) of each well-homogenized sample (Palme, Touma, Arias, Dominchin, & Lepschy, 2013). After shaking (2 min) and centrifugation (15 min, 3,000 G), we separated an aliquot (0.5 ml) of the supernatant for further use. The extracts were evaporated at 60 °C, sent to the laboratory in Vienna, resuspended in 80% methanol and dissolved in enzyme immunoassay (EIA) buffer. We ran all measurements in duplicate with an 11-oxoetiocholanolone EIA, as described by Möstl, Maggs, Schrötter, Besenfelder, and Palme (2002). This assay was previously validated for *T. tetradactyla* (Eguizábal et al., 2013). The sensitivity of the EIA for FGM measurement was 4 ng/g feces. Inter-assay coefficients of variation (CV) were 12.0% and 8.3% for a low and high concentration pool sample, respectively. Intra-assay CV were always below 10%.

## 2.4 | Statistical analyses

To analyze behavioral data, we applied a mixed general linear model (MGLM) to determine statistical differences between TA, APLD, NAT, and ABN by individual, considering day as random factor. Then, we used MGLM to determine differences by sex, considering day and individual as random factors. Because records represent count data, we assumed a Poisson error distribution. APLD for dawn could not be estimated due to scarce or null records. We applied Fisher's *a posteriori* test when the statistical analysis showed a $p \leq .05$.

Adrenocortical data did not show normal distribution according to the modified Shapiro–Wilks test. We transformed data to square root to meet normal distribution and applied a mixed linear model (MLM) to determine statistical differences between FGM per individual, considering days as random factor. Then, we used MLM to determine differences between FGM per sex, considering days and individuals as random factors. We applied Fisher's *a posteriori* test when the statistical analysis showed a $p \leq .05$.

Finally, we calculated a nonparametric Spearman's correlation coefficient for FGM versus TA, NAT, and ABN. We performed all analyses using InfoStat (Di Rienzo, Guzman, & Casanoves, 2002). Reported values are expressed as mean ± SEM, and the significant level was 5% for all tests.

## 3 | RESULTS

### 3.1 | Behavioral measurements

We detected a total of 28.8 ± 0.2% of activity per day (83/288 records), with significant differences among individuals (female 3<female 2<female 1<male 3<male 1 = male 2; $p < .0001$; $\chi^2_{(5)} = 434.04$) and between sexes (females 23.8 ± 0.2% and males 32.3 ± 0.3%; $p = .005$; $\chi^2_{(1)} = 7.87$). The activity pattern showed two peaks (13:10 and 19:10 hr); profiles for the two sexes
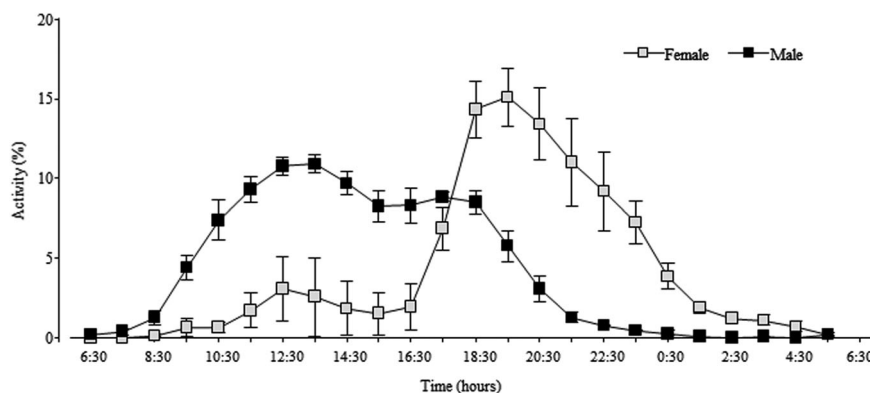


**FIGURE 2** Activity pattern of zoo-housed *Tamandua tetradactyla* in autumn according to sex. Animal behavior was registered every 5 min (288 records/individual/day), and the average of the activity per hour was calculated from the data obtained during the 29 days. Values were then averaged by sex (three males and three females). Animals were exposed to natural photoperiod (light phases: dawn 06:30-07:49 hr, day 07:50–18:39 hr, dusk 18:40–19:59 hr, night 20:00–06:25 hr)
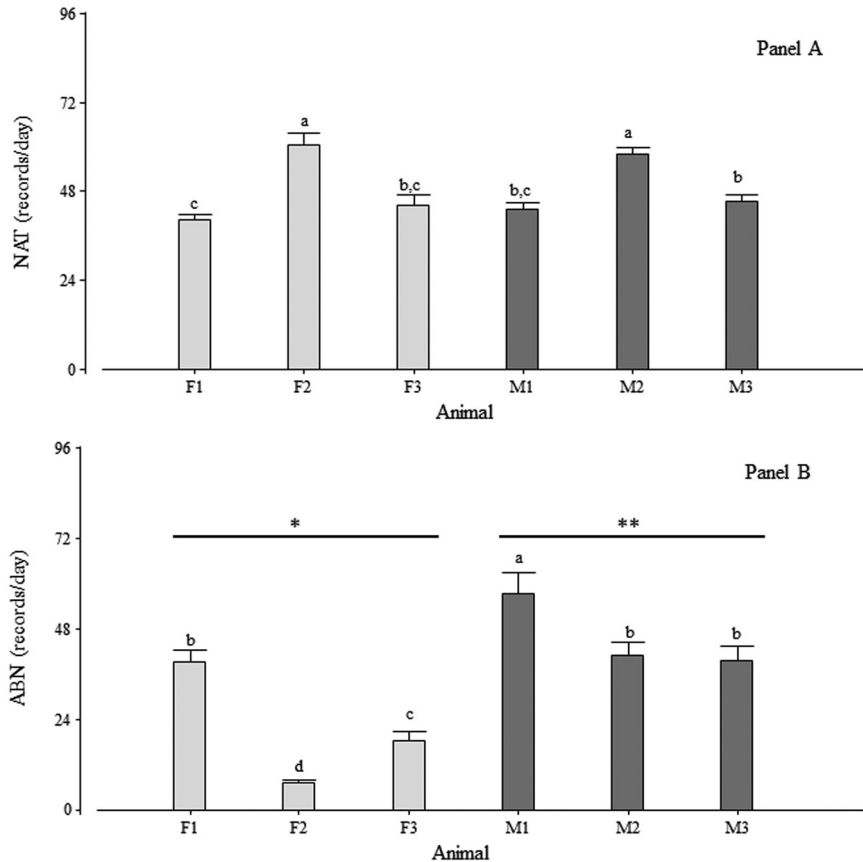
**FIGURE 3** Natural and abnormal activity of zoo-housed *Tamandua tetradactyla* (light-grey: females and dark-grey: males). Animal behavior was registered every 5 min during 29 consecutive days of autumn. Natural (a) and abnormal (b) activities were calculated as the number of records per category (considering all natural active, and abnormal active behaviors, respectively) during the day. Different letters indicate significant differences among individuals ($p < .0001$) and asterisks (* vs **) indicate significant differences between sexes ($p = .05$)

throughout the day were dissimilar (Figure 2). Moreover, APLD differed among individuals ($p < .0001$; day: $\chi^2_{(5)} = 4333.35$; dusk: $\chi^2_{(5)} = 118.06$; night: $\chi^2_{(5)} = 2359.29$) and between sexes (day: $12.2 \pm 3.5 < 73.3 \pm 20.7$, $p = .0028$, $\chi^2_{(1)} = 8.95$; dusk: $13.5 \pm 1.4 > 9.5 \pm 0.9$, $p = .0375$, $\chi^2_{(1)} = 4.33$; night: $37.2 \pm 6.6 > 7.6 \pm 1.4$, $p = .0004$, $\chi^2_{(1)} = 12.66$; females vs. males, respectively). We also found differences among individuals ($p < .0001$; $\chi^2_{(1)} = 208.51$, and 1633.56; Figure 3a,b) for NAT and ABN, respectively, and between sexes only for ABN ($p = .05$; $\chi^2_{(1)} = 3.83$; Figure 3b).

## 3.2 | Measurement of fecal glucocorticoid metabolites

We found individual differences in FGM ($p < .0001$; $F = 17.624$; Figure 4). However, we did not detect sex-related differences for FGM.

## 3.3 | FGM and behavior correlations

Spearman's correlation coefficient for daily FGM and TA gave a value of $r = 0.19$ ($p < .01$). Figure 5 shows FGM correlations with NAT
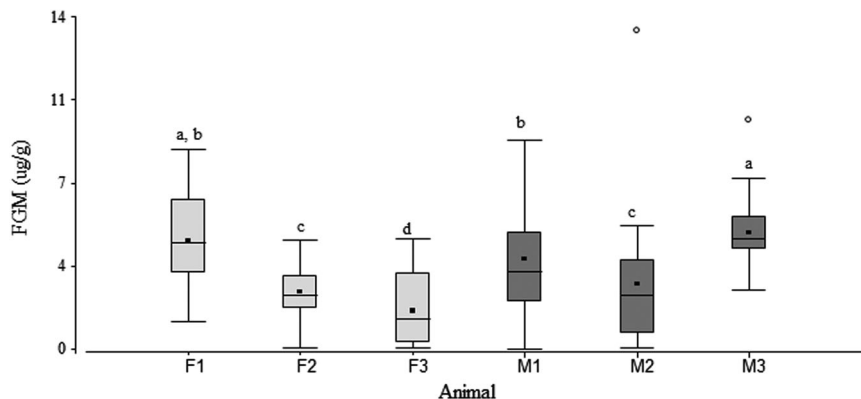


**FIGURE 4** Adrenocortical activity of zoo-housed *Tamandua tetradactyla* (light-grey: females and dark-grey: males). Fecal glucocorticoid metabolite measurements were performed in all feces collected during 29 consecutive days of autumn (3 males, 3 females, 174 samples). Box plots show median values (horizontal line), average (dark square dot), first and third quartiles (bottom and top lines), average $\pm 1 \times$ SD (whiskers) and values outside the range of average $\pm 1 \times$ SD (white circles). Different letters indicate significant differences among individuals ($p < .0001$)

**FIGURE 5** Spearman's correlations of fecal glucocorticoid metabolites (FGM) with natural active behaviors (a) and abnormal active behaviors (b) of zoo-housed *Tamandua tetradactyla*. Data were collected for 29 consecutive days in autumn. Fecal glucocorticoid metabolite measurements were performed in all feces collected and behavior was registered every 5 min using the instantaneous sampling method
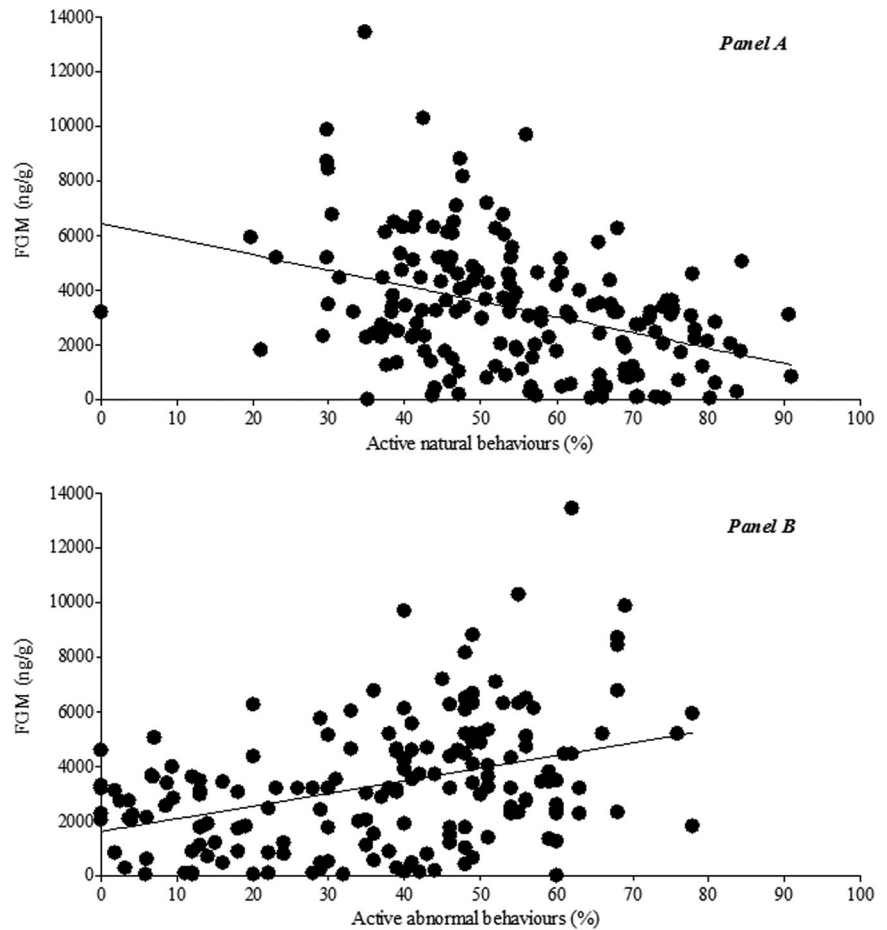
($r = −0.39$; Figure 5a) and ABN ($r = 0.38$; Figure 5b), respectively ($p < .0001$). Furthermore, when correlations were made for both sexes separately, we detected similar results.

## 4 | DISCUSSION

In this study, we characterized the behavioral and adrenocortical activity of zoo-housed *T. tetradactyla*. We detected individual differences for all variables and observed sex differences in total activity, activity pattern according to light/dark phases and abnormal activity. However, we did not detect sex differences in natural activity and adrenocortical activity. Assessment of the potential association between behavior and adrenocortical activity indicates that natural behaviors correlate with lower levels of glucocorticoid excretion, possibly signifying a lower demand for HPA activity compared to a higher adrenocortical activity associated with abnormal behaviors.

The observed behavioral patterns revealed that zoo-housed *T. tetradactyla* females exhibited less activity per day than males. Moreover, data also show that animals allocate their activity throughout the day differently, with females being mostly active at night and males almost exclusively during the day. Considering that animals were in contiguous enclosures, the results in terms of *social coupling* were unexpected. The social coupling theory suggests that prolonged cohabitation might have robust effects on the rhythmicity

of cohoused individuals that are not easily reproduced by indirect or pulsatile social contacts in the wild (Castillo-Ruiz, Paul, & Schwartz, 2012). Brown (2011) studied the activity pattern of *T. mexicana* in the wild and observed large individual differences in the percentage of total activity during different light phases. Nevertheless, taken collectively, day and dusk percentages of active records were similar and higher than those for dark and dawn. Moreover, the timing of activity for the two sexes overlapped considerably, with female peak activity (13:53 hr) averaging 2 hr earlier than male activity (15:54 hr), perhaps to avoid aggressive male conspecifics (Brown, 2011). Since both *Tamandua* species were exposed to natural variations in environmental conditions over the study periods but the captive *T. tetradactyla* were not exposed to predator risk and/or possible aggressive intraspecific encounters, it is possible that their activity was influenced by other factors (space restriction, sensitivity to human interactions, sex and individual differences, etc.) rather than by interspecific differences. Further studies are required to investigate this possibility.

In a recent review, Bennie, Duffy, Inger, and Gaston (2014) reported that the majority of mammal species are nocturnal (nocturnal 69%, diurnal 20%, cathemeral 8.5%, and crepuscular 2.5%). According to Brown (2011), the activity pattern of *T. mexicana* is best categorized as cathemeral. Similarly, translocated *Myrmecophaga tridactyla*, a species closely related to *Tamandua*, also exhibited a cathemeral activity pattern (with some seasonal changes; e.g.,

animals were more active during light phase than dark phase on cold days; Di Blanco, 2015). Another study suggests that *M. tridactyla* shows a nocturnal pattern, which also changes with the decrease in ambient temperature (Sampaio, Camilo-Alves, & de Miranda Mourão, 2006). It was not possible to categorize the activity pattern of *T. tetradactyla* on the basis of the results of the present study since the activity pattern of females resembled a nocturnal and that of males a diurnal strategy. Perhaps, the sex differences we observed in captive animals may explain the current characterization of wild *T. tetradactyla* as nocturnal to diurnal–crepuscular.

The study animals displayed both natural and abnormal active behaviors with large individual differences. Further analysis of these behaviors by sex indicated a similar proportion of natural behaviors in both sexes, and that females exhibited less abnormal locomotion than males. Differential behavioral needs between sexes could not be fully explained by adrenocortical activity in this species. We have not found any reports about such stress responses in this species or other Myrmecophagidae. Results reported by Wielebnowski et al. (2002) for *Neofelis nebulosa* indicated that animals displaying self-injuring behaviors had significantly higher FGM concentrations than conspecifics showing no self-mutilation. Interestingly, more females (63%) showed this type of abnormal behavior, suggesting increased sensitivity of females to harmful or chronic stressors in captivity. Since stereotypies are often associated with past or present suboptimal environmental conditions (Mason, 1991), *T. tetradactyla* abnormal behavior deserves more attention, particularly in males (for which values, as well as adrenocortical activity, were highest) to understand the underlying processes in individuals under human care.

Adrenocortical activity was noninvasively monitored by fecal glucocorticoid metabolite measurements. Results in the present study are similar to those reported by Eguizábal et al. (2013), which was expected because some animals were involved in both studies. The present study confirms previous findings of individual differences in adrenocortical activity in several taxa, including mammals (Palme, 2019). Because animals defecated once a day, each sample can be assumed to include glucocorticoid metabolites related to basal (ultradian and circadian rhythms) and daily HPA activities, associated with predictive homeostasis (Romero, Dickens, & Cyr, 2009). Findings revealed that *T. tetradactyla* females and males exhibited similar average levels of adrenocortical activity. Among mammalian species, sex differences in HPA activity have been studied in rodents by means of blood and fecal samples (Spencer & Deak, 2016; Touma, Palme, & Sachser, 2004); females showed higher glucocorticoid levels than males. The results of studies on wild mammalian species still show diverging results; perhaps differences in metabolism and excretion could partly explain sex differences detected in feces (Palme, Rettenbacher, Touma, El-Bahr, & Möstl, 2005). Sipari, Ylönen, and Palme (2017) observed a different pattern (females < males) in *Myodes glareolus*. In contrast, female *Neofelis nebulosa* exhibited higher FGM concentrations than males (Wielebnowski et al., 2002). The results of the present study differed from the pattern detected in

serum cortisol (ng/dl) for the same animals (females = 18.1 ± 7.4 < males = 41.3 ± 11.3; *p* = .01; Busso et al., 2017), which may be explained by the fact that measured glucocorticoids (or their metabolites) reflect different things in blood and feces (Palme, 2019). Nevertheless, further studies are necessary to gain a more precise understanding of the adrenocortical pattern of *T. tetradactyla*, exploring intrinsic and extrinsic factors as source variations, including younger animals and/or considering the exposome concept rather than intrinsic sex genetic differences (Wild, 2012; Wingfield, 2013).

Morgan and Tromborg (2007) pointed out that animals housed in artificial habitats are confronted with a wide range of potentially challenging environmental conditions. At Córdoba Zoo, *T. tetradactyla* facilities were modified and brought into line with those described by Superina et al. (2008). Despite this welfare improvement over traditional abiotic environmental resource-based habitats, enclosures still imply restriction of movement due to their size compared to natural spaces; this restriction may act as a potential stressor (Morgan & Tromborg, 2007). In the present study, adrenocortical activity was negatively associated with natural behaviors and positively correlated with abnormal locomotion. Abnormal behaviors (stereotypies) have been largely studied in zoo-housed carnivores. They are defined as repetitive, unvarying activities with no obvious goal or function. R. E. Clubb (2001) suggested several hypotheses about the possible behavioral ecology role in stereotype development in carnivores. Some results revealed that instead of relating to foraging (e.g., hunting), as often assumed, carnivore stereotypy levels are significantly predicted by natural ranging behavior (e.g., home-range size and typical daily travel distances; R. Clubb & Mason, 2007). Considering that *T. tetradactyla* are also active hunters, future studies should focus on finding strategies to reduce repetitive locomotion and stimulate natural behaviors, for instance by increasing enclosure space. According to Dawkins (2004), noninvasive welfare assessment methods could reveal animals' wants. In this first study, interaction between levels of glucocorticoids (mediators of allostasis; see e.g., Korte et al., 2007) and natural behaviors in *T. tetradactyla* appear to indicate that the welfare of females is less compromised than that of males.

Finally, the present study offers the first longitudinal noninvasive assessment of *T. tetradactyla* activity patterns and adrenocortical activity using 24 hr video-surveillance technology and fecal glucocorticoid metabolite measurements. The significance of hormone-behavior relationships is reflected in the fact that high-level performance of a specific behavior during the day appears to trigger higher levels of endocrine adrenal function. Practical implications can be drawn from these analyses, which indicate that zoo-housed *T. tetradactyla* respond to environmental housing conditions by showing both natural and abnormal behaviors. Moreover, the technological approach used in this study shows great potential for the acquisition of additional data to refine housing conditions and reduce abnormal behavior and/or to test whether the latter disappears in larger enclosures. Knowing that the translocation of small numbers of individuals into suitable habitats is possible without adverse effects on resident anteaters (Rodrigues, Marinho-Filho, &

Santos, 2001), this type of study will strengthen the role of zoos as breeding centers to recolonize areas where species have become locally extinct.

## 5 | CONCLUSION

The findings of the present study on behavioral and adrenocortical activity in zoo-housed *T. tetradactyla* show that: (a) females exhibited lower total activity than males; (b) females exhibited nocturnal activity and males a diurnal pattern; (c) females exhibited less abnormal behaviors than males; and (d) females and males had similar FGM concentrations. Remarkably, individuals showed large differences in behavioral and adrenocortical activity.

Finally, the fact that we found a negative correlation between FGM concentrations and natural behaviors, and a positive correlation between FGM concentrations and abnormal behaviors, could imply that repetitive behaviors with no apparent function or goal demand higher adrenocortical activity in zoo-housed *T. tetradactyla*.

## CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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