Hormones and Behavior 141 (2022) 105152

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Sex, not social behavior, predicts fecal glucocorticoid metabolite concentrations in a facultatively social rodent, the highland tuco-tuco (*Ctenomys opimus*)

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ARTICLE INFO

Keywords:
Ctenomyidae
Behavior
Stress physiology
Sex effects
Facultative sociality
Adrenocorticotropic hormone
Corticosterone

ABSTRACT

Social relationships may influence circulating glucocorticoid levels, particularly in group-living species in which individuals regularly engage in interactions with conspecifics. The effects of such interactions appear to vary, with greater social contact being associated with increased glucocorticoid concentrations in some species but decreased concentrations in others. These distinct responses raise intriguing questions regarding relationships among social behavior, individual phenotypes, and glucocorticoid physiology. To explore such relationships in a free-living mammal with a dynamic social organization, we quantified variation in baseline glucocorticoids in a population of highland tuco-tucos (*Ctenomys opimus*) from Jujuy Province, Argentina. These subterranean rodents are facultatively social, with lone and group-living individuals regularly occurring within the same population. To assess potential endocrine correlates of this behavioral variability, we examined differences in baseline fecal glucocorticoid metabolite (fGCm) concentrations as a function of social group size and composition as well as several metrics of social behavior derived from social network analyses. Despite marked variability in social relationships among the 37 (12 male, 25 female) free-living tuco-tucos sampled, none of the measures of social behavior examined were significant predictors of variation in fGCm concentrations. In contrast, individual variation in glucocorticoid metabolites was best explained by sex, with males having higher fGCm concentrations than females. These analyses provide the first characterization of the glucocorticoid physiology of highland tuco-tucos and underscore the potential importance of intrinsic phenotypic factors (e.g., sex) in shaping glucocorticoid variation in free-living mammals.

1. Introduction

Glucocorticoid hormones play a central role in multiple physiological processes (McMahon et al., 1988; Bartolomucci, 2007; Vegiopoulos and Herzig, 2007; de Guia et al., 2014; Cain and Cidlowski, 2017). Glucocorticoid hormone concentrations in wild animals vary over multiple time scales and in response to multiple factors, including both intrinsic properties of individuals (e.g., circadian biology, sex, age: Touma and Palme, 2005; Sopinka et al., 2015) and the extrinsic conditions that they experience (e.g., photoperiod, food availability, weather conditions: de Bruijn and Romero, 2018). Interactions with conspecifics are expected to have significant effects on circulating glucocorticoids (Goymann and Wingfield, 2004; Creel et al., 2013), particularly in group-living species in which social contact is frequent but often varies with respect to the nature and function of specific encounters (Broom et al., 2009; Kutsukake, 2009). Consistent with this, relationships between social structure and glucocorticoid physiology vary, with greater social contact being associated with increased baseline glucocorticoid concentrations in some species (Rogovin et al., 2003; Raouf et al., 2006) but decreased baseline concentrations in others (Woodruff et al., 2013; Fürtbauer et al., 2014). These outcomes suggest that interactions between social behavior and baseline measures of glucocorticoids are complex and likely reflect variation in individual phenotypes as well as differences in the social environments in which conspecifics occur. As a result, efforts to understand relationships between social behavior and glucocorticoid physiology require detailed consideration of multiple intrinsic and extrinsic factors (Bonier et al., 2009).

Use of social network analyses to characterize interactions among

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https://doi.org/10.1016/j.yhbeh.2022.105152
Received 22 October 2021; Received in revised form 28 February 2022; Accepted 2 March 2022

conspecifics has revealed considerable and sometimes unexpected complexity in social relationships – particularly in species in which groups lack clear dominance hierarchies or other conspicuous forms of social structure (Kappeler et al., 2019; Smith and Pinter-Wollman, 2021; Sosa et al., 2021). Aspects of relationships that have been examined using network analyses include the centrality of an individual within its social group as well as the extent to which each animal is directly and indirectly connected to conspecifics (Wey et al., 2008; Krause et al., 2009; Whitehead, 2009). Collectively, these metrics provide a more comprehensive and nuanced description of an individual's social environment than do singular measures such as group size or composition. Despite increasing use of network metrics to characterize variability in social relationships, few studies have examined the effects of this variability on baseline glucocorticoid concentrations. Greater understanding of the effects of social behavior on glucocorticoid hormones is critical to elucidating the effects of social environment on homeostasis and allostasis in free-living animals.

Highland tuco-tucos (*Ctenomys opimus*) are subterranean rodents that are endemic to high elevation Puna habitats in Argentina, Bolivia, and Peru (Patton et al., 2015). Unlike most species of *Ctenomys* studied to date, highland tuco-tucos are social, meaning that multiple adults share a burrow system and subterranean nest site (Lacey, 2000; O'Brien et al., 2020). Studies of a population of *C. opimus* at Laguna de los Pozuelos, Jujuy Province, Argentina, have revealed considerable variability in individual social relationships. In particular, while some members of this population live in groups, others are solitary (O'Brien et al., 2020, 2021). This variation does not appear to reflect persistent individual level differences in behavior; instead, social relationships vary markedly over time, with a general tendency for individuals to live in larger social groups in successive years (O'Brien et al., 2021). Given this behavioral variability, studies of highland tuco-tucos provide an ideal opportunity to examine the role of the social environment in shaping glucocorticoid responses in a natural population of mammals.

As part of ongoing studies of the behavioral ecology of *C. opimus*, we quantified baseline glucocorticoid concentrations in relation to multiple aspects of the social behavior of the population of this species at Pozuelos. Based on studies of the congeneric, group-living colonial tuco-tuco (*C. sociabilis*: Woodruff et al., 2010, 2013), we predicted that more social members of our study population would display lower baseline concentrations of circulating glucocorticoids. To test this hypothesis, we combined field observations of group size and composition with both network analyses of social behavior and enzyme-linked immunosorbent assays (ELISAs) of glucocorticoid metabolites in fecal samples collected from the same individuals for which social relationships were characterized. As part of these efforts, we also conducted a biochemical validation study (Touma and Palme, 2005) to confirm that fecal metabolites provide robust measures of circulating glucocorticoid concentrations in highland tuco-tucos. In addition to providing the first characterization of the glucocorticoid physiology of *C. opimus*, our analyses generate insights into the effects of social relationships on differences in glucocorticoid hormone concentrations.

2. Material and methods

2.1. Study site

The population of highland tuco-tucos (*Ctenomys opimus*) studied was located in Monumento Natural Laguna de los Pozuelos, Jujuy Province, Argentina (−22.469347, −65.994279, WGS 84; elevation: 3600 m); this is the same population of *C. opimus* studied by O'Brien et al. (2020, 2021). The ~3 ha study site was located along the western bank of the Río Cincel in open, high elevation Puna habitat that was dominated by saltgrass (*Distichlis* sp.) and needlegrass (*Stipa* sp). The population of *C. opimus* at this location had been monitored annually from 2009 to 2014 and again from 2017 to 2019. Data for this study were collected from 23 December 2017 to 9 January 2018 (2017 field season) and from 21 December 2018 to 5 January 2019 (2018 field season). This corresponds to the early austral summer, which is the primary breeding season for members of the study population.

2.2. Animal capture and handling

All procedures involving live tuco-tucos were consistent with the guidelines of the American Society of Mammalogists for the use of wild mammals in research (Sikes and Animal Care and Use Committee of the American Society of Mammalogists, 2016) and had been approved by the Animal Care and Use Committee at the University of California, Berkeley. Live-trapping was conducted on the primary study site and in surrounding portions of the habitat. Individuals were captured using Tomahawk-style live traps baited with carrots (O'Brien et al., 2020, 2021). Open traps were placed on the soil surface near active-burrow entrances, as identified based on recently excavated mounds of dirt or direct visual observations of tuco-tucos using a given entrance. All trapping was conducted during daylight hours. Traps were monitored continuously and animals were retrieved immediately upon capture. Capture locations were recorded using a hand-held GPS unit (accuracy ~6 m). In addition, capture localities for tuco-tucos trapped on the primary study site were recorded using a Cartesian coordinate system (8 m × 8 m grid cells) established on the site each year prior to the start of data collection. The same grid was used to record the localities of individuals during radio-telemetric monitoring of spatial relationships among members of the study population (see below).

Upon first capture, each animal was permanently marked with a PIT-tag (IMI-1000, Bio Medic Data Systems, Inc., Seaford, DE) inserted subcutaneously at the nape of the neck. PIT-tags were read using a hand-held scanner (DAS 4000 Pocket Scanner, Bio Medic Data Systems Inc., Seaforth, DE). Sex and body mass were recorded for each individual captured. Data on body mass and pelage coloration were used to determine the age-class (subadult or adult) of each animal (Lacey et al., in prep). For females, reproductive status was assessed based on the appearance of the external genitalia (sexually receptive), the ability to palpate fetuses (pregnant), or the presence of enlarged mammae (lactating). In contrast, because testes of males in the study population never descend externally, the reproductive status of members of this sex could not be determined based on external appearance.

The study species is unusual within *Ctenomys* in that individuals spend considerable time above ground while foraging, making it possible to observe the animals directly (O'Brien et al., 2020). To facilitate visual identification of individuals, human hair dyes (e.g., Manic Panic semi-permanent hair color) were used to mark the fur of each animal captured with a unique combination of colored patches. In addition, all adults captured on the primary study site were fitted with radio transmitters (G3-1V transmitters, AVM Instrument Company, Colfax, CA) that were affixed using plastic cable ties as collars. The weight of the transmitter and collar together (~7 g) represented <5% of the body weight of adults in the study population (Sikes and Animal Care and Use Committee of the American Society of Mammalogists, 2016; O'Brien et al., 2020). Telemetric monitoring of individuals was used to characterize spatial and social relationships among members of the study population (see below).

2.3. Field collection of fecal samples

Captured tuco-tucos typically defecated during routine handling and marking procedures, providing a convenient means of collecting fecal samples directly from known individuals. To facilitate collection of fecal pellets, captured animals were transferred from traps to cloth bags that served to restrain individuals while also allowing us to gather pellets released during handling. All pellets from the same individual were placed in a cryogenic vial and frozen in liquid nitrogen until samples could be transferred to a −80 °C freezer. To allow characterization of circadian patterns of fecal glucocorticoid metabolite (fGCm) production

by members of the study population (Touma and Palme, 2005), the time of collection was recorded for each sample. Tuco-tucos that did not defecate during handling were placed in plastic rodent cages (one animal per cage), the bottoms of which were lined with dry grass. Cages were checked regularly until fecal pellets were produced (typically <30 min), after which pellets were stored as described above. Cages used to collect fecal pellets were emptied of grass and wiped clean between uses.

Once animal marking and fecal sample collection procedures had been completed, individuals were released at the point of capture. During the 2018 field season, a subset of 12 adults (6 males, 6 females) captured outside of the primary study site was retained in captivity for use in validation studies of glucocorticoid response (see *ACTH challenge*, below). These individuals were chosen based on visual confirmation that they occupied burrow systems outside of the primary study site; as a result, temporary removal of these animals from the population should not have affected social network relationships among individuals on the primary study site. Fecal samples were collected from most captive-housed tuco-tucos at the time of capture; the few individuals that did not defecate during initial handling were checked every 30 min for the first few hours following capture, until fecal samples were obtained. Fecal pellets collected at or shortly after capture were used to evaluate potential differences in fGCm concentrations among captive tuco-tucos prior to the start of our validation study.

2.4. Characterizing social environments of free-living tuco-tucos

The number of conspecifics with which an individual lives is a key component of its social environment. To quantify social unit size (the number of individuals comprising a spatially distinct group: O'Brien et al., 2021), we used radiotelemetry to document spatial relationships among members of the study population. Locations of radio-collared tuco-tucos were determined using R1000 receivers (Communications Specialists, Inc., Orange, CA) and 3-element hand-held Yagi antennas (AVM Instrument Company, Colfax, CA). Radio fixes were collected multiple times per day, typically between 0700 and 2000 h, with a minimum of 1 h between successive recordings. For each fix, we recorded the location of each collared individual to the nearest 0.5 m using the 8 m × 8 m grid system established on the study site. Analyses of data obtained for objects placed at known locations revealed this procedure to be accurate to within 0.5 m (O'Brien et al., 2020). At the end of each field season, individuals were recaptured, and their radio collars were removed.

Spatial relationships were quantified using 95% minimum convex polygons (MCPs) generated in the R package adehabitatHR (Calenge, 2015). Percent overlap between 95% MCPs was estimated for all pairs of individuals captured on the study site during the same field season. The resulting association matrix was analyzed in SOCPROG (Whitehead, 2009) to identify spatially distinct clusters of tuco-tucos. The fit between association matrices and the resulting clusters of individuals was assessed using the cophenetic correlation coefficient, with values ≥0.8 considered indicative of a strong correspondence between these data sets (Bridge, 1993). Clusters (i.e., social units) were identified using the maximum modularity criterion, which provides a measure of the degree to which a population is divided into spatially distinct subsets; values >0.3 are generally interpreted as evidence of significant spatial clustering (Newman, 2006; Whitehead, 2008). Clusters identified by these analyses were used to determine the size of the social unit to which each member of the study population belonged.

To quantify potential variability in relationships among members of the same social unit, we also examined five metrics of social network structure generated by SOCPROG. The specific metrics considered were network strength (the sum of an individual's associations), eigenvector centrality (how well an individual is associated, as well as how well their associates are associated), affinity (the weighted average strength of an individual's associations), reach (how well an individual is connected indirectly to other members of the population), and the clustering coefficient for the network (how well an individual's associates are associated). Detailed descriptions of these parameters are provided in Whitehead (2009). Evaluation of these metrics allowed us to examine quantitatively the effects of variable social relationships within social units on differences in baseline glucocorticoid concentrations (see below).

2.5. Captive housing

The 12 tuco-tucos (6 males, 6 females) retained for use in our adrenocorticotropic hormone (ACTH) challenge study (see below) were housed in captivity for 12–17 days (mean = 13.6 ± 1.9 days per animal) in a secure, weatherproof building located near the study site. All of these individuals were adults; all females were reproductively active (perforate vaginae) but none were detectably pregnant or lactating. In captivity, these animals were housed either singly (3 males, 3 females) or in pairs (3 male-female pairs). Pairs were identified based primarily on proximity of capture localities (mean distance between captures = 7.9 ± 7.7 m), such that pairs were likely to be members of the same social unit. Tuco-tucos housed alone were placed in ventilated plastic enclosures (33 × 25 × 20 cm), the bottoms of which were lined with shredded paper. Enclosures used to house pairs were roughly double in size (58 × 40 × 18 cm) but included a mesh partition that divided containers into two sections, each of which was lined with shredded paper. Partitions allowed members of a pair to interact (see and smell each other, huddle together against the mesh divider) while keeping them physically separated for collection of fecal samples. All enclosures used to house tuco-tucos included a short (16 cm) section of PVC pipe that served as a refuge. Enclosures were cleaned daily; dirty bedding was removed and the containers were wiped down with a 1:10 bleach solution, after which clean bedding was added. The animals were fed twice daily with ad libitum quantities of salt grass (*Distichlis* sp.) that had been collected near the study site, supplemented with carrots and corn. Individuals were weighed daily to detect potential changes in body condition associated with captive housing conditions.

2.6. ACTH challenge

To validate use of enzyme immunoassay (EIA) protocols for quantifying fGCm concentrations in highland tuco-tucos, an ACTH challenge study was conducted (Touma and Palme, 2005; Woodruff et al., 2010). To allow acclimation to captive housing conditions, the study subjects were held in captivity for a minimum of 1 week (mean = 8.6 ± 1.9 days) prior to injection with synthetic ACTH; the duration of the acclimation period varied due to differences in the dates on which individuals were captured. Once the acclimation period had ended, fecal pellets were collected from all captive tuco-tucos every 6 h (06:00, 12:00, 18:00, 24:00 h) for 48 h; these samples were used to examine circadian variation in fGCm production (Dickmeis, 2009; Reppert and Weaver, 2002). At the end of this initial sampling period, 8 individuals (4 males, 4 females) were each given an intramuscular injection of 12 IU/kg body mass of Cortrosyn (Amphastar Pharmaceuticals Inc., Rancho Cucamonga, CA); doses of Cortrosyn were determined based on protocols used in similar studies of wild rodents (Woodruff et al., 2010; Hammond et al., 2015). The remaining 4 tuco-tucos (2 males, 2 females) received an equivalent volume (based on individual body mass, Table 1) of 0.9% saline as a control. All animals were injected within a 30-min period between 0700 and 0730 on 29 December 2018. Treatment versus control tuco-tucos were balanced across housing conditions (Table 1). After injection, fecal samples were collected from all individuals at 6-h intervals (see above) for 72 h. The pellets collected were placed in cryogenic vials and frozen in liquid nitrogen until they could be transferred to a −80 °C freezer.

Table 1
Summary of phenotypic attributes and experimental conditions experienced by highland tuco-tucos used in the ACTH challenge experiment. For each treatment (injection with Cortrosyn versus injection with saline control), the number of adult males and females examined is indicated, as is the housing condition (single or paired), body mass, and injection volume for each individual.

Treatment	Housing	Sex	Mass (g)	Injection volume (mg)
Cortrosyn	Single	M	325	0.16
Cortrosyn	Single	M	360	0.17
Cortrosyn	Single	F	265	0.13
Cortrosyn	Single	F	240	0.12
Cortrosyn	Pair 1	M	355	0.17
Cortrosyn	Pair 1	F	265	0.13
Cortrosyn	Pair 2	M	235	0.11
Cortrosyn	Pair 2	F	235	0.11
Saline	Single	M	225	0.11
Saline	Single	F	200	0.10
Saline	Pair 3	M	385	0.18
Saline	Pair 3	F	170	0.08

2.7. Steroid extractions and glucocorticoid assays

Following the methods of Mateo and Cavigelli (2005) as modified by Woodruff et al. (2010), frozen fecal samples were thawed and then dried in an oven at 95 °C for 4 h. After drying, samples were crushed using a mortar and pestle. For each sample, a 0.2 g aliquot of the resulting powder was transferred to a microcentrifuge tube, to which 1.5 mL of 100% ethanol was added. Tubes were vortexed and then centrifuged at 3000*g* for 45 min. The supernatant was collected from each sample, transferred to a clean microcentrifuge tube, and then frozen at −20 °C until it was assayed.

Commercially available ELISA kits (Cayman Chemical Co., Ann Arbor, MI) were used to quantify fGCm concentrations. Because fGCm concentrations had not previously been characterized for highland tuco-tucos, initial analyses of a randomly selected subset of 24 samples were conducted using assay kits for both cortisol and corticosterone. Based on these preliminary analyses (see results), remaining samples were assayed only for corticosterone. Parallelism of fecal extracts with kit standards was determined using pooled samples from the pre-ACTH injection period (N = 8 individuals) as well as the post-ACTH injection period (N = 8 individuals). Pooled samples were serially diluted from 1:2 to 1:256, after which samples were assayed in triplicate. The resulting relationships between fGCm concentrations and antibody binding were compared to those for kit standards to confirm detection of corticosterone. These preliminary analyses indicated that a 1:16 dilution (sample:kit buffer) was within the binding range (20–80%) recommended by the kit manufacturer. Replicate samples for which the coefficient of variation exceeded 20% were reanalyzed (Woodruff et al., 2013).

2.8. Statistical analyses

Throughout the text, means are reported ±1 SD. All statistical tests were performed in R v. 4.0.4 (R Core Team, 2017). For standard two-sample tests, normality of the data was assessed using Shapiro-Wilks tests, after which parametric or non-parametric analyses were used, as appropriate. When sample sizes were unequal, effect sizes were calculated using Cohen's d or Hedges' g. Parallelism between fGCm concentrations for serially diluted samples and kit standards was assessed using ANCOVAs. To examine circadian variation in fGCm production, mean fecal corticosterone concentration was calculated for each 6-hour sampling interval during the 48 h prior to injection of captive tuco-tucos with Cortrosyn. Not all individuals defecated during each 6-hour sampling interval, with the result that sample sizes varied among the time points examined. As a result, a Skillings-Mack test was used to compare fGCm concentrations across sampling intervals; this test is a modification of Friedman's ANOVA that is robust to variation in sample sizes (Chatfield and Mander, 2009), making it appropriate for our data regarding fGCm concentrations in captive highland tuco-tucos. Analyses were conducted using the 'Skillings.Mack' package in R (Srisuradetchai, 2015).

As with analyses of circadian patterns of fGCm production, not all individuals in our ACTH challenge study defecated during each 6-hour sampling period, resulting in variable sample sizes across the time points examined. For comparisons of control versus Cortrosyn-injected tuco-tucos, fGCm concentrations were assessed by binning data from samples collected 0–6 hour post capture as well as those collected 0–12, 12–24, 24–36, 36–54, and 54–72 hour post-injection. No fecal samples were detected at 42- or 66-hour post-injection, resulting in larger time intervals (18 h) for the final two temporal periods examined. Further parsing the data to examine the effects of sex and housing on response to ACTH challenge resulted in smaller sample sizes (number of individuals) per treatment combination, which increased the impact of time points for which fecal samples were not available. As a result, for the ACTH challenge study, data regarding fGCm concentrations were binned into larger temporal intervals. The intervals examined were 0–6 hour post-capture plus 0–24, 24–48, and 48–72 hour post-injection; these intervals were chosen because each represents one 24-h period of data collection. Again, a Skillings-Mack test was used to compare fGCm concentrations for control versus Cortrosyn-injected tuco-tucos across all sampling intervals. In contrast, Mann-Whitney *U* tests were used to compare concentrations for these treatment groups during individual sampling intervals.

To explore relationships among fGCm concentrations and social behavior within the free-living population, we constructed linear mixed-effect models using the R package 'lme4' (Bates et al., 2007). Aspects of sociality examined included data on social unit composition (number of adults, number of adult males, number of adult females) as well as the five metrics obtained from social network analyses (strength, Eigenvector centrality, reach, clustering coefficient, affinity). Prior to model construction, a Q-Q plot was used to determine the underlying distribution of data regarding fGCm concentrations. Separate models were constructed for each metric of sociality examined. Each model included sex as a fixed effect, with animal ID and time of fecal sample collection included as random effects; because estimates of social network metrics were based on group-specific attributes, social group ID was not included in our models. All models were run with and without interactions between the predictor variables. The best-fit model for each set of predictor variables was identified using the Akaike information criterion (AIC). Post-hoc type III Wald Chi-square tests were then used to determine which explanatory variables in the best-fit model were significant predictors of fGCm concentrations; these post hoc tests were completed using the R package 'car' (Fox et al., 2007).

3. Results

3.1. Cortisol versus corticosterone

Analyses of fGCm concentrations from a randomly selected subset of 24 samples (10 males, 14 females) revealed concentrations that were above the manufacturer's reported limit of detection for corticosterone and cortisol (30 and 35 pg/mL at 80% binding, respectively). Sensitivity of the assay at 50% binding was 269 pg/mL for corticosterone and 85 pg/mL for cortisol. Paired comparisons of fGCm concentrations for the 24 tuco-tucos sampled revealed a significant tendency for corticosterone concentrations to be greater than those for cortisol (Wilcoxon signed-rank test, $V = 0$, $N = 24$, $P < 0.001$, Cohen's $d = 1.79$; Fig. 1a). Accordingly, all subsequent analyses examined corticosterone metabolites only. Intra- and inter-assay coefficients of variation for fecal corticosterone metabolites were 10.45% and 13.74% (N = 12 plates), respectively.

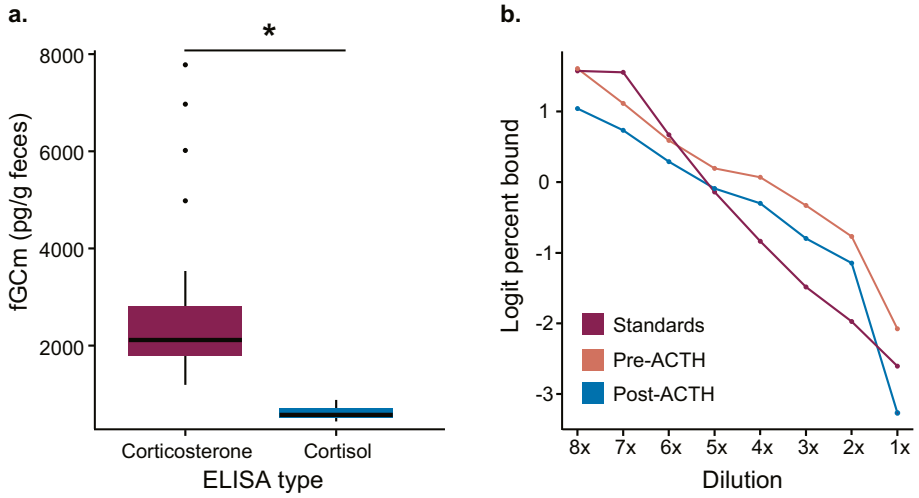

Fig. 1. Biochemical validation of assays for fecal glucocorticoid metabolite (fGCm) concentrations in highland tuco-tucos. In (a), concentrations of corticosterone versus cortisol metabolites are compared for a subset of 24 randomly selected members of the study population. In (b), logit-transformed slopes for 8 pooled, serially diluted samples collected pre- and post-injection with ACTH are compared with the slope for kit standards. Significant contrasts based on Wilcoxon Signed Rank tests are denoted with an asterisk (*).

3.2. Biochemical validation

The logit-transformed slopes for serial dilutions of pooled fecal samples did not differ from those for kit standards for either pre- or post-ACTH injection samples (ANCOVA, $F_{2,18} = 2.70$, $P = 0.09$, $\eta_p^2 = 0.23$; Fig. 1b), providing no evidence that detection of glucocorticoids differed between kit standards and fecal samples collected from the highland tuco-tucos.

3.3. Circadian variation

Among the 12 tuco-tucos housed in captivity, fGCm concentrations were generally lowest in the morning and increased over the course of the day (Fig. 2); this tendency was significant (Skillings-Mack test, $X^2 = 25.77$, df = 11, $P = 0.007$). In contrast, among free-living tuco-tucos captured on the primary study site, there was no correlation between fGCm concentrations and time of fecal sample collection (Kendall's rank correlation, $Z = -0.46$, $Tau = -0.05$, $P = 0.65$).

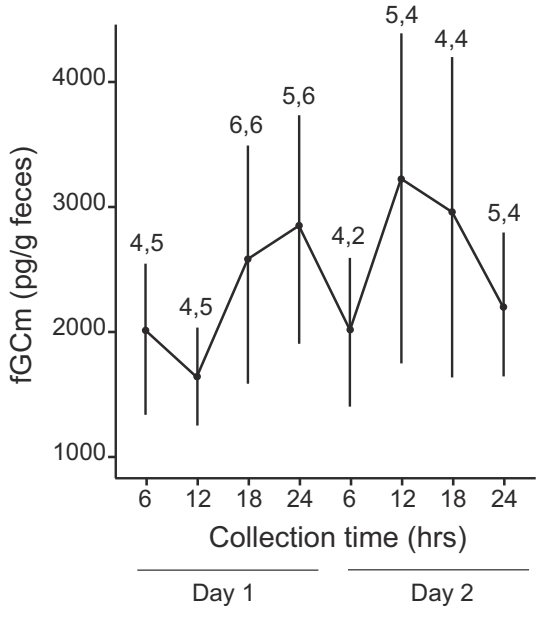

Fig. 2. Patterns of circadian variation in fGCm concentrations. Mean (± SD) concentrations are shown for 12 captive highland tuco-tucos from which samples were collected every 6 h for a total of 48 h. Sample sizes are indicated above each time point; the number of males sampled is given first, followed by the number of females sampled.

3.4. ACTH challenge

Analyses of fecal samples collected within the first 6 h that tuco-tucos were housed in captivity revealed no significant differences in fGCm concentrations between individuals later assigned to saline versus Cortrosyn treatment groups (Mann-Whitney *U* test, W = 34, P = 0.92, Hedges' g = 0.31; Fig. 3a). Similarly, there were no differences between initial fGCm concentrations for males versus females (Mann-Whitney U test, W = 57, P = 0.16, Cohen's d = 0.82) or between concentrations for individuals subsequently assigned to solitary versus paired housing (Mann-Whitney *U* test, W = 52, P = 0.31, Hedges' g = 0.29).

Post injection, there was significant variation in fGCm concentrations among Cortrosyn-treated but not among control individuals (Skillings-Mack tests, Cortrosyn: $X^2 = 33.24$, df = 14, $P = 0.002$, control: $X^2 = 3.09$, df = 7, P = 0.88). Comparisons of data from Cortrosyn-treated versus control individuals revealed significant differences between these groups for samples collected 0–12 hour post-injection (Mann-Whitney U test, W = 8, P = 0.03, Hedges' g = 0.85; Fig. 3a), with Cortrosyn-treated individuals having higher mean fGCm concentrations (3681.24 ± 2648.02 pg/g feces) than control individuals (1631.05 ± 709.00 pg/g feces). None of the other post-injection time intervals examined revealed significant differences between treatment groups (Mann-Whitney *U* tests; 12–24 hour post-injection: W = 20, P = 0.25, Hedges' g = 0.73; 24–36 hour post-injection: W = 37, P = 0.15, Hedges' g = 0.75; 36–54 hour post-injection: W = 16, P = 0.31, Hedges' g = 0.68; 54–72 hour post-injection: W = 29, P = 0.31, Hedges' g = 0.56).

When these data were re-analyzed using larger (24 h) temporal bins, no significant variation in fGCm concentrations was detected for either Cortrosyn-treated or control animals (Skillings-Mack tests, Cortrosyn: $X^2 = 30.72$, df = 23, P = 0.13, control: $X^2 = 12.39$, df = 10, P = 0.26). Despite this, significant differences between treatment groups were evident for samples collected 0–24 hour post-injection (Mann-Whitney *U* test, W = 63, P = 0.01, Hedges' g = 0.74; Fig. 3b), with Cortrosyn-treated individuals having higher mean fGCm concentrations (3768.37 ± 2561.43 pg/g feces) than control individuals (2119.91 ± 1008.62 pg/g feces). Neither of the other sample collection intervals examined revealed significant differences between treatment groups (24–48 hour post-injection: Mann-Whitney U test, W = 43, P = 0.16, Hedges' g = 0.69; 48–72 hour post-injection: Mann-Whitney *U* test, W = 81, P = 0.13, Hedges' g = 0.66).

When these larger temporal bins were used to analyze fGCm

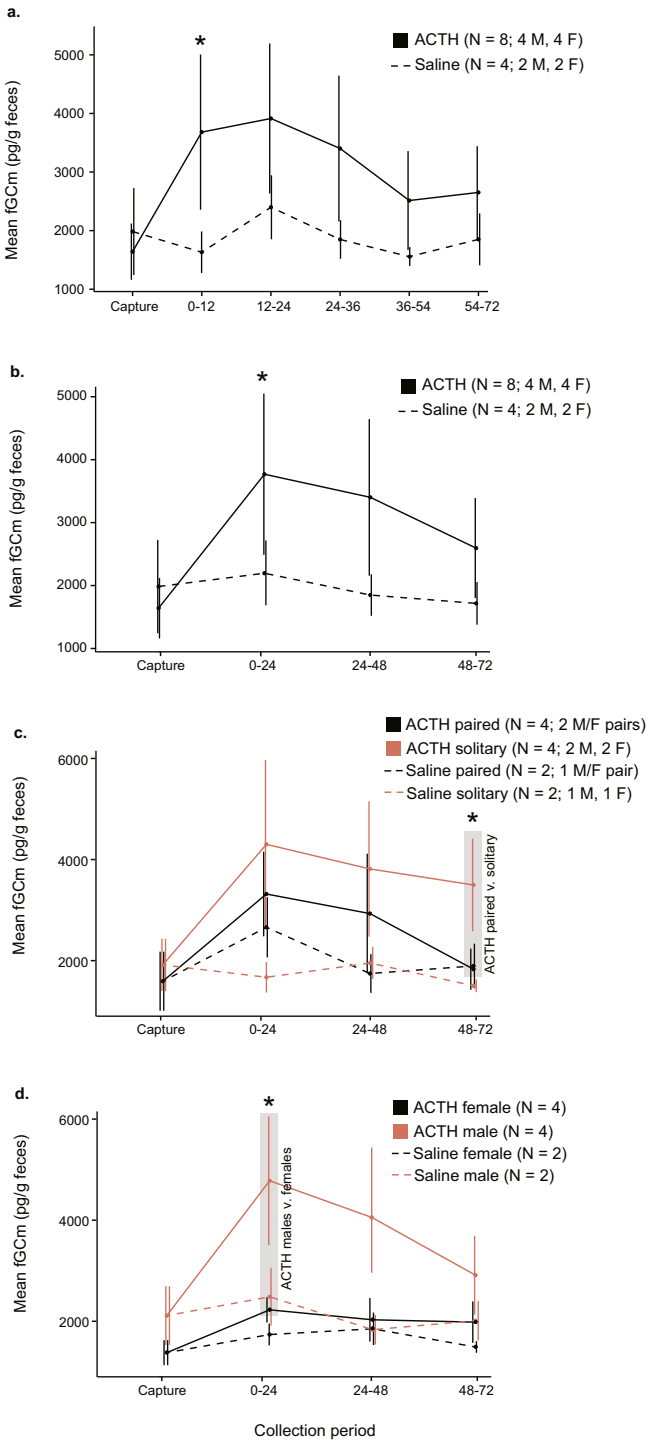

Fig. 3. Results of ACTH challenge study. In (a), comparisons of fGCm concentrations are shown for ACTH- and saline-treated highland tuco-tucos at capture and at the specified time intervals continuing until 72 h after injection. In (b), the same data are shown but with samples pooled over the larger time periods used to assess the effects of housing and sex on fGCm concentrations. In (c), these data are presented as a function of the housing condition (solitary or paired) under which individuals were held in captivity. In (d), these data are shown as a function of sex. For each panel, sample sizes are denoted in the upper right. Significant contrasts are denoted with an asterisk (*); details of the statistical analyses are given in the text.

concentrations as a function of captive housing conditions, no significant differences were detected between solitary versus pair-housed members of the control group for any of the time intervals examined (Mann-Whitney *U* tests, 0–24 hour post-injection: W = 24, P = 0.13, Hedges' g = 1.08; 24–48 h: W = 6, P = 0.69, Cohen's d = 0.30; 48–72 h: W = 18, P = 0.31, Cohen's d = 0.78; Fig. 3c). In contrast, among individuals injected with Cortrosyn, mean fGCm concentrations were significantly higher for solitary (3497.13 ± 1833.77 pg/g feces) versus pair-housed tuco-tucos (1892.78 ± 902.61 pg/g feces) for samples collected 48–72 hour post-injection (Mann-Whitney U test, W = 15, P = 0.03, Hedges' g = 1.03; Fig. 3c). No differences between solitary versus pair-housed Cortrosyn-treated individuals were detected for the other time intervals examined (Mann-Whitney *U* tests; 0–24 hour post-injection: W = 66, P = 0.78, Hedges' g = 0.38; 24–48 hour post-injection: Mann-Whitney U test, W = 25, P = 0.32, Hedges' g = 0.45).

When the same larger temporal bins were used to examine fGCm concentrations as a function of sex, no significant differences were detected between control males and females for any of the time intervals considered (Mann-Whitney *U* tests, 0–24 hour post-injection: W = 22, P = 0.25, Hedges' g = 0.91; 24–48 hour post-injection: W = 10, P = 0.69, Cohen's d = 0.03; 48–72 hour post-injection: W = 24, P = 0.13, Hedges' g = 0.95; Fig. 3d). In contrast, among tuco-tucos injected with Cortrosyn, fGCm concentrations for males (5243.97 ± 2938.18 pg/g feces) were significantly greater than those for females (2292.77 ± 578.52 pg/g feces) in samples collected 0–24 hour post-injection (Mann-Whitney U test, W = 129, P = 0.0005, Cohen's d = 1.39; Fig. 3d). No differences between Cortrosyn-treated males and females were detected for any of the other sampling intervals examined (Mann-Whitney U tests; 24–48 hour post-injection: W = 53, P = 0.08, Hedges' g = 0.90; 48–72 hour post-injection W = 87, P = 0.08, Hedges' g = 0.88).

3.5. *Social relationships among free-living tuco-tucos*

A total of 33 individuals (10 adult males, 20 adult females, 2 subadult males, 1 subadult female) were captured on the primary study site during the 2017 field season; a total of 17 individuals (4 adult males, 10 adult females, 3 subadult males, 0 subadult females) were captured on the primary site during the 2018 field season. Subsequent observations revealed no evidence of unmarked tuco-tucos on the primary site, suggesting that all animals resident in this area had been caught and identified with respect to sex, age, and (for females) reproductive status. As a result, our analyses should have captured information regarding all spatial relationships in which these individuals engaged. Sufficient spatial data for social network analyses plus fecal samples were available for a subset of 23 individuals (6 adult males, 14 adult females, 2 subadult males, 1 subadult female) from the 2017 field season and 14 individuals (4 adult males, 10 adult females, 0 subadult males, 0 subadult females) from the 2018 field season. A summary of the tuco-tucos captured as well as the spatial data and fecal samples obtained during each field season is provided in Supplementary Table 1. The number of subadults captured (N = 3) was too small to allow statistical evaluation of the effects of age. However, fGCm concentrations for these individuals (1114.14–2054.34 pg/g feces) fell within the range of values recorded for adults (387.86–3008.29 pg/g feces) and thus age was not considered a factor in subsequent analyses of social network metrics or glucocorticoid concentrations.

Social network analyses of association matrices based on 95% minimum convex polygons revealed the presence of both lone and group living adults in both years of the study (Supplementary Fig. S1). Cophenetic correlation coefficients for these analyses were >0.93, indicating a strong correspondence between the association matrix and the degree of overlap of individual home ranges. Maximum modularity was >0.56 based on an association matrix cut-off value of 0.08. Multiple distinct clusters of tuco-tucos were identified for each year of the study. Although mean social unit size did not differ significantly between years (2017: 5.0 ± 4.7 individuals per social unit, N = 5 units; 2018: 2.0 ± 1.2

individuals per social unit, N = 7 units; Mann Whitney *U* test, W = 22, P = 0.447, Hedges' g = 0.96), the range of social unit sizes was markedly greater in 2017 (N = 1–11 individuals) compared to 2018 (N = 1–4 individuals), which may have affected within-group social interactions. During the 2017 field season, all multi-animal social units (N = 3) contained adults of both sexes. In contrast, one of four multi-animal social units identified during the 2018 field season contained only adult females (Supplementary Fig. S1). Five of the individuals (1 adult male, 4 adult females) included in our analyses were captured in both field seasons; between-year comparisons of social unit composition revealed that none of these animals lived with the same conspecifics in both years of the study (Fig. 4) and thus data collected from these individuals in successive years were treated as independent for analyses of relationships between social behavior and fGCm concentrations.

Mean values for four (strength, reach, clustering coefficient, affinity) of the five social network metrics examined (Supplementary Table 2) differed significantly between years of the study (Mann-Whitney *U* tests, all P < 0.03; Supplementary Table 3); the sole exception was eigenvector centrality, mean values for which did not differ between 2017 and 2018 (Mann-Whitney *U* test, W = 181.5, N = 23, 14, P = 0.46, Hedges' g = 0.06). In contrast, no differences between mean values for males versus females were detected for any of the network metrics considered, either within years or when data from both years were pooled (Mann-Whitney U tests, all P > 0.08; Supplementary Table 3).

3.6. Effects of social relationships on glucocorticoids

When all data from free-living tuco-tucos were considered, mean fGCm concentrations did not differ between years of the study (2017: 1352.43 ± 597.89 pg/g feces, range = 387.86–3008.29, N = 23; 2018: 1316.64 ± 694.06 pg/g feces, range = 525.75–2818.99, N = 14; Mann-Whitney U test, W = 173, P = 0.72, Hedges' g = 0.06; Fig. 4a). Within years, mean fGCm concentrations for males and females did not differ significantly (Mann-Whitney U tests, both P > 0.08, 2017 Hedges' g = 0.62, 2018 Hedges' g = 1.09). However, when data for both years were pooled, the mean fGCm concentration for males (1662.72 ± 564.73 pg/g feces, N = 12) was significantly greater than that for females (1183.44 ± 604.41 pg/g feces, N = 25: Mann-Whitney U test, W = 75, P = 0.01, Hedges' g = 0.81; Fig. 4b). Among females, fGCm concentrations did not differ with reproductive status (i.e., pregnant, lactating, or neither; Kruskal-Wallis test, X^2 = 4.50, df = 2, P = 0.10). Based on these outcomes, glucocorticoid data from 2017 and 2018 were pooled for subsequent analyses and sex was included as a factor in linear models exploring the effects of social behavior on fGCm concentrations.

In both years of the study, fGCm concentrations varied within and among the social units identified by our social network analyses (Fig. 5). Use of linear mixed-effects models to explore relationships between measures of social unit size (number of adults), composition (number of adult males, number of adult females) and individual fGCm concentrations revealed that the best fit model included the interaction between sex and the number of adult males per social unit as predictor variables (AIC = 542.0275, df = 7; Table 2). Post-hoc tests indicated that sex was a significant explanatory variable in this model (Wald Chi-square type III, F = 11.09, df = 1, p = 0.0009). In contrast, neither overall social unit size nor the number of adult females per social unit appeared to affect individual fGCm concentrations.

Analyses of relationships between social network metrics and glucocorticoid metabolites revealed two models that, based on AIC values, were equally predictive of individual variation in fGCm concentrations (Table 2). One of these models included the interaction between sex and Eigenvector centrality as explanatory variables (AIC = 533.6091, df = 7; Table 2); the other included the interaction between sex and the clustering coefficient as explanatory variables (AIC = 535.3422, df = 7; Table 2). Models including the remaining three network metrics examined (strength, reach, affinity) received considerably less support (Table 2). Post-hoc tests of the two best-fit models revealed that sex was a significant explanatory variable in both (Wald Chi-square type III tests, F = 7.18, df = 1, p = 0.007; F = 14.86, df = 1, p = 0.0001). In contrast, neither Eigenvector centrality nor the clustering coefficient appeared to affect individual fGCm concentrations.

4. Discussion

Our analyses of the population of highland tuco-tucos at Laguna de los Pozuelos indicate that sex is an important determinant of fecal glucocorticoid metabolites in these animals, with males having higher baseline fGCm concentrations than females. In contrast, neither social unit composition (size, sex ratio) nor the social network metrics examined were significant predictors of differences in individual fGCm concentrations. Experimental treatment of a subset of individuals with Cortrosyn confirmed that measures of fGCm concentrations were responsive to exogenous ACTH, thereby validating use of these data to quantify baseline glucocorticoid levels in the study population. Among Cortrosyn-treated tuco-tucos, peak fGCm concentrations for males were significantly greater than those for females, again revealing an effect of sex on glucocorticoid response. Collectively, these findings suggest that sex may be more important than social environment in shaping hypothalamic-pituitary-adrenal axis (HPA) activity in highland tuco-

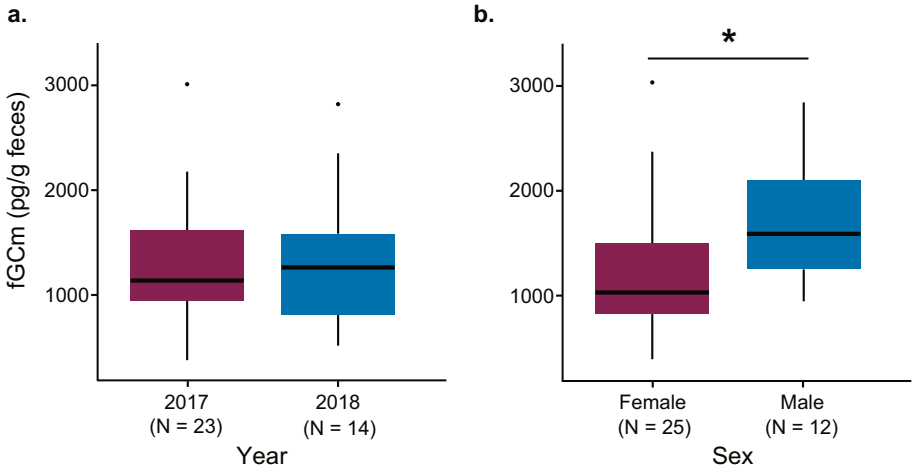

Fig. 4. Comparisons of fGCm concentrations for free-living highland tuco-tucos as a function of (a) year and (b) sex. Mean and quartile values are depicted for each subset of individuals examined; sample sizes for each comparison are shown. Significant contrasts (Mann-Whitney *U* tests) are indicated with asterisks (*).

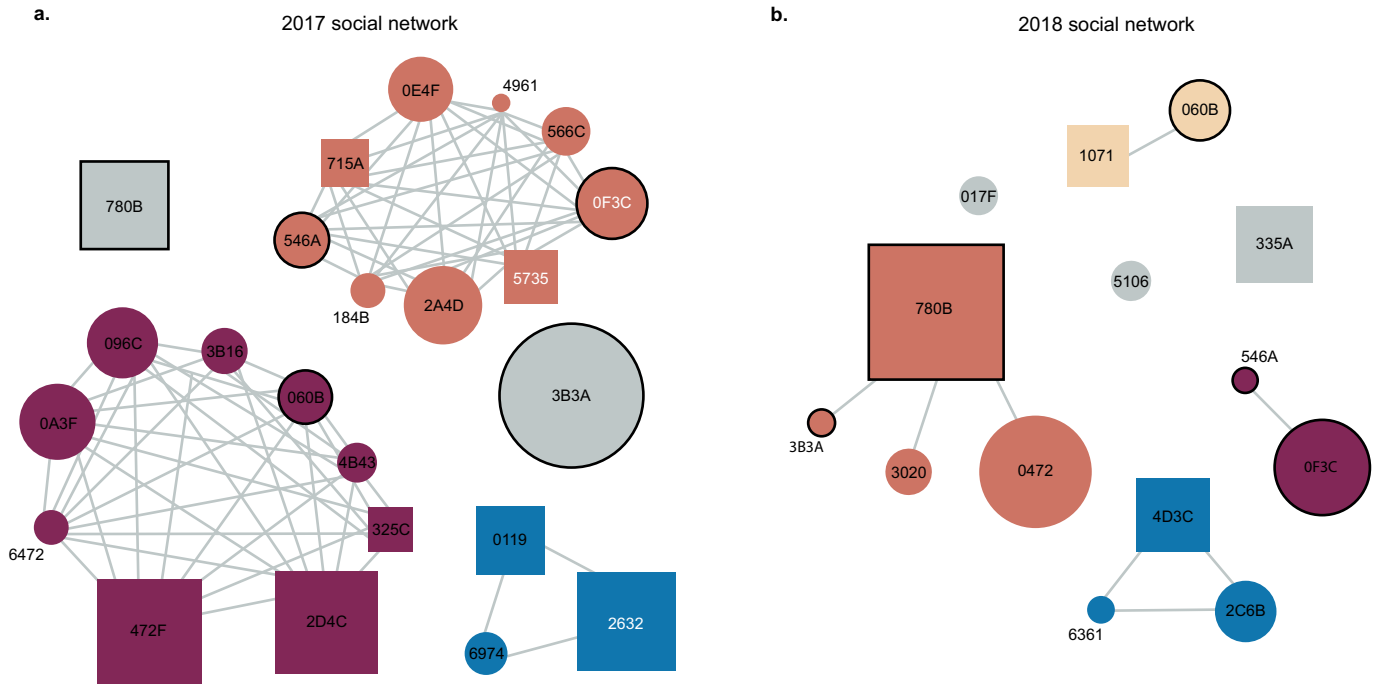

Fig. 5. Relative fGCm concentrations within social units of free-living highland tuco-tucos. Data are from individuals monitored during (a) 2017 and (b) 2018; colors are the same as those used in Fig. S1 to depict distinct social units. Males are indicated by squares and females are indicated with circles. Symbols used to denote individuals are sized proportionately to the fGCm concentration for each animal, with larger symbols indicating higher concentrations of these metabolites. The alphanumeric code associated with each symbol denotes the animal ID; black text indicates adults (N = 34) while white text denotes subadults (N = 3). Symbols for individuals captured in both years (N = 3) are outlined in black.

tucos. In addition to providing the first information regarding glucocorticoid physiology in this species, our analyses underscore the importance of considering both intrinsic and extrinsic factors when evaluating the factors affecting HPA response in free-living mammals.

It is possible that the number of free-living tuco-tucos sampled during this study was not sufficient to detect relationships between social network metrics and variation in fGCm concentrations. As is common in studies of wild mammals, our sample sizes were determined by the number of animals present on the study site each year and by our ability to collect the required behavioral and endocrine data from each of these individuals. Sample sizes for our analyses are comparable to those for other studies of relationships between social behavior and fGCm concentrations in wild populations of small mammals (e.g., Ebensperger et al., 2011; Woodruff et al., 2013). While larger sample sizes would potentially have increased our ability to detect effects of social network metrics on glucocorticoid metabolites, it seems unlikely that such expanded analyses would have altered the finding that sex was more important than social behavior in predicting differences in fGCm concentrations among members of our free-living study population.

4.1. Response to exogenous ACTH

Corticosterone was the predominant glucocorticoid metabolite in our study subjects, with peak response to exogenous ACTH occurring within 12 h of injection. Corticosterone is also the predominant baseline metabolite in the colonial tuco-tuco (*C. sociabilis*), although peak response in this species is slower, not occurring until 16–24 h after injection (Woodruff et al., 2010). Multiple factors may affect the time to peak response, including diet (White et al., 2015; Shively et al., 2020), life history stage (Lattin et al., 2012; Ensminger et al., 2014), and environmental conditions at the time of injection (Reeder and Kramer, 2005). In *C. sociabilis*, response to injection with Cortrosyn was assessed using captive-reared tuco-tucos that were fed the same diet on which they were typically maintained in the lab (Woodruff et al., 2010). In contrast, our analyses of highland tuco-tucos were based on individuals that had been housed in captivity for only ~2 weeks prior to injection, where they were fed a mixture of natural and recently introduced food items. These environmental changes may have influenced both digestive physiology (Karasov and Diamond, 1988; Hilton et al., 2000) and response to stressors (Romero, 2004; Dantzer et al., 2010), with associated impacts on the timing of maximum fecal glucocorticoid metabolite production. Nevertheless, peak response time for our study subjects was within the range reported for other rodent species (Montiglio et al., 2012; Sheriff et al., 2012), thereby underscoring the suitability of fecal metabolites as a source of information regarding baseline glucocorticoid concentrations in *C. opimus*.

Experimental administration of ACTH revealed potentially important differences in response as a function of sex and social environment. Among Cortrosyn-injected highland tuco-tucos, peak response was significantly greater for males than for females. No comparable difference was evident among control (saline injected) individuals, suggesting a possible interaction between sex and response to physiological challenge. Similarly, only Cortrosyn-injected highland tuco-tucos demonstrated an effect of housing, with solitary individuals displaying significantly greater fGCm concentrations than pair-housed individuals during our final sample collection interval (54–72 h post injection). This corresponds to the timeline for return to baseline fGCm concentrations in other rodent species (Dantzer et al., 2010; Woodruff et al., 2010; Hammond et al., 2015), suggesting that highland tuco-tucos housed in pairs returned to pre-manipulation glucocorticoid concentrations more quickly than conspecifics that were housed alone. More generally, the results of our ACTH manipulation suggest that both intrinsic (e.g., sex) and extrinsic (e.g., housing environment) factors may contribute to individual variation in fGCm concentrations in *C. opimus*.

4.2. Social relationships and glucocorticoids

The social environments experienced by members of our free-living

Table 2
Summary of the linear mixed-effect models used to examine variation in fGCm concentrations among members of the free-living population of highland tuco-tucos studied. Models based on measures of social unit size and composition are shown, as are models based on estimates of social network metrics. For each model considered, the predictor variables included are indicated, as are the associated degrees of freedom (DF) and Akaike Information Criterion (AIC). Best-fit models based on AIC values are indicated in bold. Models with differences in AIC values of ≤2 were interpreted as equally good at predicting variation in fGCm concentrations.

Type of explanatory variable	Equation	DF	AIC
Social unit size and composition	fGCm ~ sex + social unit size + (1|collection time) + (1|ID)	6	554.2386
	fGCm ~ sex + adult males + (1|collection time) + (1|ID)	6	551.9521
	fGCm ~ sex + adult females + (1|collection time) + (1|ID)	6	553.4476
	fGCm ~ sex * social unit size + (1|collection time) + (1|ID)	7	546.0504
	fGCm ~ sex * adult males + (1|collection time) + (1|ID)	**7**	**542.0275**
	fGCm ~ sex * adult females + (1|collection time) + (1|ID)	7	544.5902
Social network statistics	fGCm ~ sex + strength + (1|collection time) + (1|ID)	6	550.6
	fGCm ~ sex + eigenvector + (1|collection time) + (1|ID)	6	547.5827
	fGCm ~ sex + reach + (1|collection time) + (1|ID)	6	554.5715
	fGCm ~ sex + clustering + (1|collection time) + (1|ID)	6	548.0356
	fGCm ~ sex + affinity + (1|collection time) + (1|ID)	6	549.9214
	fGCm ~ sex * strength + (1|collection time) + (1|ID)	7	539.9269
	fGCm ~ sex * eigenvector + (1|collection time) + (1|ID)	**7**	**533.6091**
	fGCm ~ sex * reach + (1|collection time) + (1|ID)	7	547.6048
	fGCm ~ sex * clustering + (1|collection time) + (1|ID)	**7**	**535.3422**
	fGCm ~ sex * affinity + (1|collection time) + (1|ID)	7	536.1339

study population varied with respect to multiple parameters. For example, the number of highland tuco-tucos per social unit ranged from one to eleven, indicating the presence of both lone and group-living individuals in the population (O'Brien et al., 2020, 2021). Within social units comprised of multiple adults, the ratio of males to females differed. Finally, most of the social network metrics examined varied among groups, presumably reflecting differences in the range of social unit sizes present during each year of the study (Naug, 2009). Despite this variation, none of these measures of social environment emerged as significant predictors of fGCm concentrations in our free-living study population. This is in apparent contrast to data from our captive, Cortrosyn-injected study animals, among which fGCm concentrations were greater for lone versus pair-housed individuals. These distinct outcomes may reflect differences in the number or saliency of challenges experienced in each setting. For example, natural environments likely present a greater array of challenges than captive settings, with the result that effects of social environment on free-living animals may be more difficult to detect because they occur in concert with responses to other stimuli (Reeder and Kramer, 2005). In contrast, at least for our study subjects, the novelty of the captive environment may have rendered contact with conspecifics more important than it would be among free-living individuals (DeVries et al., 2003), resulting in a particularly pronounced effect of housing condition during our ACTH challenge study. Studies of multiple species have revealed different patterns of glucocorticoid response in captive versus free-living individuals (Calisi and Bentley, 2009), thereby underscoring both the importance of environmental conditions on glucocorticoid physiology and the need for additional analyses that compare data from captive and free-living conspecifics.

Our finding that social environment was not a significant predictor of fGCm concentrations in free-living highland tuco-tucos contrasts with data from *C. sociabilis*, for which studies of captive and free-living animals indicate that fGCm concentrations are significantly higher for lone versus group-living individuals (Woodruff et al., 2010, 2013). Although both *C. sociabilis* and *C. opimus* have been characterized as group-living (Lacey et al., 1997; O'Brien et al., 2020), the social organizations of these species differ in several potentially important ways, including the extent to which lone individuals are isolated from conspecifics. In particular, while lone *C. sociabilis* do not overlap spatially with other adults (Lacey et al., 1997; Lacey and Wieczorek, 2004), home ranges for lone *C. opimus* may overlap with those of multiple conspecifics (O'Brien et al., 2020), suggesting that the distinction between lone and group-living tuco-tucos is less extreme in the latter species. This difference may in turn contribute to interspecific differences in relationships between social environment and glucocorticoid regulation (Schoepf and Schradin, 2013; Hill et al., 2021). Future studies that combine analyses of naturally occurring variation in social behavior with experimental manipulation of specific behavioral parameters should help to clarify the role of social relationships in shaping baseline glucocorticoid concentrations in highland and other species of tuco-tucos.

4.3. Sex and glucocorticoids

The primary predictor of differences in fGCm concentrations among members of our study population was sex, with males having higher baseline corticosterone metabolite concentrations than females. This difference was evident in our analyses of free-living highland tuco-tucos as well as in the results of our ACTH challenge experiment. Sex-based differences in baseline glucocorticoids have been reported for several other species of rodents, including Syrian hamsters (Chelini et al., 2010), Siberian hamsters (Bilbo and Nelson, 2003), yellow-bellied marmots (Smith et al., 2012), and spiny mice (Nováková et al., 2008). In contrast, no intersexual differences were detected for degus (Soto-Gamboa et al., 2009) or arctic lemmings (Fauteux et al., 2017). Among those species for which sex-based differences are evident, the directionality of these relationships differs, with glucocorticoid concentrations being higher for males in some species but higher for females in others (Tilbrook et al., 2000; Touma and Palme, 2005). Thus, interactions between sex and glucocorticoid physiology likely reflect the effects of multiple factors, including environmental as well as phenotypic parameters (von der Ohe and Servheen, 2002).

Among the factors that may contribute to contrasting glucocorticoid levels in males versus females are intersexual differences in reproductive behavior. Our studies of free-living highland tuco-tucos were conducted during the spring breeding season for this species, raising questions regarding the role of male versus female behavior in shaping baseline fGCm concentrations in these animals. For example, in species in which males compete aggressively to gain access to females, the energetic demands of competition combined with the potential for injuries and the need to mount an associated immune response may render reproduction more challenging for males (Berger et al., 2005; Ancona et al., 2010), leading to the expectation that baseline glucocorticoids should be higher among members of this sex (Girard-Buttoz et al., 2014; Hudson et al., 2019). Potentially consistent with this, home ranges for males in our study population are larger than those for females (O'Brien et al., 2020, 2021) and aggressive interactions between males but not females are observed at home range boundaries (Lacey et al., unpublished data). Relationships between these differences in behavior and baseline fGCm concentrations, however, may not be straightforward; studies of other seasonally breeding mammals indicate that while males have higher baseline glucocorticoid concentrations during reproduction in some species (Lynch et al., 2002; Fichtel et al., 2007), in others it is females

with higher baseline concentrations (Schradin, 2008; Dantzer et al., 2010). Because our analyses were limited to data collected during the breeding season, we were unable to assess seasonal variation in fGCm concentrations or to examine intersexual differences in these levels during other portions of the year. Studies that examine temporal changes in baseline glucocorticoid concentrations within and between the sexes are needed to understand how these factors interact to shape the differences in fGCm concentrations reported here. Additionally, studies that examine the relationship between fGCm concentrations and sex steroids may also prove useful, particularly regarding how variation in concentrations of these hormones influence group dynamics (Dakin et al., 2021).

4.4. Implications for GC physiology

Although we had expected that differences in social environment – including differences in both social unit composition and social network metrics – would be important contributors to baseline glucocorticoid concentrations in our study population, none of the behavioral parameters examined emerged as significant predictors of fGCm concentrations. Instead, the only significant predictor of fGCm concentrations in our study animals was sex. Because analyses of fGCm concentrations in *C. sociabilis* did not include males (Woodruff et al., 2010, 2013), the relative contributions of sex versus social behavior have not been assessed for this species of tuco-tuco. More generally, few studies of free-living, non-primate populations have attempted to distinguish the effects of intrinsic factors such as sex from those of extrinsic variation in social environment. Those efforts that have considered social behavior have typically focused on specific forms of interactions such as position in a dominance hierarchy (Gesquiere et al., 2011; van Kesteren et al., 2012; reviewed in Creel, 2001 and Creel et al., 2013), rather than more general variation in social environment. An exception to this pattern is a study of common degus that, in keeping with our findings, revealed reproductive status to be more important than social context in predicting baseline fGCm concentrations in a free-living population of this burrow dwelling rodent (Ebensperger et al., 2011). Collectively, available data suggest that the effects of social environment on glucocorticoid physiology are complex and are likely to vary situationally as well as among species. Future studies that combine experimental manipulation of extrinsic conditions with analyses of fGCm variation among free-living animals should help to clarify the roles of intrinsic phenotypic differences versus social environment in shaping glucocorticoid physiology.

5. Conclusion

Our analyses of free-living highland tuco-tucos revealed that sex was a significant predictor of individual differences in fGCm concentrations. In contrast, although multiple aspects of an individual's social environment varied within our study population, none of the behavioral metrics examined were associated with variation in fGCm concentrations. Treatment of captive highland tuco-tucos with synthetic ACTH indicated that corticosterone is the dominant glucocorticoid metabolite in this species and confirmed that fGCm concentrations are responsive to external conditions, suggesting that the absence of relationships between behavioral parameters and glucocorticoid metabolites was not due to limitations of the EIA procedures used. Although we expect that larger samples sizes would have increased our ability to detect such relationships, it seems unlikely that this difference in outcomes would have altered the relative importance of sex versus social behavior in determining individual fGCm concentrations. Our findings differ from those for the only other social species of tuco-tuco (*C. sociabilis*) for which glucocorticoid data are available, in which social environment is associated with significant differences in fGCm concentrations (Woodruff et al., 2013). Thus, in addition to providing the first characterization of glucocorticoid physiology in highland tuco-tucos, our results – particularly when compared to those for *C. sociabilis* — underscore both the complexity of the factors affecting HPA function and the importance of considering both intrinsic and extrinsic variables when exploring glucocorticoid variation in free-living mammals.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2022.105152.

Declaration of competing interest

None.

Acknowledgments

For permits to conduct fieldwork, we thank the Delegacion Técnica NOA de Parques Nacionales Argentinas, particularly Maria Elena Sanchez and Juliana de Gracia. Permission to export samples for endocrine analyses was provided by the Dirección Nacional de Biodiversidad in Buenos Aires, Argentina. For housing and logistic support, we thank the Intendente and park guards at Monumento Natural Laguna de los Pozuelos, in particular Cristian Mamani, Walter Arias, Marcos Bernuci, and Sergio Ariel Carzon. For assistance in the field, we thank Florencia Dosil, Juan Amaya, and Josefina Menendez. For assistance with sample storage prior to export, we thank Pablo A. Cuello and Ricardo A. Ojeda from IADIZA-CONICET in Mendoza, Argentina. Mattina Alonge kindly provided advice regarding ELISA assays. This work was supported by the American Society of Mammalogists, the Animal Behavior Society, and the Museum of Vertebrate Zoology.

References

Ancona, S., Drummond, H., Zaldívar-Rae, J., 2010. Male whiptail lizards adjust energetically costly mate guarding to male–male competition and female reproductive value. Anim. Behav. 79 (1), 75–82. https://doi.org/10.1016/j.anbehav.2009.10.005.

Bartolomucci, A., 2007. Social stress, immune functions and disease in rodents. Front. Neuroendo. 28 (1), 28–49. https://doi.org/10.1016/j.yfrne.2007.02.001.

Bates, D., Sarkar, D., Bates, M.D., Matrix, L., 2007. In: The lme4 package. R package version, 2(1), p. 74.

Berger, S., Martin II, L.B., Wikelski, M., Romero, L.M., Kalko, E.K., Vitousek, M.N., Rödl, T., 2005. Corticosterone suppresses immune activity in territorial Galapagos marine iguanas during reproduction. Horm. Behav. 47 (4), 419–429. https://doi.org/10.1016/j.yhbeh.2004.11.011.

Bilbo, S.D., Nelson, R.J., 2003. Sex differences in photoperiodic and stress-induced enhancement of immune function in Siberian hamsters. Brain Behav. Immun. 17 (6), 462–472. https://doi.org/10.1016/S0889-1591(03)00063-1.

Bonier, F., Martin, P.R., Moore, I.T., Wingfield, J.C., 2009. Do baseline glucocorticoids predict fitness? Trends Ecol. Evol. 24 (11), 634–642. https://doi.org/10.1016/j.tree.2009.04.013.

Bridge, P.D., 1993. Classification. In: Fry, J.C. (Ed.), Biological Data Analysis. Oxford University Press, Oxford, pp. 219–242.

Broom, M., Koenig, A., Borries, C., 2009. Variation in dominance hierarchies among group-living animals: modeling stability and the likelihood of coalitions. Behav. Ecol. 20 (4), 844–855. https://doi.org/10.1093/beheco/arp069.

Cain, D.W., Cidlowski, J.A., 2017. Immune regulation by glucocorticoids. Nat. Rev. Immun. 17 (4), 233–247. https://doi.org/10.1038/nri.2017.1.

Calenge, C., 2015. Home range estimation in R: the adehabitatHR package. https://cran.rproject.org/web/packages/adehabitatHR/vignettes/adehabitatHR.pdf.

Calisi, R.M., Bentley, G.E., 2009. Lab and field experiments: are they the same animal? Horm. Behav. 56 (1), 1–10. https://doi.org/10.1016/j.yhbeh.2009.02.010.

Chatfield, M., Mander, A., 2009. The Skillings-Mack test (Friedman test when there are missing data). Stata J. 9 (2), 299–305. https://doi.org/10.1177/1536867X0900900208.

Chelini, M.O.M., Otta, E., Yamakita, C., Palme, R., 2010. Sex differences in the excretion of fecal glucocorticoid metabolites in the Syrian hamster. J. Comp. Phys. B 180 (6), 919–925. https://doi.org/10.1007/s00360-010-0467-9.

Creel, S., 2001. Social dominance and stress hormones. Trends Ecol. Evol. 16 (9), 491–497. https://doi.org/10.1016/S0169-5347(01)02227-3.

Creel, S., Dantzer, B., Goymann, W., Rubenstein, D.R., 2013. The ecology of stress: effects of the social environment. Func. Ecol. 27 (1), 66–80. https://doi.org/10.1111/j.1365-2435.2012.02029.x.

Dakin, R., Moore, I.T., Horton, B.M., Vernasco, B.J., Ryder, T.B., 2021. Testosterone-mediated behaviour shapes the emergent properties of social networks. J. Anim. Ecol. 90 (1), 131–142. https://doi.org/10.1111/1365-2656.13305.

Dantzer, B., McAdam, A.G., Palme, R., Fletcher, Q.E., Boutin, S., Humphries, M.M., Boonstra, R., 2010. Fecal cortisol metabolite levels in free-ranging North American red squirrels: assay validation and the effects of reproductive condition. Gen. Comp. Endo. 167 (2), 279–286. https://doi.org/10.1016/j.ygcen.2010.03.024.

de Bruijn, R., Romero, L.M., 2018. The role of glucocorticoids in the vertebrate response to weather. Gen. Comp. Endo. 269, 11–32. https://doi.org/10.1016/j.ygcen.2018.07.007.
de Guia, M., Rose, A.J., Herzig, S., 2014. Glucocorticoid hormones and energy homeostasis. Horm. Molec. Bio. Clinic. Invest. 19 (2), 117–128. https://doi.org/10.1515/hmbci-2014-0021.
DeVries, A.C., Glasper, E.R., Detillion, C.E., 2003. Social modulation of stress responses. Phys. Behav. 79 (3), 399–407. https://doi.org/10.1016/S0031-9384(03)00152-5.
Dickmeis, T., 2009. Glucocorticoids and the circadian clock. J Endo. 200 (1), 3–22. https://doi.org/10.1677/joe-08-0415.
Ebensperger, L.A., Ramírez-Estrada, J., León, C., Castro, R.A., Tolhuysen, L.O., Sobrero, R., Hayes, L.D., 2011. Sociality, glucocorticoids and direct fitness in the communally rearing rodent, Octodon degus. Horm. Behav. 60 (4), 346–352. https://doi.org/10.1016/j.yhbeh.2011.07.002.
Ensminger, D.C., Somo, D.A., Houser, D.S., Crocker, D.E., 2014. Metabolic responses to adrenocorticotropic hormone (ACTH) vary with life-history stage in adult male northern elephant seals. Gen. Comp. Endo. 204, 150–157. https://doi.org/10.1016/j.ygcen.2014.04.024.
Fauteux, D., Gauthier, G., Berteaux, D., Bosson, C., Palme, R., Boonstra, R., 2017. Assessing stress in arctic lemmings: fecal metabolite levels reflect plasma free corticosterone levels. Phys. Biochem. Zool. 90 (3), 370–382. https://doi.org/10.1086/691337.
Fichtel, C., Kraus, C., Ganswindt, A., Heistermann, M., 2007. Influence of reproductive season and rank on fecal glucocorticoid levels in free-ranging male Verreaux's sifakas (Propithecus verreauxi). Horm. Behav. 51 (5), 640–648. https://doi.org/10.1016/j.yhbeh.2007.03.005.
Fox, J., Friendly, G.G., Graves, S., Heiberger, R., Monette, G., Nilsson, H., Suggests, M.A. S.S., 2007. The car package. R Foundation for Statistical Computing.
Fürtbauer, I., Heistermann, M., Schülke, O., Ostner, J., 2014. Low female stress hormone levels are predicted by same-or opposite-sex sociality depending on season in wild Assamese macaques. Psychoneuroendo. 48, 19–28. https://doi.org/10.1016/j.psyneuen.2014.05.022.
Gesquiere, L.R., Learn, N.H., Simao, M.C.M., Onyango, P.O., Alberts, S.C., Altmann, J., 2011. Life at the top: rank and stress in wild male baboons. Science 333 (6040), 357–360. https://doi.org/10.1126/science.1207120.
Girard-Buttoz, C., Heistermann, M., Rahmi, E., Agil, M., Fauzan, P.A., Engelhardt, A., 2014. Costs of mate-guarding in wild male long-tailed macaques (Macaca fascicularis): physiological stress and aggression. Horm. Behav. 66 (4), 637–648. https://doi.org/10.1016/j.yhbeh.2014.09.003.
Goymann, W., Wingfield, J.C., 2004. Allostatic load, social status and stress hormones: the costs of social status matter. Anim. Behav. 67 (3), 591–602. https://doi.org/10.1016/j.anbehav.2003.08.007.
Hammond, T.T., Palme, R., Lacey, E.A., 2015. Contrasting stress responses of two co-occurring chipmunk species (Tamias alpinus and T. speciosus). Gen. Comp. Endo. 211, 114–122. https://doi.org/10.1016/j.ygcen.2014.11.013.
Hill, D.L., Pillay, N., Schradin, C., 2021. Glucocorticoid levels predict subsequent social tactic in females of a facultatively social mammal. Func. Ecol. 35 (3), 650–662. https://doi.org/10.1111/1365-2435.13744.
Hilton, G.M., Furness, R.W., Houston, D.C., 2000. The effects of diet switching and mixing on digestion in seabirds. Func. Ecol. 14 (2), 145–154. https://doi.org/10.1046/j.1365-2435.2000.00403.x.
Hudson, S.B., Robertson, M.W., Wilcoxen, T.E., 2019. Fecal glucocorticoid response to periodic social stress in male green anoles,Anolis carolinensis. Copeia 107 (4), 653–660. https://doi.org/10.1643/CP-19-192.
Kappeler, P.M., Clutton-Brock, T., Shultz, S., Lukas, D., 2019. Social complexity: patterns, processes, and evolution. Behav. Ecol. Sociobio. 73, 5. https://doi.org/10.1007/s00265-018-2613-4.
Karasov, W.H., Diamond, J.M., 1988. Interplay between physiology and ecology in digestion. BioSci. 38 (9), 602–611.
Krause, J., Lusseau, D., James, R., 2009. Animal social networks: an introduction. Behav. Ecol. Sociobio. 63 (7), 967–973. https://doi.org/10.1007/s00265-009-0747-0.
Kutsukake, N., 2009. Complexity, dynamics and diversity of sociality in group-living mammals. Ecol. Res. 24 (3), 521–531. https://doi.org/10.1007/s11284-008-0563-4.
Lacey, E.A., 2000. Life Underground: The Biology of Subterranean Rodents. University of Chicago Press.
Lacey, E.A., Braude, S.H., Wieczorek, J.R., 1997. Burrow sharing by colonial tuco-tucos (Ctenomys sociabilis). J. Mamm. 78 (2), 556–562. https://doi.org/10.2307/1382907.
Lacey, E.A., Wieczorek, J.R., 2004. Kinship in colonial tuco-tucos: evidence from group composition and population structure. Behav. Ecol. 15 (6), 988–996. https://doi.org/10.1093/beheco/arh104.
Lattin, C.R., Bauer, C.M., de Bruijn, R., Romero, L.M., 2012. Hypothalamus–pituitary–adrenal axis activity and the subsequent response to chronic stress differ depending upon life history stage. Gen. Comp. Endo. 178 (3), 494–501. https://doi.org/10.1016/j.ygcen.2012.07.013.
Lynch, J.W., Ziegler, T.E., Strier, K.B., 2002. Individual and seasonal variation in fecal testosterone and cortisol levels of wild male tufted capuchin monkeys,Cebus apella nigritus. Horm. Behav. 41 (3), 275–287. https://doi.org/10.1006/hbeh.2002.1772.
Mateo, J.M., Cavigelli, S.A., 2005. A validation of extraction methods for noninvasive sampling of glucocorticoids in free-living ground squirrels. Phys. Biochem. Zool. 78 (6), 1069–1084. https://doi.org/10.1086/432855.
McMahon, M., Gerich, J., Rizza, P., 1988. Effects of glucocorticoids on carbohydrate metabolism. Diabetes Metab. Rev. 4 (1), 17–30. https://doi.org/10.1002/dmr.5610040105.
Montiglio, P.O., Pelletier, F., Palme, R., Garant, D., Réale, D., Boonstra, R., 2012. Noninvasive monitoring of fecal cortisol metabolites in the eastern chipmunk (Tamias striatus): validation and comparison of two enzyme immunoassays. Phys. Biochem. Zool. 85 (2), 183–193. https://doi.org/10.1086/664592.
Naug, D., 2009. Structure and resilience of the social network in an insect colony as a function of colony size. Behav. Ecol. Sociobio. 63 (7), 1023–1028. https://doi.org/10.1007/s00265-009-0721-x.
Newman, M.E., 2006. Modularity and community structure in networks. Proc. Natl. Acad. Sci. USA 103, 8577–8582. https://doi.org/10.1073/pnas.0601602103.
Nováková, M., Palme, R., Kutalová, H., Janský, L., Frynta, D., 2008. The effects of sex, age and commensal way of life on levels of fecal glucocorticoid metabolites in spiny mice (Acomys cahirinus). Phys. Behav. 95 (1–2), 187–193. https://doi.org/10.1016/j.physbeh.2008.05.017.
O'Brien, S.L., Tammone, M.N., Cuello, P.A., Lacey, E.A., 2020. Facultative sociality in a subterranean rodent, the highland tuco-tuco (Ctenomys opimus). Bio. J. Linn. Soc. 129 (4), 918–930. https://doi.org/10.1093/biolinnean/blaa011.
O'Brien, S.L., Tammone, M.N., Cuello, P.A., Lacey, E.A., 2021. Multi-year assessment of variability in spatial and social relationships in a subterranean rodent, the highland tuco-tuco (Ctenomys opimus). Behav. Ecol. Sociobio. 75 (6), 1–13. https://doi.org/10.1007/s00265-021-03034-z.
Patton, J.L., Pardiñas, U.F., D'Elía, G., 2015. Mammals of South America, Vol. 2 Rodents. University of Chicago Press, Chicago.
R Core Team, . R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. . URL. https://www.R-project.org/.
Raouf, S.A., Smith, L.C., Brown, M.B., Wingfield, J.C., Brown, C.R., 2006. Glucocorticoid hormone levels increase with group size and parasite load in cliff swallows. Anim. Behav. 71 (1), 39–48. https://doi.org/10.1016/j.anbehav.2005.03.027.
Reeder, D.M., Kramer, K.M., 2005. Stress in free-ranging mammals: integrating physiology, ecology, and natural history. J. Mamm. 86 (2), 225–235. https://doi.org/10.1644/BHE-003.1.
Reppert, S.M., Weaver, D.R., 2002. Coordination of circadian timing in mammals. Nature 418 (6901), 935–941. https://doi.org/10.1038/nature00965.
Rogovin, K., Randall, J.A., Kolosova, I., Moshkin, M., 2003. Social correlates of stress in adult males of the great gerbil, Rhombomys opimus, in years of high and low population densities. Horm. Behav. 43 (1), 132–139. https://doi.org/10.1016/S0018-506X(02)00028-4.
Romero, L.M., 2004. Physiological stress in ecology: lessons from biomedical research. Trends Ecol. Evol. 19 (5), 249–255. https://doi.org/10.1016/j.tree.2004.03.008.
Schoepf, I., Schradin, C., 2013. Endocrinology of sociality: comparisons between sociable and solitary individuals within the same population of African striped mice. Horm. Behav. 64 (1), 89–94. https://doi.org/10.1016/j.yhbeh.2013.04.011.
Schradin, C., 2008. Seasonal changes in testosterone and corticosterone levels in four social classes of a desert dwelling sociable rodent. Horm. Behav. 53 (4), 573–579. https://doi.org/10.1016/j.yhbeh.2008.01.003.
Sheriff, M.J., Wheeler, H., Donker, S.A., Krebs, C.J., Palme, R., Hik, D.S., Boonstra, R., 2012. Mountain-top and valley-bottom experiences: the stress axis as an integrator of environmental variability in arctic ground squirrel populations. J. Zool. 287 (1), 65–75. https://doi.org/10.1111/j.1469-7998.2011.00888.x.
Shively, C.A., Appt, S.E., Chen, H., Day, S.M., Frye, B.M., Shaltout, H.A., Register, T.C., 2020. Mediterranean diet, stress resilience, and aging in nonhuman primates. Neurobio. Stress 13, 100254. https://doi.org/10.1016/j.ynstr.2020.100254.
Sikes, R.S., Animal Care and Use Committee of the American Society of Mammalogists, 2016. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. J. Mamm. 97 (3), 663–688. https://doi.org/10.1093/jmammal/gyw078.
Smith, J.E., Monclús, R., Wantuck, D., Florant, G.L., Blumstein, D.T., 2012. Fecal glucocorticoid metabolites in wild yellow-bellied marmots: experimental validation, individual differences and ecological correlates. Gen. Comp. Endo. 178 (2), 417–426. https://doi.org/10.1016/j.ygcen.2012.06.015.
Smith, J.E., Pinter-Wollman, N., 2021. Observing the unwatchable: integrating automated sensing, naturalistic observations and animal social network analysis in the age of big data. J. Anim. Ecol. 90 (1), 62–75. https://doi.org/10.1111/1365-2656.13362.
Sopinka, N.M., Patterson, L.D., Redfern, J.C., Pleizier, N.K., Belanger, C.B., Midwood, J. D., Cooke, S.J., 2015. Manipulating glucocorticoids in wild animals: basic and applied perspectives. Cons. Phys. 3 (1), 1–16. https://doi.org/10.1093/conphys/cov031.
Sosa, S., Jacoby, D.M., Lihoreau, M., Sueur, C., 2021. Animal social networks: towards an integrative framework embedding social interactions, space and time. Methods Ecol. Evol. 12, 4–9. https://doi.org/10.1111/2041-210X.13539.
Soto-Gamboa, M., Gonzalez, S., Hayes, L.D., Ebensperger, L.A., 2009. Validation of a radioimmunoassay for measuring fecal cortisol metabolites in the hystricomorph rodent, Octodon degus. J. Exp. Zool. Part A: Ecol. Gen. Phys. 311 (7), 496–503. https://doi.org/10.1002/jez.546.
Srisuradetchai, P., 2015. The Skillings-Mack Test Statistic for Block Designs With Missing Observations. R Foundation for Statistical Computing.
Tilbrook, A.J., Turner, A.I., Clarke, I.J., 2000. Effects of stress on reproduction in non-rodent mammals: the role of glucocorticoids and sex differences. Rev. Repro. 5 (2), 105–113. https://doi.org/10.1530/ror.0.0050105.
Touma, C., Palme, R., 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. Ann. N. Y. Acad. Sci. 1046 (1), 54–74. https://doi.org/10.1196/annals.1343.006.
Vegiopoulos, A., Herzig, S., 2007. Glucocorticoids, metabolism and metabolic diseases. Mol. Cell. Endo. 275 (1–2), 43–61. https://doi.org/10.1016/j.mce.2007.05.015.
van Kesteren, F., Sillero-Zubiri, C., Millar, R., Argaw, K., Macdonald, D.W., Paris, M., 2012. Sex, stress and social status: patterns in fecal testosterone and glucocorticoid metabolites in male Ethiopian wolves. Gen. Comp. Endo. 179 (1), 30–37. https://doi.org/10.1016/j.ygcen.2012.07.016.

von der Ohe, C.G., Servheen, C., 2002. Measuring stress in mammals using fecal glucocorticoids: opportunities and challenges. Wildlife Soc. Bull. 1215–1225.
Wey, T., Blumstein, D.T., Shen, W., Jordán, F., 2008. Social network analysis of animal behaviour: a promising tool for the study of sociality. Anim. Behav. 75 (2), 333–344. https://doi.org/10.1016/j.anbehav.2007.06.020.
White, B.C., Taylor, S.R., Franklin, J.A., Burns, R., Kozlowski, C., 2015. Faecal glucocorticoid concentrations during ACTH challenge tests in captive grizzly bears (Ursus arctos horribilus) and polar bears (Ursus maritimus). J. Zoo Aqua. Res. 3 (2), 59–62.
Whitehead, H., 2008. Analyzing Animal Societies: Quantitative Methods for Vertebrate Social Analysis. University of Chicago Press, Chicago.
Whitehead, H., 2009. SOCPROG programs: analysing animal social structures. Behav. Ecol. Sociobio. 63 (5), 765–778. https://doi.org/10.1007/s00265-008-0697-y.
Woodruff, J.A., Lacey, E.A., Bentley, G., 2010. Contrasting fecal corticosterone metabolite levels in captive and free-living colonial tuco-tucos (Ctenomys sociabilis). J. Exp. Zool. Part A: Ecol. Gen. Phys. 313 (8), 498–507. https://doi.org/10.1002/jez.621.
Woodruff, J.A., Lacey, E.A., Bentley, G.E., Kriegsfeld, L.J., 2013. Effects of social environment on baseline glucocorticoid levels in a communally breeding rodent, the colonial tuco-tuco (Ctenomys sociabilis). Horm. Behav. 64 (3), 566–572. https://doi.org/10.1016/j.yhbeh.2013.07.008.