The foraging ecology of banded mongooses (*Mungos mungo*): Epidemiological and human-wildlife conflict implications

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ABSTRACT

Free-ranging banded mongooses (*Mungos mungo*) in northeastern Botswana are infected by a novel *Mycobacterium tuberculosis* complex pathogen, *M. mungi*, which putatively infects mongooses through lesions in the skin (often the *planum nasale*) from an environmental reservoir. To understand the epidemiology of the yearly and highly seasonal outbreaks of *M. mungi* in this population of banded mongooses, researchers need to understand what factors influence banded mongoose exposure to *M. mungi* and banded mongoose susceptibility to *M. mungi* infection.

Researchers have no baseline data on the behavioral ecology of this population of banded mongooses — such as home range dynamics, denning ecology, movement ecology, and foraging ecology, all of which may play a role in banded mongoose exposure to *M. mungi*. Further, researchers have highlighted the potential role of prolonged elevations of glucocorticoids in impairing cell-mediated immunity, which would play a significant role in determining susceptibility to a mycobacterium such as *M. mungi*, however, researchers have no data on the endocrinology of banded mongooses. Finally, researchers have not detected *M. mungi* infection in any other population of banded mongooses. Our study population has a gradient of troops (social groups) that vary from troops with extremely close association with humans in a town, to troops associated with humans at tourist lodges within the Chobe National Park, to troops with no discernible association with humans within the national park and surrounding forest reserve. Researchers have few data on how synanthropy (living with humans) affects banded mongoose behavioral ecology and no data on how synanthropy affects banded mongoose endocrinology. Researchers do not know whether or how the high level of synanthropy in this population of banded mongooses plays a role in the epidemiology of *M. mungi* outbreaks.

Thus, we document here some aspects of banded mongoose home range dynamics, movement metrics, denning ecology and foraging behavior for our study population in northeastern Botswana. We present a novel method for screening data from global positioning system (GPS) collars for large measurement error and we present a detailed home range study. We also document the spatio-temporal dynamics of glucocorticoid production among several banded mongoose study troops across our study site, using a non-invasive assay for fecal glucocorticoid metabolites, which we validated and also present here. We tested to see which factors, including nutritional limitation, predation risk, and reproduction (and associated competition, agonistic encounters, and predation), best explained the variation in glucocorticoid production among our study troops over several years.

We found that the metrics traditionally used to screen data from GPS collars, horizontal dilution of precision (HDOP) or fix dimension (2-D or 3-D), performed poorly relative to a new screening metric that we propose, the estimated elevation error (EEE). We propose that researchers use our screening method, which combines test data and a model-averaging information-theoretic framework that uses *a priori* candidate models of telemetry measurement error. Although we recommend including EEE in *a priori* candidate models, it may not describe telemetry error in other systems as well as it did in our own.

Banded mongooses in our study population formed troops of a median of 13 adults (IQR: 11 to 21 adults) and these troops used home ranges of a median of 68 ha (IQR: 39 ha to 134 ha) with core areas of a median of 15 ha (IQR: 9 ha to 28 ha). These cores (statistically-clumped space use) occurred at a median volume contour of 66 % (IQR: 58 % to 71 %). Synanthropic troops showed more clumped area use than apoanthropic troops (those living away from humans). Synanthropic troops also used man-made structures for den sites in 81 % of nights, fed from refuse sites in 13 % of foraging observations, and drank from anthropogenic water sources in 78 % of drinking observations.

From our conducted adrenocorticotropic hormone challenge, we detected valid increases in fecal glucocorticoid metabolite concentrations in mongoose feces using our four tested enzyme-immunoassays. An 11-oxoetiocholanolone assay detecting 11,17-dioxoandrostanes (11,17-DOA) performed best. Using this assay, we detected expected decreases in fecal glucocorticoid metabolite concentrations 48 h after administering dexamethasone sodium phosphate. We also validated this assay using biological events as challenges, in which captive mongooses showed higher fecal glucocorticoid metabolite concentrations during reproductive activity, agonistic encounters, and depredation events. The time delay of fecal glucocorticoid metabolite excretion approximately corresponded with food transit time, at a minimum of approximately 24 h. Fecal glucocorticoid metabolite metabolism was minimal up to 8 h post-defecation.

Reproduction and its associated challenges dramatically increased glucocorticoid production, which otherwise remained low and stable in a captive troop with a constant food supply and lowered predation risk. Variation in glucocorticoid production in free-ranging banded mongooses was best explained by food limitation as described by current nutritional limitation (proportion of fecal organic matter), recent rainfall (which increases soil macrofauna availability), and access to concentrated anthropogenic food resources. Habitat differences in soil macrofauna density and reproductive events also explained variation in glucocorticoid production in free-ranging mongooses, but to a much lower degree. Predation risk, as measured by canopy cover (escape from aerial predators) and group size (decreased per capita vigilance) explained very little of the variation in glucocorticoid production. In the late dry season, banded mongooses in our population may face a "perfect storm" of nutritional limitation, agonistic encounters at concentrated food resources, aggressive evictions, estrus, competition for mates, parturition, and predation pressure on pups. We suspect that this prefect storm may push glucocorticoid responses into homeostatic overload and may impair cell-mediated immunity in banded mongooses.

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Dedication

I dedicate this dissertation to the memory of Tshimologo Njonjo (1984–2011) who helped me tirelessly in the field and lab.

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Chapter 1

Introduction

1.1 Introduction

1.1.1 Public health in the southern African context

Infectious diseases are the leading cause of human death worldwide (Binder *et al.*, 1999), most of which are from exposure to zoonotic pathogens (Daszak *et al.*, 2000, Woolhouse & Gowtage-Sequeria, 2005). In addition, introduction of novel pathogens into naïve wildlife populations through anthropogenic activities has resulted in significant (though underestimated) impacts on biodiversity (Daszak *et al.*, 2000). Emergence of pathogens is usually linked to ecological change (Woolhouse, 2002, Woolhouse & Gowtage-Sequeria, 2005) and specifically, species jumps by pathogens depend upon exposure of a new host to the pathogen, which in turn depends upon the ecology and behavior of the old and new hosts, and on the ecology of the pathogen (Woolhouse *et al.*, 2005).

Between 1990 and 2001, tuberculosis (TB) was one of the ten leading causes of death worldwide (Lopez *et al.*, 2006). In 2004 there were 8.9 million new cases and 1.7 million deaths from tuberculosis worldwide with increasing prevalence in African countries with a high human immunodeficiency virus (HIV) burden (Dye, 2006) for which there were over 2 million TB cases (Zignol *et al.*, 2006). In 2004 in South Africa, Botswana, and Zambia, more than half of tuberculosis-infected people were co-infected with HIV (Dye, 2006). Further, Multidrug-resistant (MDR) tuberculosis has emerged as a major threat to public health, and in 2004 there were over 48 000 cases in African countries with a high HIV burden (Zignol *et al.*, 2006). Extensively drug-resistant (XDR) tuberculosis has also recently become a significant threat in South Africa (Koenig, 2008). Current estimates suggest that in 2006 there were 10 230 cases and 1696 deaths associated with tuberculosis in Botswana and in 2005 there were between 260 000 and 350 000 people in Botswana living with HIV/AIDS (World Health Organization, 2008).

In addition to the public health issues that sub-Saharan Africa faces, poverty is a major issue, with approximately 60 % of the population of this region living in rural areas (United Nations, 2008c). In Botswana, a relatively wealthy country in the region, 30 % of the population are living below the poverty line (United Nations, 2008a) and approximately 40 % of the population are living in rural areas (United Nations, 2008b). The region also has some of the largest concentrations of wildlife in Africa. The combination of large wildlife populations and large rural human populations results in significant rates of human-wildlife conflict. Wildlife-based tourism-related activities are some of the common pathways for increases in the human-wildlife interface in Botswana (e.g. Vanderpost (2006)). Human-wildlife conflict can occur through direct agonistic interactions, damage to property, and disease transmission. Given the large pool of immunocompromised people and the large pool of poor people in Botswana, human-wildlife conflict from all three of the latter pathways has potentially serious implications for public health and poverty in Botswana.

1.1.2 Anthropogenic change and synanthropy

Anthropogenic change to wildlife habitat forces some wildlife species to seek alternative habitat and imperils or extirpates populations of others (Rosenzweig, 1999). However, modifying habitat may cause or allow some species to live in close association with humans — synanthropy. Although synanthropy may sometimes benefit or sometimes harm humans or wildlife, humans may have to learn how to promote mutually-beneficial synanthropy (i.e. reconciliation ecology) to prevent future mass extinction (Rosenzweig, 2003a,b).

Unfortunately, for many species, we do not understand how animals respond to synanthropy. Synanthropy can alter animal behavior, such as changing the duration of activity periods (Beckmann & Berger, 2003), or changing the timing of activity periods (Riley *et al.*, 2003). Further, synanthropy causes human-wildlife conflict worldwide in the form of direct predation (Packer *et al.*, 2005), competition for resources from herbivores (Guerbois *et al.*, 2012) and carnivores (Valeix *et al.*, 2012), and disease transmission to humans or domesticated animals (Gortázar *et al.*, 2011, McFarlane *et al.*, 2012).

For many species we have limited understanding of how synanthropy affects group sizes, movement ecology and foraging ecology. Animals may alter their movement and foraging ecology in response to anthropogenic resources in species-specific and site-specific ways. In some species, observational studies suggest that synanthropic populations have smaller home ranges than apoanthropic populations (i.e. populations living away from or without association with humans) (Brearley *et al.*, 2011, Davison *et al.*, 2009, Prange *et al.*, 2004, Rotem *et al.*, 2011, Wright *et al.*, 2012). In other species, synanthropic populations have larger home ranges than apoanthropic populations (Gese *et al.*, 2012, Riley *et al.*, 2003) or have home ranges of the same size (Bellantoni & Krausman, 1993, Gilchrist & Otali, 2002, Hellgren & Polnaszek, 2011, Herr *et al.*, 2009). Further, a single species may respond to anthropogenic resources differently depending on the site and circumstances.

Experimental manipulation of food resources suggests similar complexity. Some species increase home range size in response to the loss of anthropogenic resources (Kolowski & Holekamp, 2008). Some species maintain the same home range size in response to food supplementation (López-Bao *et al.*, 2010). Some species respond to clumped versus dispersed food supplementation by increasing overlap with conspecifics but maintaining the same home range size (Wehtje & Gompper, 2011). Finally, some species may respond to anthropogenic resources by decreasing core range size but not home range size (Gilchrist & Otali, 2002, López-Bao *et al.*, 2010). Other species respond by reducing several measures of range use, including home range, core range and day range (Rotem *et al.*, 2011).

From the perspective of disease transmission, synanthropy has facilitated some of the worst historic epidemics, such as plague (Drancourt & Raoult, 2011) and continues to facilitate some important infectious diseases worldwide, including malaria (Shetty, 2012), H5N1 influenza A virus (Newman *et al.*, 2012), West Nile virus (Kilpatrick *et al.*, 2006), and rabies (Russell *et al.*, 2006). To better manage pathogen transmission in modified landscapes, we need to understand how

changes in habitat quality affect susceptibility and the movement and behavior of infected and susceptible animals — movement and behavior that mediate exposure. We also need to understand how modified landscapes may change group sizes and wildlife density to better understand the effect of synanthropy on density-dependent pathogen transmission (McCallum *et al.*, 2001).

1.1.3 A novel *Mycobacterium tuberculosis*-complex pathogen

A novel pathogen in the *Mycobacterium tuberculosis* complex, *M. mungi* causes repeated outbreaks in free-ranging banded mongooses *Mungos mungo* living at the human-wildlife interface in northeastern Botswana (Alexander *et al.*, 2010). Outbreaks of *M. mungi* occur in multiple mongoose troops, with up to 17 % of troop members becoming infected. *M. mungi* presents a pathogen closely related to *M. tuberculosis* that researchers could not previously differentiate from *M. tuberculosis* using conventional molecular techniques (Alexander *et al.*, 2010). Currently, we do not know the route of infection or mode of transmission for this emerging pathogen.

This population of banded mongooses lives in close contact with humans in the town of Kasane, Botswana, and at tourist lodges in and around the Chobe National Park. We need to understand the epidemiology of *M. mungi* outbreaks to aid conservation of this population of banded mongooses and because we do not know if other species, including humans, are susceptible to *M. mungi* infection. If *M. mungi* can infect humans, it could have potentially disastrous consequences in light of the high number of immuno-compromised people in the region.

1.1.4 Exposure, susceptibility, and disease emergence

For infectious disease to emerge in any animal (including humans), the animal needs exposure to a pathogen and susceptibility to that pathogen. In this preliminary investigation into the epidemiology of *M. mungi* outbreaks in banded mongooses, we explored both of these aspects. We found from preliminary histopathologic investigation that *M. mungi* appeared to infect mongooses via surface abrasions and lesions on the *planum nasale* (the hairless portion of a mongoose's nose) and possibly via lesions elsewhere on the body (Alexander *et al.*, 2010). Although this is not definitive, it does suggest that banded mongooses may become infected with *M. mungi* from environmental sources rather than from horizontal transmission, as is common in other *Mycobacterium tuberculosis*-complex pathogens. To date we have found only one infected animal with a predominantly respiratory presentation (K.A. Alexander, *personal communication*) while most cases with fulminating disease have presented infection in multiple organ systems.

If *M. mungi* does infect mongooses from an environmental reservoir, then it is important to understand the factors that predispose mongooses to foraging in areas that could have a high environmental *M. mungi* load. There is, however, a paucity of information on mongoose foraging ecology in general and almost no literature on the foraging ecology of this species in the context of the human-wildlife interface where *M. mungi* infection poses the greatest threat. It is

also important to understand the behaviors such as opportunistic scavenging that bring banded mongooses into contact with humans in case humans are also susceptible to *M. mungi* infection. Thus, the first aspect of our investigation was to characterize banded mongoose movement and home range behavior.

The second aspect of *M. mungi* emergence, susceptibility, is important in other diseases caused by *Mycobacterium tuberculosis*-complex pathogens. For instance, researchers have estimated that up to one third of the world's human population is infected with *M. tuberculosis*, but most of these infections do not lead to disease emergence in the host (Flynn & Chan, 2001). Thus, the immune response is important in mediating susceptibility to *M. tuberculosis* infection in humans. In mycobateria in general, as with other intracellular pathogens, this immune response is cell-mediated (Cooper, 2009, Flynn & Chan, 2001). In our population of banded mongooses, if up to 17 % of troop members become infected with *M. mungi*, and if these infections are caused by exposure to an environmental reservoir of *M. mungi*, then it is also likely that other troop members have similar exposure to the same reservoir, because banded mongooses forage and den communally. It seems likely that susceptibility, and the cell-mediated immune response in particular could be important in the epidemiology of *M. mungi* outbreaks. We chose to investigate the potential role of glucocorticoids in the cell-mediated immune response in banded mongooses. The effect of glucocorticoids and catecholamines on immune function is complex and involves the suppression of cellular immunity and activation of humoral immunity, mediated by a glucocorticoid-induced change in the T-helper cell (Th1/Th2) balance (Elenkov & Chrousos, 1999). Further, chronic elevations of glucocorticoids may lower skin immunity in particular (Dhabhar, 2000, Dhabhar & McEwen, 1999) and may be important given our preliminary histopathologic findings (Alexander *et al.*, 2010). Thus, the second aspect of our investigation was to determine if banded mongooses experience chronic elevations of glucocorticoids and to determine what factors lead to these elevations.

The final aspect of our investigation was to determine what role anthropogenic change may have in *M. mungi* outbreaks, through its effect on banded mongoose exposure to potential environmental reservoirs of *M. mungi* and through its effect on banded mongoose immune function and hence susceptibility to *M. mungi*. Our population of banded mongooses is the only population currently known to suffer from *M. mungi* outbreaks and it is also the only study population that lives in close association with humans, along a gradient from urban troops to troops living in a national park. Because of this close association and the potential for immune-compromised humans to become infected with *M. mungi*, we also wanted to investigate what factors facilitated or encouraged this close association between banded mongooses and humans.

1.1.5 Banded mongoose movement and home range ecology

To characterize animal movements and home range behavior, researchers typically use very high frequency (VHF) radio telemetry or increasingly, global positioning system (GPS) telemetry. Here, we present a novel method for screening GPS telemetry data for large errors (Chapter 2).

We deployed both VHF and GPS collars on free-ranging banded mongooses in our study area. We used the VHF telemetry to home in on mongoose troops for direct behavioral and clinical observation. We used the screened data from the GPS telemetry to obtain location estimates for troops that we could not visit with high frequency. We used both the location estimates from the GPS telemetry and the location estimates from our direct behavioral observations to perform a detailed home range study (Chapter 3).

1.1.6 Banded mongoose glucocorticoid production

To test for chronic elevations of glucocorticoids we first developed and validated an enzyme immunoassay for banded mongoose fecal glucocorticoid metabolites (Laver *et al.*, 2012) (Chapter 4). Using this technique we then conducted a large-scale field study, which we paired with experimental manipulations in a captive troop of banded mongooses (Chapter 5).

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Chapter 2

Screening for measurement error in GPS telemetry data

Screening GPS telemetry data for locations having large error

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2.1 Abstract

1. Technological improvements in GPS telemetry have increased the number of locations one can collect which increases the number of locations with large measurement error.
2. We propose and show examples of a new method for screening data for locations with large error.
3. We also propose a new screening metric: estimated elevation error (EEE).
4. EEE identifies *xy*-coordinate error in some cases better than do methods that use horizontal dilution of precision (HDOP) or fix dimension (2-D or 3-D).
5. Our screening method combines test data and a model-averaging information-theoretic framework that uses *a priori* candidate models of telemetry measurement error.
6. One can adapt this screening method to any GPS data.

2.2 Introduction

Increasing numbers of researchers studying terrestrial wildlife deploy telemetry collars that use global navigation satellite systems such as the NAVSTAR Global Positioning System (GPS) to collect wildlife location data (Moorcroft, 2012, Tomkiewicz *et al.*, 2010). Technological improvements in GPS telemetry allow remote data collection at an unprecedented rate. Without effective methods for screening these large amounts of data, one can easily include many locations with large measurement error in analyses.

Both data with measurement error and biased data caused by missed locations (or "fixes"), are problems for GPS telemetry (Frair *et al.*, 2010). These problems are caused by variations in canopy closure across a landscape, by rugged topography, by changes in collar orientation with respect to GPS satellites, by fix interval, and by behavior of collared animals (Belant, 2009, Cain III *et al.*, 2005, D'Eon & Delparte, 2005, D'Eon *et al.*, 2002, Jiang *et al.*, 2008, Lewis *et al.*, 2007, Mattisson *et al.*, 2010). Missed fixes pose major problems but one can identify them from the null, default, or illogical values representing them in the data. Conversely, fixes with measurement error are insidious and can evade current data screening and censoring methods. In analyses, data with measurement error can produce spurious inference about behavioral states (Ganskopp & Johnson, 2007), habitat use (Montgomery *et al.*, 2010, Visscher, 2006), home range (Moser & Garton, 2007) and animal movements (Hurford, 2009, Jerde & Visscher, 2005).

Researchers currently screen GPS data by using only 3-dimensional locations (versus 2-D) (D'Eon *et al.*, 2002), by using only locations having values of dilution of precision (DOP) that do not exceed an arbitrary level (D'Eon & Delparte, 2005), by combining dimensional and DOP limits (Lewis *et al.*, 2007), and by using only locations having movement metrics, such as turning angle and speed, that meet subjective limits (Bjorneraas *et al.*, 2010). Screening data leads to a necessary trade-off between positional accuracy and loss of data (Bjorneraas *et al.*, 2010, D'Eon & Delparte, 2005, Hurford, 2009, Lewis *et al.*, 2007). Removing inaccurate data from a dataset

is worth the loss of data if such removal does not introduce bias, such as that caused by deleting data with non-random inaccuracies (D'Eon & Delparte, 2005). Screening may thus decrease positional inaccuracy but increase bias. These evaluations only partly considered bias because they ignored the additional bias of pre-screening missed fixes. Further, these evaluations did not differentiate random from systematic data reduction, thereby confounding accuracy and bias. Finally, the roles of positional accuracy and data reduction were subjectively weighted in these evaluations.

In addition to subjective and arbitrary limits and a lack of appropriate evaluation, current screening methods have further problems. For example, before May 2000 the United States Department of Defense artificially degraded GPS satellite signals for non-military applications and high values of DOP may have been effective then, but not now, at identifying inaccurate locations (Milbert, 2009). Further, movement metrics such as step length (distance between consecutive GPS fixes) and turning angle (angle between consecutive inferred trajectories) are only useful if the time between fixes is short relative to animal movement patterns. For example, step length and turning angle at a daily fix interval may be appropriate for seasonally migrating animals but inappropriate for central-place foragers.

We evaluated a new approach to GPS data screening by replacing arbitrary screening criteria with a modeling framework. This approach evaluates several screening models and imposes no subjective trade-off between positional accuracy and data reduction. Using field data we show how to construct and evaluate the best descriptive model for test data and how to apply the model to screen GPS data generated by collars fit to animals. Thus, we show how other researchers can build their own best parameterized models (Table 2.1). We also propose and evaluate a new screening metric: estimated elevation error (EEE).

2.3 Methods

2.3.1 A new screening method

Each combination of brand of GPS collar, collar model, study species, and study site will generate unique errors in location data and, therefore, one should not expect rule-of-thumb, discriminatory thresholds for screening metrics to apply universally. In addition, researchers usually have an *a priori* tolerance for location error unique to their objectives and study configuration. Current screening methods ignore these study-specific error tolerances. We propose that researchers abandon single-metric thresholds in screening GPS data and, instead, use an information-theoretic modeling approach. We recommend modeling the measurement error specific to each study configuration using test data that provide known *xy*-coordinate error. Using our approach one develops candidate models for GPS location error; tests GPS collars in important habitats in the specific study area, generating test data for model selection; evaluates the candidate models using an appropriate information-theoretic approach (for example, Akaike's information criterion, or

AIC); performs model averaging if appropriate; deploys GPS collars on study animals; records representative animal locations simultaneous with scheduled GPS collar fixes (generates data for independent model validation); retrieves GPS collar data; using the screening model (the highest ranked model or the model-averaged model, whichever is appropriate), screens data for locations potentially having large error; evaluates model performance using independent validation data (Table 2.1).

2.3.2 A new screening metric: estimated elevation error (EEE)

Ideally, one would screen GPS fixes based on *xy*-coordinate error but true *xy*-coordinates of fixes are usually unknown for deployed GPS collars. Fortunately, GPS fixes are 3-dimensional. GPS centers spheres on the positions of satellites with radii inferred from the time signals take to travel to a collar. Each inferred radius is a pseudo-range, or the estimated distance between the satellite and the receiver. *z*-coordinate error should correlate with *xy*-coordinate error because pseudo-range positioning approximates trilateration, using the intersection of spheres in 3-D Cartesian space (Meyer *et al.*, 2006b). One can improve screening by using *z*-coordinate error, a metric inherent to GPS fixes, rather than relying solely on ancillary data such as DOP, fix dimension, or number of satellites. While one cannot estimate *z*-coordinate error at true locations, one can estimate *z*-coordinate error at GPS-estimated locations: estimated elevation error (EEE). True elevation at estimated locations can be approximated using reference elevation data such as digital-elevation-model data. If GPS and reference-elevation height systems differ, one must convert GPS elevations to match reference data. One will often convert from ellipsoid heights to orthometric heights (Figure 2.1).

2.3.3 Error-tolerance threshold

In 2010 and 2011, we applied our new metric and new screening approach to screen GPS telemetry data collected on banded mongooses (*Mungos mungo*) in Kasane, Botswana. The first step in our screening method is to choose the level of tolerance for GPS error that is appropriate for the objectives and hypotheses of a study (Table 2.1, 1[a]). We chose *xy*-coordinate error tolerance of 20 m based on error ratio analysis (Moser & Garton, 2007) and on our intended use of the GPS collar data: home range analysis using kernel density estimation, and habitat analysis. Our error ratio analysis produced a maximum allowable median telemetry error of 21.85 m. The error ratio relates positioning error to home range size. At a ratio of ≥ 0.01, positioning error affects utilization distributions estimated using a kernel density estimator (Moser & Garton, 2007). To estimate home range size, we used 2761 direct observations of mongooses in nine troops from 2008/05/16 to 2011/04/25 (range: 67 – 675 observations per troop, median = 250 observations) and a fixed, bi-weight, kernel density estimator. We defined home ranges using 95 % volume contouring, unit variance standardization, and least-squares-cross-validation smoothing functions (selected separately for each troop) using ArcGIS 9.3.1 (Environmental Systems Research Institute, Redlands,

CA) and ABODE (Laver, 2005).

2.3.4 Model building

We developed 16 candidate models for accepting or rejecting GPS fixes (Table 2.1, 1[b]; Table 2.2). We used the “accept" or “reject" dichotomy (1,0) as the binomial response variable (y_i's, Eqn 2.1) and evaluated all models as generalized linear mixed effects models with the ‘logit' link function (Eqn 2.2) using *glmer* in Package ‘lme4' (Bates *et al.*, 2011) in R (R Core Team, 2012). Our candidate models were plausible subsets of our global model, which had as fixed effects (β_j's, Eqn 2.3) the estimated elevation error, horizontal DOP, number of satellites, and fix dimension. The fixed effects relate to GPS satellite geometry, which researchers consider a major source of GPS positioning error. No interaction terms were plausible. We included location as a random effect (1|Loc) in all candidate models (u_{ik}'s, Eqn 2.3) with test locations as levels (Z_{ik}'s, Eqn 2.3). We assumed that the parameters of the random effect were normally distributed (u_{ik}'s, Eqn 2.4), and we assumed that GPS units performed similarly without random effects. We investigated multicolinearity by assessing variance inflation factors (VIFs) (Anderson *et al.*, 2001).

$$y_i = Bin\left(n_i, p_i\right) \tag{2.1}$$

$$logit\left(p_i\right) = ln\left(\frac{p_i}{1-p_i}\right) = \eta_i \tag{2.2}$$

$$\eta_i = \sum_{j=1}^{s} \beta_j x_{ij} + \sum_{k=1}^{r} Z_{ik} u_{ik} \tag{2.3}$$

$$u_{ik} \sim N\left(0, \sigma_k^2\right) \tag{2.4}$$

2.3.5 GPS collars, study animals and study area

We field-tested six Quantum 4000 Enhanced collars (75 g, Telemetry Solutions, Concord, CA, USA) placed together at five sites in Kasane, Botswana (Table 2.1, 2, 4). Our field study area was centered at 25.163° E and 17.828° S, described by Laver *et al.* (2012) (Chapter 4). We located five test sites in a low-density residential area in microhabitats ranging from open ground to complete canopy cover. These test sites represented habitat conditions typical for mongooses in our study area and sites under complete canopy cover represented worst-case scenarios for GPS satellite reception.

We attached the six collars $\sim$ 5 cm apart to a raised cylinder, to replicate their placement on active mongooses. At this spacing, collars may have interfered with one another. We recommend a

spacing of $\geq$1 m. From 2010/08/02 to 2010/08/04, we placed this configuration of collars at the five sites, keeping collars at four sites for five fixes at 5 min intervals and at the final site for two sets of five fixes, two hours apart. We had a total of 174 GPS collar fixes.

2.3.6 Test-data analysis — response variable

We projected *xy*-coordinates from geodesic latitude and longitude to the Universal Transverse Mercator (UTM) system (Zone 35 South). We converted ellipsoid heights (h) to orthometric heights (H) using geoid heights (N) for the Earth Gravitational Model 1996 (EGM96) supplied by the GeoidEval utility in GeographicLib (Karney, 2012) and using the approximation $H \approx h - N$ (Meyer *et al.*, 2006a)(Table 2.1, 3[a]). The mean geoid height for our study area was 8.62 m (range for all GPS fixes: 8.49 m – 8.67 m). We obtained reference elevation data from Google Earth (Google Inc., Mountain View, CA, USA) for each GPS-estimated *xy*-coordinate pair (Table 2.1, 3[b]). In July 2012 we accessed elevation data using Google Maps Application Programming Interface and *xy*-coordinates in geodesic latitude and longitude (WGS 84). Here we embedded one *xy*-coordinate pair in the call:

$$http://maps.googleapis.com/maps/api/elevation/json?locations = -17.8428870203,25.0764870522\&sensor = false$$

We computed estimated elevation error as the absolute difference between Google Earth orthometric height (EGM96) at GPS-estimated *xy*-coordinates and the elevation recorded by GPS collars (converted from ellipsoidal to orthometric height)(Table 2.1, 3[c]). For model-building, we used *xy*-coordinates of test sites to determine actual *xy*-coordinate error (Table 2.1, 3[d]). We rejected (assigned zero to) any fix with $>$ 20 m *xy*-coordinate error (Table 2.1, 3[e]).

2.3.7 Model selection

We evaluated candidate screening models using an information-theoretic approach (Anderson, 2008) using Akaike's Information Criterion (Akaike, 1974) with small sample size correction (AICc) (Anderson, 2008) (Table 2.1, 3[f]). Conditional AICc (cAICc) was unnecessary. Our objective was inference about population parameters and marginal likelihood (Vaida & Blanchard, 2005). We used multimodel inference and model averaging (Burnham & Anderson, 2002) based on Akaike weights (w_i) of all candidate models using Package 'MuMIn' (Barton, 2012) to build a descriptive screening model (Table 2.1, 3[g]).

2.3.8 Model fit

After model selection, one can assess model fit using frequentist measures such as goodness of fit and parameter estimates with 85 % confidence intervals (Anderson, 2008, Anderson *et al.*, 2001, Arnold, 2010). We assessed fit of the global model using pseudo coefficient of determination (R^2) analogues with *LogRegR2* in Package 'descr' (Aquino, 2011) (Table 2.1, 3[h]). To improve interpretability of parameter estimates (as changes on the logit scale), we standardized numeric variables to $\bar{x} = 0, \sigma = 0.5$ and binary variables to $\bar{x} = 0$ with a difference of 1 between categories (Gelman, 2008), using Package 'arm' (Gelman *et al.*, 2012). For binary discriminatory models, one can assess performance across the entire range of thresholds using receiver operating characteristic (ROC) curves (Swets, 1988), and select a threshold using the prevalence-dependent threshold or minimum distance methods (Jiménez-Valverde & Lobo, 2006, 2007, Liu *et al.*, 2005). We selected a discrimination threshold for our model-averaged model using the minimum distance method on an unsmoothed receiver operating characteristic curve (Table 2.1, 3[i]).

2.3.9 Field-data analysis

We deployed collars on three free-ranging, male banded mongooses (1354 g, 1373 g, and 1498 g) from different troops within our study area. GPS collars were ~ 5 % of collared animals' masses. We deployed collar 1 from 2010/08/30 to 2011/05/31 (registering 562 attempted fixes), collar 2 from 2010/09/13 to 2010/11/25 (145 attempted fixes), and collar 3 from 2010/12/15 to 2011/02/10 (117 attempted fixes). We conducted our study under approval of Virginia Tech's Institutional Animal Care and Use Committee (07-146-FIW).

We classified GPS fixes from the three collars deployed on mongooses using our screening model to report fix rate and error rate (percentage of rejected fixes) for each collar and to remove from our data fixes with potentially large error (Table 2.1, 5[b]). We classified fixes as failures if the GPS obtained no fix and as "rejected" or "accepted" according to the screening model. We also collected 2869 direct observations of mongooses between 2008/05/16 and 2011/04/14 for 39 troops in our study area. We removed data for June, July and August, for which we had no corresponding GPS collar data, leaving 1727 observations of individual mongooses from 30 troops. We classified observations as active (foraging, moving, social interactions) or inactive (resting, denning). Finally, we used 816 attempted fixes by the three collars deployed on mongooses. We expected a higher proportion of rejections and failures at times when our mongooses rested (usually inside dens or under structures and dense vegetation).

We assessed our screening model with field data in three ways (Table 2.1, 5[d]): we observed mongooses when GPS collars were scheduled to obtain fixes and compared collar-derived and directly-observed coordinates (Table 2.1, 4[a]); we examined plots of GPS collar fixes for implausible fixes (Table 2.1, 5[a]); we compared missed GPS collar fixes and model rejections to mongoose activity budgets.

2.4 Results

For our test collars, median telemetry error was 7.3 m (range: 0 m to 506 m) with a median circular error probable ($CEP_{0.50}$) of $CEP_{0.50} = pi * (7.3\,\mathrm{m})^2 = 167\,\mathrm{m}^2$ (Table 2.1, 3[d]). Median home range size for mongoose troops in our study site was 61 ha (range: 15 ha to 214 ha). The error ratio for a median circular error of $167\,\mathrm{m}^2$ and minimum home range size of 15 ha was 0.001. Using an error ratio of 0.01, the home range size at which our telemetry error would have been problematic was 1.6 ha and the maximum allowable median telemetry error would have been 21.85 m for the smallest home range.

Figure 2.2 shows telemetry error at 2 scales (fine scale, Figure 2.2a, coarse scale Figure 2.2b) for our test collars. At a fine scale, *xy*-coordinate errors caused mis-categorizations of microhabitat use (Figure 2.2a). At a coarse scale, *xy*-coordinate errors caused broad mis-categorizations of habitat use, with errors of up to 506 m (Figure 2.2b). The three largest errors altered habitat use from low-density residential to high-density residential or commercial (Figure 2.2b).

Using 20 m of *xy*-coordinate error as an *a priori* threshold, the global model performed moderately well according to pseudo R^2 analogues for the generalized linear model (not the mixed model): Cox and Snell's $R^2 = 0.19$, Nagelkerke's $R^2 = 0.47$, McFadden's $R^2 = 0.41$, and $\chi^2 = 35.8$ with 4 degrees of freedom and $p < 0.0001$. We correctly assigned 157 fixes and incorrectly assigned 17 fixes. Multicolinearity was not problematic in the global model with variance inflation factors < 5.

The eight top-ranked models (ΔAICc < 5) all included estimated elevation error (Table 2.2). Summed Akaike weight (Σw_i) was 1 for estimated elevation error (Figure 2.3). The remaining descriptors (horizontal DOP, number of satellites, and fix dimension) each had $\Sigma w_i \sim 0.5$ (Figure 2.3). Estimated elevation error had the largest effect size (standardized model) while other descriptors were close to zero (Figure 2.3). The standardized coefficients, except number of satellites, differed significantly from zero (85 % confidence interval). Models with number of satellites, horizontal DOP and fix dimension as single fixed effects performed poorly relative to the model with estimated elevation error as a single fixed effect (Table 2.2). For the global model, ΔAICc = 2.0, but model fit (log-likelihood: log$L$) was virtually unimproved by adding number of satellites — we might consider nSat an uninformative parameter (Arnold, 2010).

Receiver operating characteristic curves for the models indicate that the model-averaged screening model performed with higher sensitivity and higher specificity at all thresholds and had more area under the curve (0.89) (Figure 2.4a-f). Using the minimum distance method, 0.983 was the appropriate discrimination threshold for our model (Figure 2.4e).

For the test data, rejections occurred across a range of estimated elevation errors (Figure 2.5a). Although the fitted model correctly rejected fixes with the largest *xy*-coordinate errors and estimated elevation errors (Figure 2.5b), the model failed to reject several fixes with low but unacceptable *xy*-coordinate error. Several model failures occurred with low horizontal DOP values (Figure 2.5c). Several model failures had a low number of satellites, but there were as many fail-

ures with five or six satellites (Figure 2.5d). Both 3-D and 2-D fix dimensions were associated with model failures (Figure 2.5e).

The three field-deployed GPS collars had a fix rate of 68 % (51, 71, and 72 % each). Using the model-averaged descriptive model we rejected 129 of 557 fixes at an acceptance rate of 77 % (85, 73, and 90 %, each). We observed mongooses during GPS collar fixes 17 times. GPS-collar-derived coordinates matched direct observations on these occasions and the model-averaged model correctly accepted them. GPS collars reported fixes in three implausible locations. One was 292 km from our study site. A second was in Namibia, across the Chobe River, which is 182 m wide near the fix. A third was in the Chobe River, 204 m from the Botswana riverbank. Banded mongooses swim poorly. The model-averaged model correctly rejected these fixes. The proportion of fixes accepted by the model-averaged model approximately matched the hourly activity pattern of mongooses in our study area (Figure 2.6). The highest proportion of GPS collar failures and model-rejected fixes (Figure 2.6b) occurred when mongooses tended to be inactive (Figure 2.6a).

Post hoc analysis suggests that estimated elevation error might also be a linear descriptor of *xy*-coordinate error (Figure 2.7a), but noise at low *xy*-coordinate error diminishes its utility at a fine scale (Figure 2.7b). Horizontal DOP performed poorly in this analysis (Figure 2.7c).

2.5 Discussion

Models with single metrics describing GPS location error performed poorly relative to models using a combination of metrics. Model averaging of plausible candidate models was a better strategy for screening our GPS collar data. Our new metric, estimated elevation error (EEE), performed well compared to other screening metrics and had the largest effect size. Horizontal DOP performed poorly, with a low effect size, even when used in combination with fix dimension. Our model-averaged screening model derived from test data and stationary collars performed well under field conditions with collars deployed on mongooses — it correctly accepted and rejected the fixes that we could verify from field observations.

The modeling framework we propose will show whether estimated elevation error is an informative variable for any study configuration for which our approach is used. We expect that estimated elevation error will not work for benthic and demersal species in aquatic systems. One might apply modified versions of estimated elevation error to pelagic species found at the surface or that breach regularly. In terrestrial systems, estimated elevation error may not work for arboreal or flying species unless movement zones are stratified and one knows them *a priori*. Depending on how GPS collars deal with 2-D fixes, estimated elevation error may be bounded and have low utility at low elevation sites. Estimated elevation error would be problematic at flat study sites near mean sea level if GPS collars defaulted to 0 m elevation for 2-D fixes. Finally, estimated elevation error may not be useful where the scale of animal movement is considerably finer than the available grain of externally-sourced elevation data. These instances may in-

clude polar regions without Shuttle Radar Topography Mission (SRTM) or other digital elevation model coverage.

Potential error sources within estimated elevation error include GPS measurement error, mainly from suboptimal pseudo-range positioning; systematic error from using reference and GPS elevations in different height systems; and error inherent in elevation data derived from remote sensing or digital elevation models. Digital elevation models such as data from SRTM provide ~ 6 m relative vertical accuracy at horizontal resolutions of 30 m or 90 m (Rabus *et al.*, 2003). Google Earth uses SRTM data as a baseline and augments these data with other data sources and interpolation. In some areas, elevation data from Google Earth provide less vertical error and finer resolution than raw SRTM data, even comparing favorably to high-resolution data from stereo photogrammetry (Hoffmann & Winde, 2010).

Our approach may be particularly useful for researchers studying small-bodied animals. Improvements in GPS sensor size and technology, battery size, cost, and removal of selective availability resulted in increased use of GPS telemetry on small-bodied animals in particular (Tomkiewicz *et al.*, 2010). We define 'small-bodied' animals as having a mass ≤ 2 kg, at which telemetry collars or backpacks need to be ≤ 100 g to remain below 5 % of animal mass. As sizes of GPS collars and collared animals decrease, erroneous GPS fixes will become more important. Small-bodied terrestrial animals generally have smaller home ranges than aquatic, marine, or flying animals of a similar size and smaller home ranges than large bodied animals (Carbone *et al.*, 2005, Jetz *et al.*, 2004). Given the fine scale of their movements, *xy*-coordinate error may affect this group significantly. GPS antenna placement and orientation may affect GPS bias and precision (Belant, 2009, D'Eon & Delparte, 2005, Jiang *et al.*, 2008) and collaring smaller animals may compound errors caused by antenna placement. Further, canopy closure and topography may have more pronounced effects on satellite reception for small animals that are low to the ground, for which canopy closure can occur at a much lower height, and that can move behind objects that block satellite reception.

We believe that our approach has broad applicability to GPS telemetry. We limited our discussion to wildlife telemetry but one could apply our screening methodology and estimated elevation error metric to telemetry systems used on other GPS-tracked objects. One could also apply the general information-theoretic modeling approach to systems beyond GPS, in which one acquires data remotely and then screens for error. Further, our approach to formulating the estimated elevation error metric should be useful in data screening situations where one simultaneously records two or more variables (e.g. A and B) that should be associated. One can then compare the recorded value for B with the value predicted for B by the recorded value A.

Our data suggest that estimated elevation error may often be useful for screening GPS telemetry data and that multimodel inference provides a robust methodology for eliminating GPS fixes with important measurement error. Researchers must acknowledge the importance of error, and not just fix rate, in preventing spurious inference from GPS telemetry data. GPS telemetry is powerful but it is also prone to error and one should avoid indiscriminate use of GPS data or use of suboptimal screening methods.

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Table 2.1. Steps for a proposed methodology for screening GPS telemetry data based on an information-theoretic framework and model-averaging.

Steps for a new GPS data-screening methodology

1. Model development
   (a) Determine tolerance threshold for GPS-positioning error
   (b) Develop plausible candidate models describing GPS-positioning error
2. Deploy stationary collars
   (a) Use representative and “worst-case" habitat
   (b) Place collars in a representative configuration (height, neck analogue)
3. Test-data analysis
   (a) Convert GPS ellipsoid heights to orthometric heights
   (b) Obtain reference elevation data for study site
   (c) Derive estimated elevation error
   (d) Compute *xy*-coordinate error
   (e) Assign a response (accept, reject) to each fix based on 1(a)
   (f) Run candidate models with response data using an appropriate information-theoretic approach
   (g) Perform model averaging if appropriate
   (h) Assess model fit informally using pseudo coefficient of determination (R^2) analogues, and receiver operating characteristic (ROC) curves
   (i) Select a discrimination threshold for the final model using ROC curves
4. Deploy collars on animals
   (a) Record *xy*-coordinates for collared animals simultaneous to scheduled GPS fixes
5. Field-data analysis
   (a) Plot data — look for implausible fixes using known restrictions to movement or habitat use
   (b) Assign model response (accept, reject) to fixes using 3(i)
   (c) Assign true response (accept, reject) to simultaneously observed fixes using 1(a) and 4(a)
   (d) Evaluate the model by comparing true and model-assigned responses

Table 2.2. Model selection for 16 mixed effects candidate models modeling the acceptance (≤ 20 m) or rejection (> 20 m) of Global Positioning System (GPS) collar fixes (n = 174) based on *xy*-coordinate error. We modeled location as a random effect (1|Loc) in all models. Fixed effects were estimated elevation error (EEE), horizontal dilution of precision (HDOP), number of satellites (nSat), and fix dimension (3-D).

	Model	log$L$	K	AICc	Δ	w_i
1	Accept ~ EEE + HDOP + 3-D + (1 | Loc)	-25.4	5	61.1	0.0	0.22
2	Accept ~ EEE + nSat + (1 | Loc)	-26.5	4	61.3	0.1	0.21
3	Accept ~ EEE + 3-D + (1 | Loc)	-27.0	4	62.2	1.1	0.13
4	Accept ~ EEE + nSat + 3-D + (1 | Loc)	-25.9	5	62.2	1.1	0.13
5	Accept ~ EEE + HDOP + nSat + (1 | Loc)	-26.2	5	62.7	1.6	0.10
6	Accept ~ EEE + HDOP + (1 | Loc)	-27.3	4	62.9	1.7	0.09
7	Accept ~ EEE + HDOP + nSat + 3-D + (1 | Loc)	-25.3	6	63.1	2.0	0.08
8	Accept ~ EEE + (1 | Loc)	-29.8	3	65.7	4.6	0.02
9	Accept ~ HDOP + 3-D + (1 | Loc)	-31.0	4	70.2	9.1	0.00
10	Accept ~ HDOP + nSat + 3-D + (1 | Loc)	-30.8	5	72.0	10.8	0.00
11	Accept ~ HDOP + nSat + (1 | Loc)	-32.2	4	72.6	11.4	0.00
12	Accept ~ nSat + (1 | Loc)	-34.4	3	75.0	13.8	0.00
13	Accept ~ nSat + 3-D + (1 | Loc)	-33.8	4	75.8	14.7	0.00
14	Accept ~ HDOP + (1 | Loc)	-35.1	3	76.3	15.2	0.00
15	Accept ~ 3-D + (1 | Loc)	-36.8	3	79.7	18.5	0.00
16	Accept ~ (1 | Loc)	-43.6	2	91.3	30.2	0.00

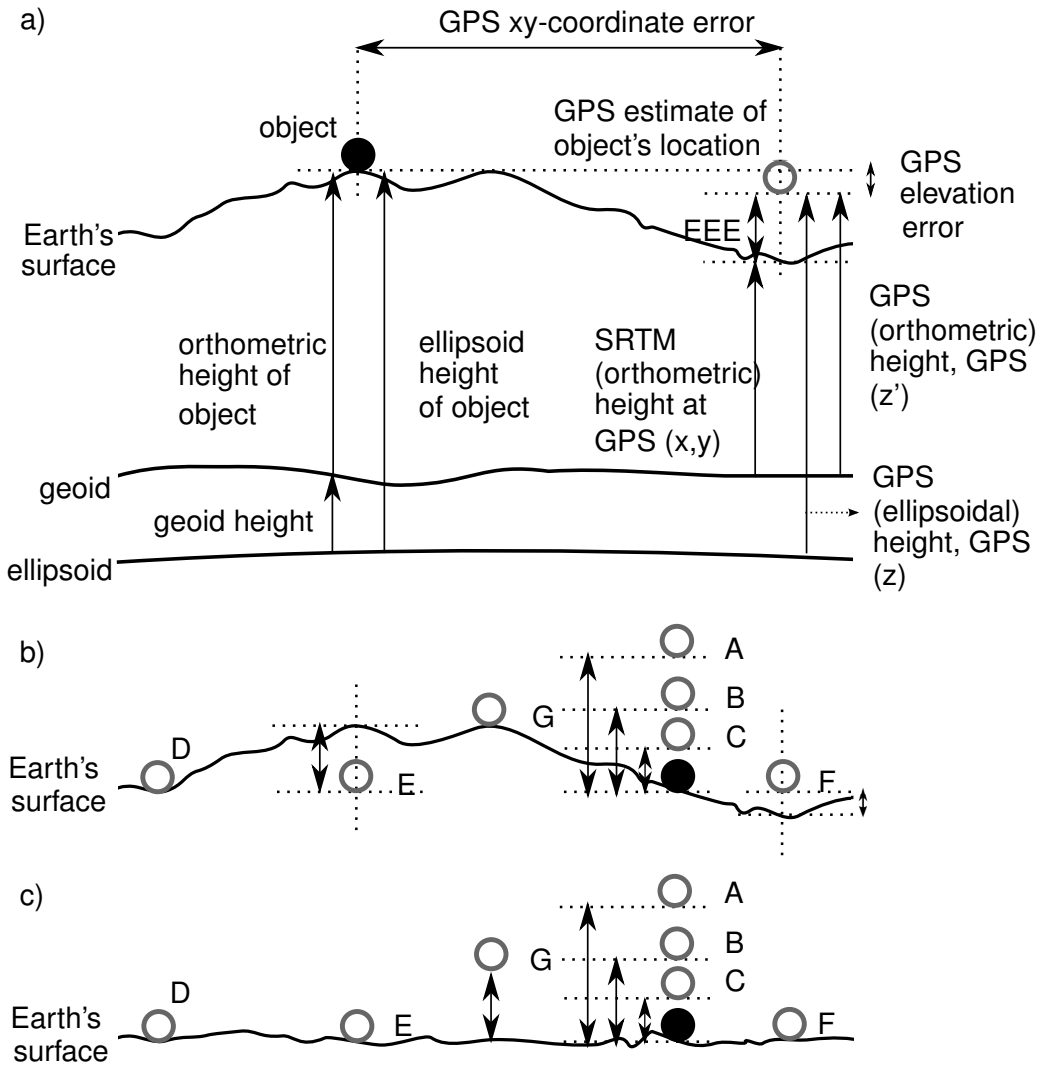

Figure 2.1. (a) Height measurements for objects (filled circles) on the Earth's surface and estimated elevation error (EEE) for Global Positioning System (GPS) fixes (open circles). One should convert ellipsoidal GPS heights (*z*) to orthometric heights (*z'*). Estimated elevation error is the difference between *z'* and reference height (e.g. Shuttle Radar Topography Mission [SRTM] data) at GPS locations (*x,y*). (b and c) In scenarios for estimated elevation error under (b) rugose or (c) level topography, GPS-estimated locations with no *xy*-coordinate error but with variable elevation error (A – C) are possible (estimated elevation error would incorrectly reject them). (b) Under rugose conditions estimated elevation error can identify erroneous locations (E, F, but not D). (c) Under level conditions, GPS-estimated locations with substantial *xy*-coordinate error (D – F) but no elevation error are unlikely because GPS has more control in the horizontal than vertical dimension. GPS estimates will likely display both *xy*-coordinate error and elevation error (G).

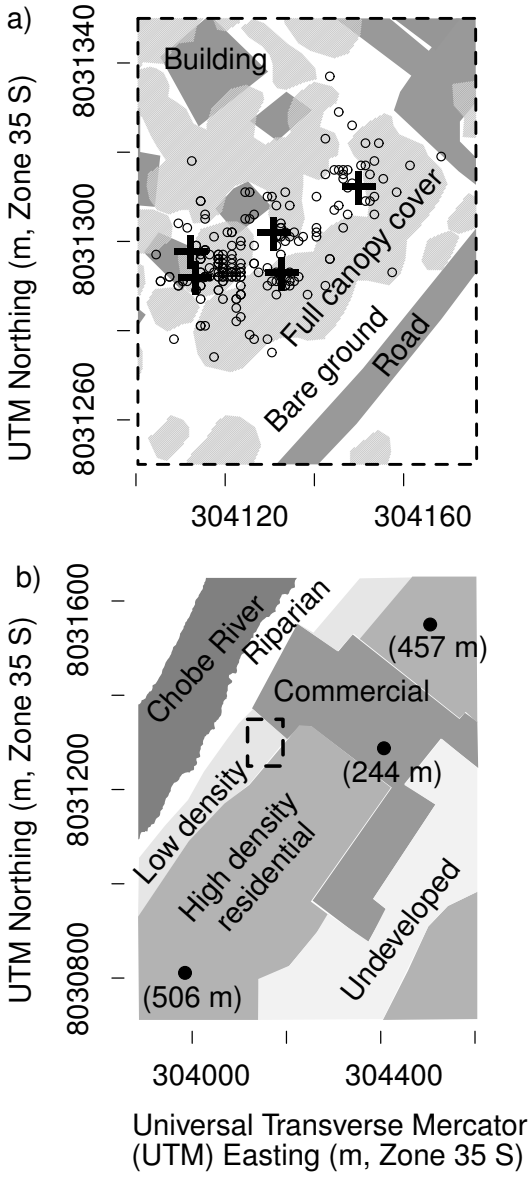

Figure 2.2. *xy*-Coordinates (n = 174) for six Global Positioning System (GPS) collars in Kasane, Botswana. (a) GPS collar fixes (open circles) with < 60 m *xy*-coordinate error (for plotting convenience) relative to five test-collar locations (large crosses) in low-density residential habitat. All test locations yielded fixes with incorrect microhabitat assignments. (b) Most GPS collar fixes had low *xy*-coordinate error (~ 10 m, in the inset). We assigned them to the same broad habitat zone, but we would have erroneously assigned three fixes (black dots, with errors of 244 m, 457 m, and 506 m) to high-density residential and commercial zones.

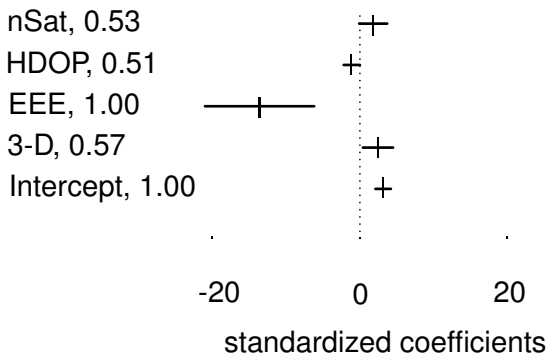

Figure 2.3. Intercept and effect sizes for standardized parameters (Gelman, 2008) with 85 % confidence intervals (Arnold, 2010), after model averaging of all candidate models. Parameters were estimated elevation error (EEE), horizontal dilution of precision (HDOP), number of satellites (nSat), and fix dimension (3-D). After parameter labels, we report relative importance — sum of Akaike weights (Σw_i) over all models that include the parameter.

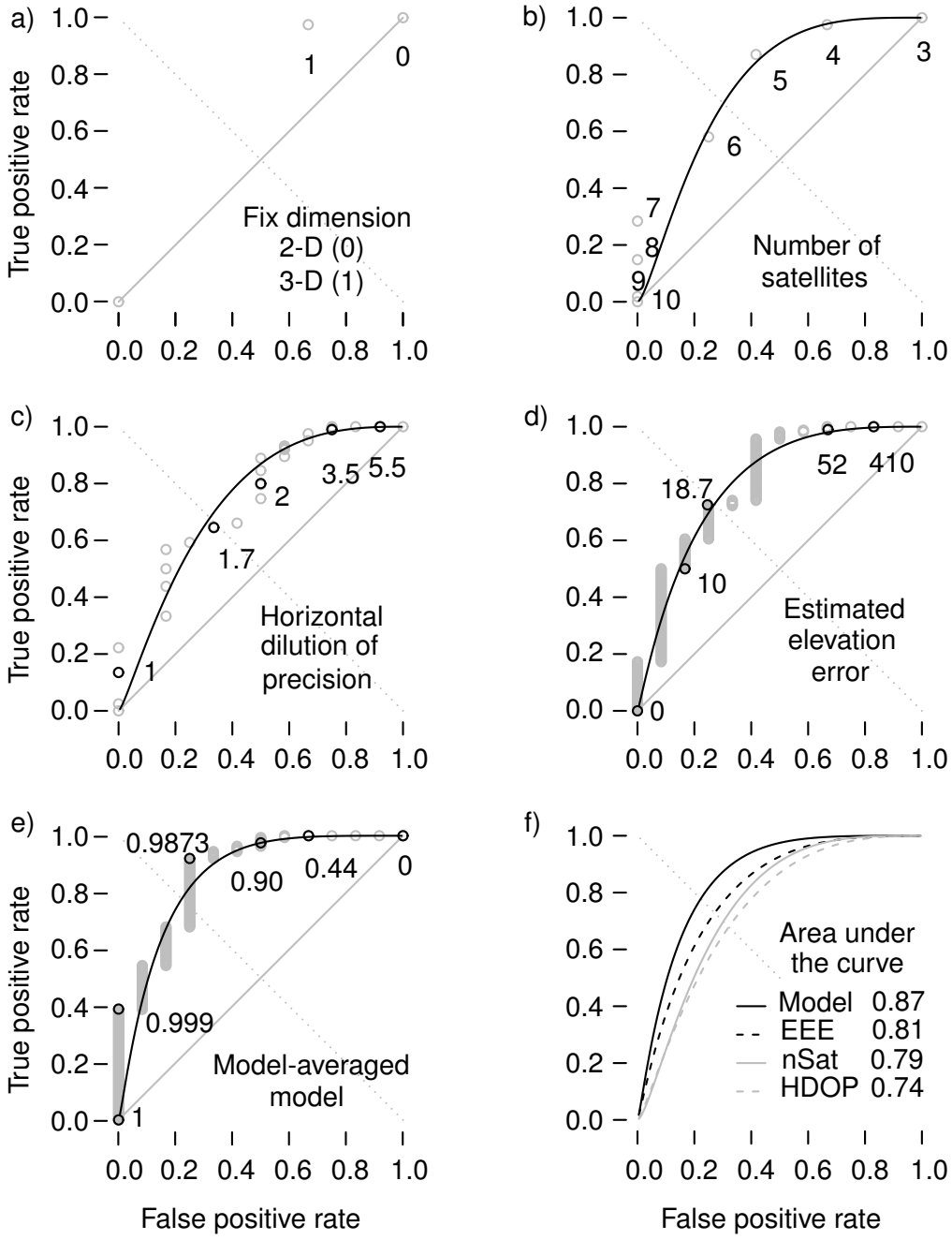

Figure 2.4. Receiver operating characteristic (ROC) curves (black curves) for model predictions based on raw data for (a) fix dimension (2-D or 3-D), (b) number of satellites (nSat), (c) horizontal dilution of precision (HDOP), (d) estimated elevation error (EEE), and (e) model predictions from the model-averaged model. (f) Receiver operating characteristic curves (a – e) combined. We depict data points as grey circles with thresholds (black circles) labeled numerically. Diagonals (0,0 to 1,1) indicate performance of random models. Better models show improved performance across relevant thresholds, lying closer to 0,1 (100 % true positive rate and 0 % false positive rate), at the intersection with the line perpendicular to the no discrimination or random model diagonal (grey dotted diagonal).

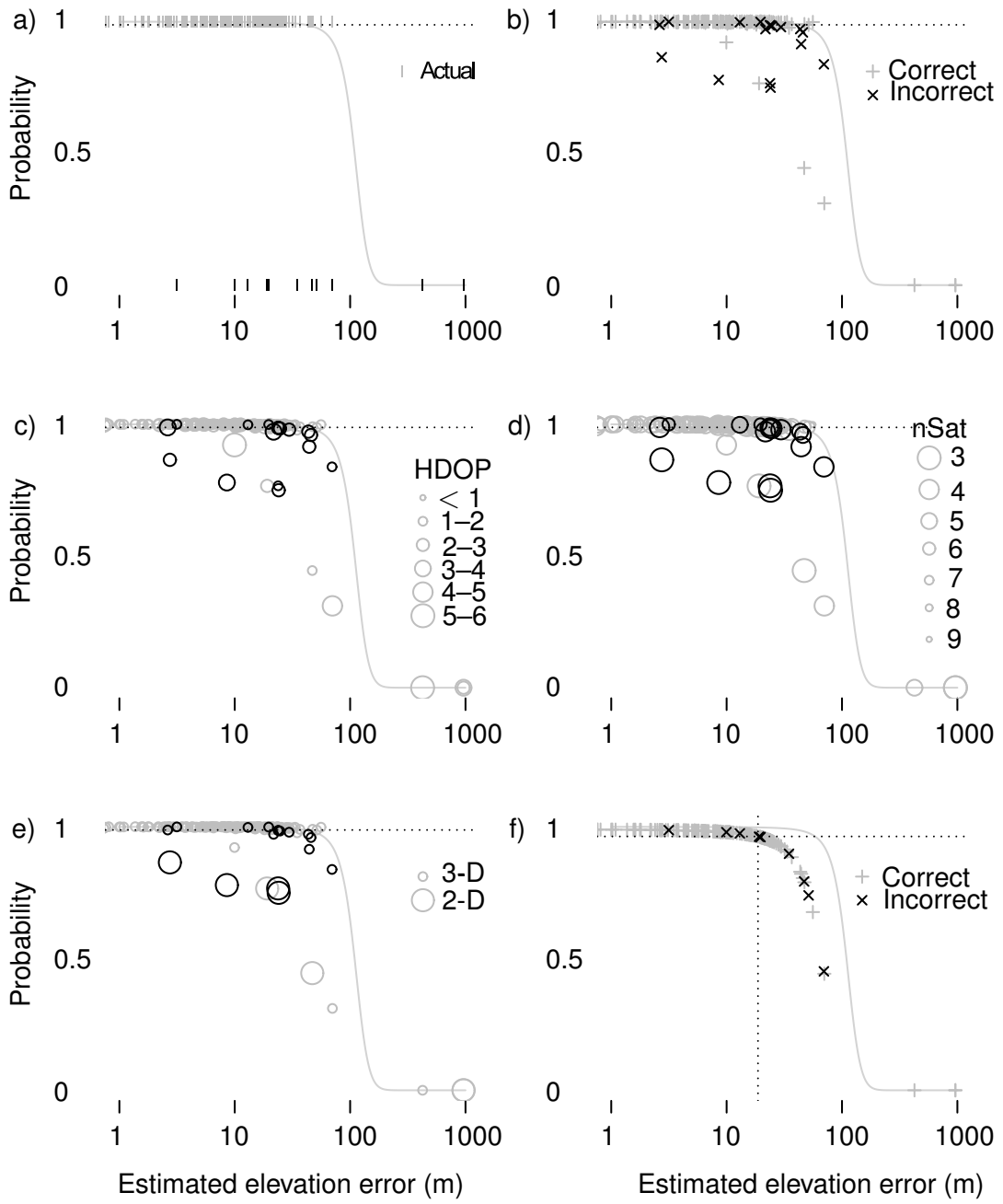

Figure 2.5. The final model-averaged model (fitted to mean parameter values: grey line) with probability of accepting a Global Positioning System (GPS) fix (with ≤ 20 m *xy*-coordinate error) plotted against the main fixed effect, estimated elevation error. (a) Raw data of acceptance ($p = 1$) and rejection ($p = 0$) of GPS fixes. (b – e) Model-predicted probability of acceptance with correct (grey symbols) and incorrect assignment (black symbols). Dotted horizontal lines indicate the discrimination threshold of 0.9873. We scaled symbols proportionally for (c) horizontal dilution of precision (HDOP), (d) number of satellites (nSat), and (e) fix dimension (3-D or 2-D). (f) Model-predicted values using a single-fixed-effect model with estimated elevation error. Rejected fixes had HDOP values ranging from low to high (c) and fix dimensions of both 3-D and 2-D (e). Most rejected fixes had few satellites (d) and high estimated elevation error (f). The model failed for a range of HDOP values, both fix dimensions, but generally few satellites and high estimated elevation error.

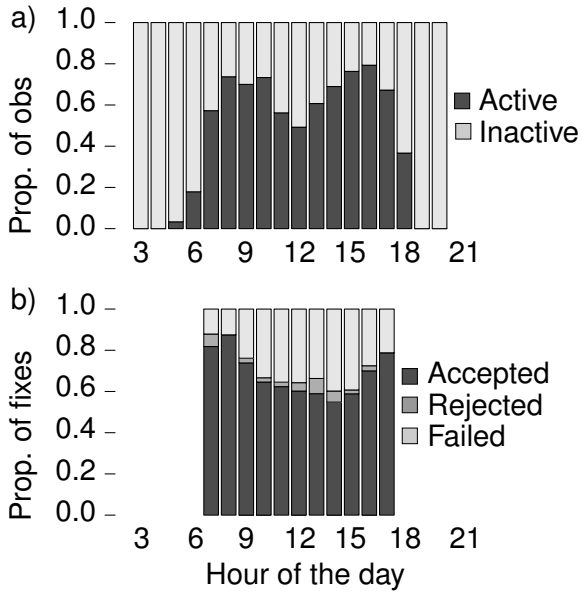

Figure 2.6. (a) Activity budget of banded mongooses (*Mungos mungo*) in Kasane, Botswana, from 1727 observations of 30 troops (2008/05/16 to 2011/04/14, excluding June, July and August for which we have no corresponding GPS collar data). During inactive periods, mongooses typically rested and denned inside structures (hollow logs, termite mounds, manmade structures) or rested under dense vegetation, behavior that should obstruct satellite reception. (b) The inferred activity budget from 816 attempted GPS fixes for three mongoose troops in the same study area (2010/08/30 to 2011/05/31) approximates the directly-observed activity budget. We programmed GPS collars to attempt fixes between 07h00 and 18h00.

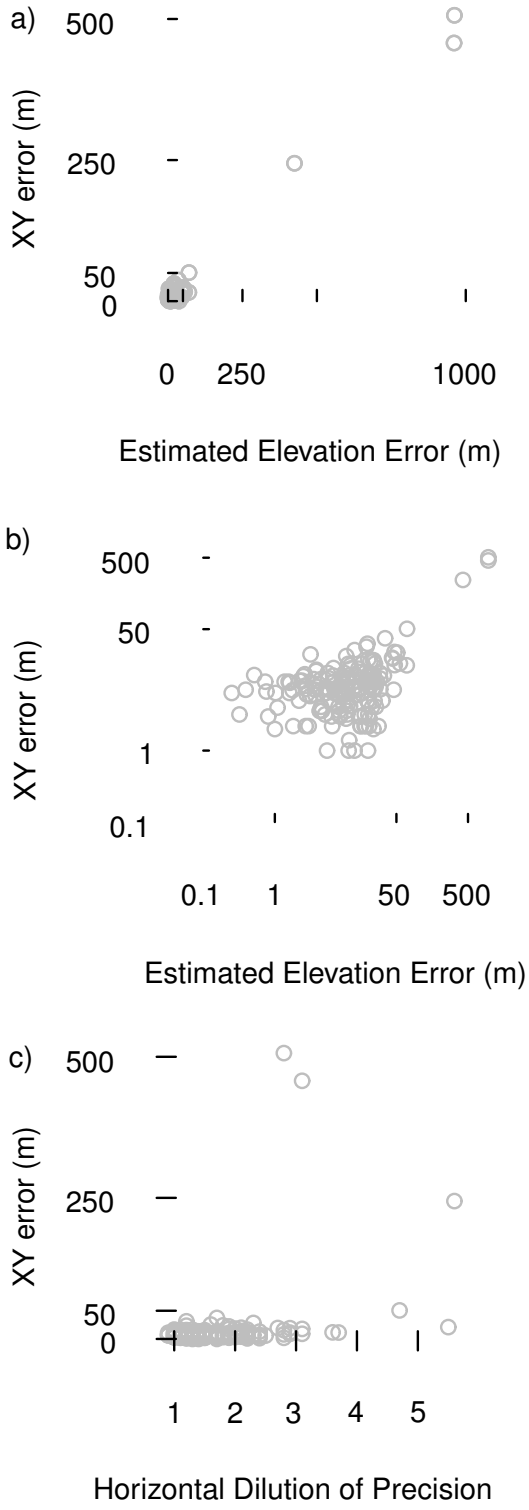

Figure 2.7. *Post hoc* investigation of linear and log-linear relationships between *xy*-coordinate error and potential screening variables, (a) estimated elevation error, (b) estimated elevation error (log-log scale), and (c) horizontal dilution of precision.

Chapter 3

Banded mongoose home ranges

Banded mongoose home ranges

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3.1 Abstract

Home ranges and associated movement metrics can shed light on where, when, and how an animal uses the landscape to maximize its fitness. Valid inferences such as these depend upon rigorous and well-reported methods, but few home range studies report their methods with enough detail to facilitate comparative studies. Here, we provide an example of detailed home range estimation. We estimated movement metrics and home range, core range, and seasonal range using rigorous kernel density estimation for a population of banded mongooses (*Mungos mungo*) in northeastern Botswana, an ecosystem lacking previous research on banded mongoose ranging behavior. We also characterized banded mongoose denning behavior and compared choice of den type among banded mongoose troops with different levels of association with humans. Banded mongooses in northeastern Botswana used home ranges of 68 ha with core ranges of 15 ha. Troops living in close association with humans used predominantly man-made structures such as buildings, permanent structures, building material, and scrap to den in (81 %, n = 1203 observations), while troops living away from humans used hollow logs and termite mounds to den in. Troops living away from humans also showed more diffuse use of their home ranges, with area-probability curves closer to random use than those of troops living with humans. Troops living with humans appear to concentrate their home ranges and core ranges around lodges and refuse sites. Access to anthropogenic resources also altered mongoose foraging behavior. Banded mongooses foraged in refuse in 110 of 850 (13 %) foraging observations and in 78 % of all observations of mongooses drinking, they drank from anthropogenic water sources. Of these anthropogenic water sources, mongooses drank most often from greywater and sewage (21 %) and lawn sprinklers (17 %). Researchers should characterize banded mongoose spacing, group size, and ranging behavior in other ecosystems and should to investigate what factors drive the within- and among-ecosystem differences in these three aspects of banded mongoose ecology.

3.2 Introduction

Humankind's most important challenges to biodiversity conservation include habitat modification, disease, and invasive species (Wilson, 1992), all of which humans may exacerbate with anthropogenic climate change (Monzón *et al.*, 2011).

To address some of these challenges for certain species, researchers need to understand where, when, and how animals use landscapes (Kostyack *et al.*, 2011, Schick *et al.*, 2008). At a basic level, each animal requires resources to survive and reproduce within the context of a surrounding community of conspecifics and heterospecifics, all trying to achieve the same goal of maximizing their own fitnesses. The resources and reproductive opportunities that an animal needs and the competition, predation, and parasitism that it faces, all occur in finite space and time. Researchers typically characterize an animal's use of this space using a probabilistic utilization distribution (Laver & Kelly, 2008), generated with a kernel density estimator (Seaman & Powell,

1996, Worton, 1989), although many other powerful techniques also exist (Kie *et al.*, 2010), such as mechanistic models (Mitchell & Powell, 2004), Brownian bridges (Horne *et al.*, 2007), local convex hulls (Getz *et al.*, 2007), state-space models (Patterson *et al.*, 2008), and artificial neural networks (Dalziel *et al.*, 2008). Researchers model time implicitly in utilization distributions although new methods may allow researchers to model time and behavior explicitly (Benhamou, 2011, Benhamou & Cornélis, 2010, Benhamou & Riotte-Lambert, 2012, Keating & Cherry, 2009). With utilization distributions researchers can then investigate additional ecologically- and conservation-relevant issues, such as resource selection (Millspaugh *et al.*, 2006, Rittenhouse *et al.*, 2008), determinants of home range size (Börger *et al.*, 2006), and animal fitness (Mitchell & Powell, 2003, Morales *et al.*, 2010). Utilization distributions and other representations of resource and patch selection may serve as rapid-response behavioral indicators for critical long-term demographic changes in populations (Morris *et al.*, 2009). Nonetheless, researchers need to ensure that their ecological inferences from home range studies stem from differences in an animal's or a population's ecology, rather than from a difference in their quantitative methods.

Unfortunately, researchers have done a relatively poor job at both reporting and using rigorous methods in studies of home range behavior (Laver & Kelly, 2008). Failing to apply rigor to home range studies can lead to spurious inferences about the ecology and behavior of a species, which may ultimately lead to poor conservation and management decisions (Chapter 2). Failing to report methods appropriately can render comparative studies of home range behavior invalid because some home range estimators are highly sensitive to sample size (Fieberg & Börger, 2012, Gautestad & Mysterud, 1995) or user-selected options such as bandwidth selection (Gitzen & Millspaugh, 2003, Gitzen *et al.*, 2006, Hemson *et al.*, 2005, Horne & Garton, 2006).

Rigorous home range estimation requires several steps (Laver & Kelly, 2008) for which we provided a detailed example here. These steps include (1) screening GPS collar data for large errors (Chapter 2), (2) testing for serial autocorrelation and determining time to statistical independence, (3) testing for site fidelity (or the non-random use of a home range), (4) testing for home range asymptotes using area-observation plots with objective methods of delineating where an asymptote is approached, (5) reporting relevant details about the software used and about the home range estimator, and (6) delineating core ranges using an objective and area-independent method. To satisfy some of the necessary considerations in reporting and implementing a home range study here, we follow the recommendations of Laver & Kelly (2008) for a population of banded mongooses (*Mungos mungo*) in northeastern Botswana. We also applied a novel method for eliminating fixes with large error from global positioning system (GPS) collar data (Chapter 2).

Banded mongoose are small-bodied (< 2 kg), diurnal carnivorans in the family Herpestidae. They have a relatively wide habitat tolerance and range through large parts of sub-Saharan Africa, but generally avoid desert, semi-desert, and lowland equatorial rainforest. Banded mongooses breed communally exhibiting a variation of cooperative breeding (Gilchrist, 2004, Hodge, 2005, Rood, 1974), but they exhibit limited social dominance (Cant, 2000, Gilchrist, 2006) and they exhibit low reproductive skew (De Luca & Ginsberg, 2001). Estimates of group sizes and banded mongoose densities from three ecosystems suggest that banded mongooses exhibit some variability

among populations and ecosystems in their movement ecology, group size limits, and inter-group spacing (Table 3.1).

For instance, in the Kruger National Park, South Africa, banded mongooses have troops of up to 75 animals (Pienaar, 1964), while in Queen Elizabeth National Park, Uganda, the largest reported group size from studies spanning 30 years (Cant, 2000, De Luca & Ginsberg, 2001, Gilchrist & Otali, 2002, Neal, 1970, Rood, 1975) was 44 animals (Cant, 2000). In the Serengeti National Park, Tanzania, banded mongooses may have troops of up to 29 animals (Waser *et al.*, 1995). Further, banded mongoose density in Queen Elizabeth National Park varies between 17 km^{-2} (Rood, 1975) and 28 km^{-2} (Cant *et al.*, 2002) but between 0.5 km^{-2} in the short-grass plains and 2 km^{-2} in the woodlands of the Serengeti National Park (Waser *et al.*, 1995). Social group size, home range size, and degree of home range overlap are related and are important in understanding resource use and sociality. Thus, if banded mongooses exhibit a high degree of territorial defense and exhibit a low degree of home range overlap, then a banded mongoose population at a low population density presumably has troops of small group size with large home ranges, or the habitat can support mongoose troops only in localized and sparsely-distributed areas.

Researchers have estimated home range size for banded mongooses in only one ecosystem to date. Based on studies done at different times and using different home range estimators, banded mongooses in Queen Elizabeth National Park, Uganda, used home ranges averaging 76.4 ha (Gilchrist & Otali, 2002) (Interquartile range (IQR): 61 ha to 101 ha for the 15 home ranges reported by Rood (1975) and Gilchrist & Otali (2002)). Home range size increased with increasing troop size in one study, which used minimum convex polygons (Cant *et al.*, 2001), but not in another study on the same population, which used kernel density estimation (Gilchrist & Otali, 2002). Disparate inferences such as this could stem from using different home range estimators, especially if researchers compare minimum convex polygon home ranges within or among studies.

Here, we present a detailed home range study for banded mongooses in an ecosystem for which no prior baseline ecological data exist. Our study site offers the additional opportunity to incorporate two important elements that potentially affect banded mongooses: endemic disease and anthropogenic change. Unlike the populations of banded mongooses studied previously (Table 3.1), our study population is infected by a novel pathogen, *Mycobacterium mungi*, a species in the *Mycobacterium tuberculosis* complex (Alexander *et al.*, 2010). We do not know the reservoir of, or the host range for this pathogen. An understanding of the foraging behavior and home range and movement dynamics of troops in this population may shed light on possible sources of *M. mungi* infection, population impacts of pathogen persistence, and exposure risk for other potentially-susceptible hosts, including humans.

Our study population also included troops that exhibited various levels of association with humans (synanthropy, i.e. living with, or benefitting from an association with humans), from complete home range overlap with modified landscape, to troops with little or no use of modified landscape. For ease of comparison, we suggest and hereafter use the term “apoanthropic” to describe wildlife living away from or without association with humans. An understanding of the

foraging behavior and home range dynamics of banded mongooses along a gradient of synanthropy may shed additional light on the epidemiology of *M. mungi* infections in banded mongooses.

3.3 Methods

3.3.1 Study area

We conducted our study in a dystrophic, or nutrient-poor, savanna woodland ecosystem in northeastern Botswana from October 2007 to November 2011. We opportunistically monitored 41 banded mongoose troops in our greater study area (Figure 3.1a). We focused our behavioral observations on 13 troops in the northeastern corner of the Chobe National Park ($\sim 30\,\mathrm{km}^2$), in the northern Kasane Forest Reserve ($\sim 73\,\mathrm{km}^2$), and in the towns of Kasane and Kazungula ($\sim 17\,\mathrm{km}^2$) at 25.163° E, 17.828° S (Figure 3.1b). Botswana has a subtropical climate with annual mean (standard deviation) rainfall in Kasane (1975 – 2005) of 574 mm (162 mm) (Batisani & Yarnal, 2010). Based on monthly rainfall data for our study area from 1994 to 2006, we delineated a wet season (November, December, January, February, March), a dry season (May, June, July, August, September), and transition months (April, October). The transition months in our study area may receive spatially-sporadic rain in some years but not in others and even with daily rainfall data from the local meteorological station we could not definitively characterize these months as either wet or dry across our study area during our study period.

Our study area has gleysol, fluvisol, luvisol, and arenosol soil groups (Aarrestad *et al.*, 2011) at an elevation of 927 to 1012 m above mean sea level. Vegetation in our study area was riparian woodland along the Chobe River, dominated by *Acacia nigrescens*, *Croton megalobotrys*, *Kigelia africana*, *Ficus sycomorus*, *Trichilia emetica*, *Capparis tomentosa*, *Garcinia livingstonei*, *Combretum elaeagnoides*, and *Dichrostachys cinerea*, and adjacent woodland dominated by *Baikiaea plurijuga* (Mosugelo *et al.*, 2002). Field layer vegetation (herbaceous plants, grasses) included four main plant communities dominated by *Panicum maximum*, *Tribulus terrestris*, *Chloris virgata*, and *Cynodon dactylon*, respectively (Aarrestad *et al.*, 2011). Soil macrofauna in our study area consisted of arachnids, hymenopterans (formicids from the subfamilies Myrmicinae and Ponerinae), coleopterans (larvae of Elateridae and Scarabaeidae and adults of Tenebrionidae, Staphylinidae, Curculionidae, and Coprinae), and isopterans (*Microtermes* spp., *Cubitermes* spp., *Macrotermes michaelseni* and *Hodotermes massambicus*) (Dangerfield, 1997). The towns of Kasane and Kazungula had an estimated 9008 and 4133 residents in 2011, respectively (Botswana Central Statistics Office, 2011). In the dry season, the combined population of these two towns increased by > 600 people per day during the peak of the tourist season (estimate based on the monthly sales figures from ten lodges for 2008 – 2011).

3.3.2 Trapping and immobilization

We telemetry-collared 36 animals in 13 troops (for a median of 158 days each, IQR = 67 to 338) from December 2007 to December 2011. We captured and immobilized additional animals for clinical and hemotological assessment of *M. mungi* infection (Alexander *et al.*, 2010), and for marking individuals for behavioral observations (Fairbanks *et al.*, In prep.). We trapped mongooses in rigid Tomahawk live traps (Tomahawk Inc., Hazelhurst, Wisconsin, USA) measuring 81.3 cm x 25.4 cm x 30.5 cm, baited with chicken or canned dog food.

We immobilized animals most effectively using 1.0 mg ml^{-1} injectable medetomidine hydrochloride (Domitor, Pfizer Inc., New York, NY, USA) at doses of 1.0 mg kg^{-1}. We estimated animal masses prior to injection by sight or by using a luggage scale on traps containing animals. For 65 animals, we effectively estimated their mass in this manner and achieved a median dose of 1.1 mg kg^{-1} (IQR: 0.97 mg kg^{-1} to 1.28 mg kg^{-1}). We reversed anesthesia for all animals with a medetomidine-equivalent dose of injectable atipamezole hydrochloride (Antisedan, Pfizer Inc., New York, NY, USA). For each immobilized animal we determined sex, estimated age, assessed body condition and tooth wear, counted ectoparasites, assessed reproductive status and took standard measurements of total, tail, and hind foot length, skull and neck circumference, and mass. We marked animals using colored collars, tattoos, hair clipping, and ear tags.

3.3.3 Radio and GPS telemetry

We collared animals with very high frequency (VHF) radio collars and GPS collars. We used three Telonics MOD-080 VHF transmitters (46 g) (Telonics, Mesa, AZ, USA), nine Sirtrack two-stage VHF transmitters (27 g) (Sirtrack Ltd., Havelock North, New Zealand), ten Telemetry Solutions TS-37 VHF transmitters (41 g) (Telemetry Solutions, Concord, CA, USA), and four Telemetry Solutions Quantum 4000 GPS/VHF transmitters (75 g). In some cases, collared animals died (e.g. from predation, as roadkill, as dogkill) before the collar batteries died, and we re-deployed these collars on other animals. In some cases we recaptured collared mongooses with dead or dying collars and replaced the collars. In some cases, collared animals dropped their collars within a few days of deployment. We collared adult mongooses with a median mass of 1341 g (IQR: 1280 g to 1515 g). The collars measured a median of 3 % of a collared animal's body mass (IQR: 2.1 % to 3.4 %). We located troops using a Communications Specialists R-1000 scanner and receiver (Communications Specialists, Orange, CA, USA), an AF Antronics folding three-element yagi antenna (AF Antronics, Urbana, IL, USA), and an Antenex omni-directional roof-mounted antenna (Laird Technologies, St Louis, MO, USA) and then approached the animals on foot or in a vehicle.

We obtained many of our location estimates (n = 1811) from direct observation, for which we estimated the center of the mongoose troop, noted a distinctive landscape or habitat feature at that point and then collected a GPS fix from a Garmin Foretrex 201 handheld GPS (Garmin Southern Africa, Honeydew, South Africa) once the troop had moved away from the point. For these 1811

observations we waited for the handheld GPS to obtain a 3-dimensional fix and we had a median GPS-estimated vertical accuracy of 9 m (IQR: 7 m to 10 m). In 1271 observations we estimated the spread of the group as the distance between the two animals with the farthest linear separation. For these 1271 observations we obtained a median group spread of 15 m (IQR: 5 m to 25 m).

For most of our location estimates (n $\approx$ 18 000) we directly observed the mongoose troops repeatedly at known locations for which we had already obtained GPS locations, or we observed mongooses at locations that we could reliably describe and subsequently find and obtain coordinates for, using satellite imagery (Google Earth, Mountain View, CA, USA).

In some cases, even with direct observation, we could not walk up to the point where the mongooses were. In these cases (n = 371 observations), we took a handheld GPS fix from our observation point, we estimated the azimuth to the mongoose troop using a Silva Ranger handheld compass with a split-sighting mirror (Johnson Outdoors Inc., Racine, WI, USA), and we estimated the offset to the mongoose troop using a visual estimate for short offsets (within $\sim$ 20 m) and using a Swarovski Optik 8x30 monocular laser range finder (Swarovski Optik, Absam, Tyrol, Austria) for large offsets (more than $\sim$ 20 m). For these 371 observations our median offset from the troop was 20 m (15 m to 30 m) and we corrected for these offsets prior to data analysis.

In a small percentage of observations we could not drive up to a mongoose troop due to inaccessable terrain and impassable vegetation, and we could not walk up to the troop due to a potential encounter with African elephants (*Loxodonta africana*), African buffalo (*Syncerus caffer*), or African lions (*Panthera leo*), in relatively closed habitat. In these cases we estimated the troop location by triangulation (n = 25 observations). For these triangulations we estimated the location of the troop and the associated 95 % error ellipse using Locate II (Nams, 1990). For triangulations with five or more bearings we used Tukey's method (Nams, 1990) and for triangulations with three or four bearings we used Lenth's (Lenth, 1981) maximum likelihood estimator. For these 25 observations we had a median 95 % error ellipse of 2.5 hectare (IQR: 1.2 ha to 11.9 ha). Because we anticipated only needing triangulation for very few location estimates, we did not employ the location error method (Zimmerman & Powell, 1995), but researchers should consider this method if they anticipate relying more heavily on triangulation.

From our four deployed GPS collars, one collar stopped transmitting a VHF signal at only half of its estimated battery life. This animal disappeared from its troop before we could trap it to retrieve the collar. From the remaining three deployed GPS collars we obtained 555 successful GPS fixes. From these collars, we rejected 127 GPS telemetry locations with large error using our novel screening method (Chapter 2), and we accepted 428 fixes.

3.3.4 Den use

To characterize banded mongoose den use, we found mongoose den sites, which were the sites where mongooses rested overnight and where they raised their litters in the breeding season. For the majority of our denning observations we found the den sites by homing in on troops that had

a radio-collared animal before dawn, before the mongooses awoke and emerged from the den. In a small percentage of observations we followed the same homing method after sunset, after the mongooses had entered their dens. For an additional small percentage of observations we observed troops emerging from their evening dens just after dawn, or entering and remaining in their evening dens at around sunset. We used this method exclusively for den observations for troops in which we did not have a radio-collared animal. For a small percentage of observations we also found adult mongooses guarding pups at dens (Rood, 1974) or adult mongooses returning to dens to provision pups during the day. We classified dens that the mongooses used by their structure and then grouped dens as being man-made (buildings, building materials, scrap materials, French drains, slash piles, overturned boats) or natural structures (termite mounds, holes in standing trees, holes in the ground, hollow logs, rock piles). In many of the observations in which the mongooses noticed us as we observed them either entering or emerging from a den, the troop used the same den as they had the previous day (when they noticed us as they approached and entered a den in the late afternoon) or used the same den the following day (when they noticed us as they emerged from a den in the morning). We do not believe that our presence affected where the mongooses chose to den, but we did not test for this and cannot exclude this as a possibility.

3.3.5 Group size counts

We counted troop members by observing them directly. For an estimate of the population size and median troop size in our study area, we used counts of adult troop members during the dry season, before mating and potential troop evictions had begun. This season also provided the most reliable troop counts because leaf cover was generally sparse, grass cover was virtually absent, and no adults remained at dens because juveniles had by then joined the adults on all foraging bouts. For our core study troops we counted troop members whenever possible during troop observations throughout the year. For these troops we had multiple counts for each month of each year of the study and we used the modal count for a troop in a month. We labeled every troop count at each observation as certain or uncertain and typically ignored the uncertain counts in our analysis unless count data were sparse for a troop in a given month. We denoted a count as certain if we observed the troop in a large open space where we had unobstructed views of the group for several minutes or if we observed the troop moving past an arbitrary landmark where we could count each animal as it passed. At every count we attempted to classify every animal as juvenile ($\sim$ 0 to 6 months), subadult ($\sim$ 6 to 12 months) or adult (approx. $>$ 12 months) based on their body size, but we report troop sizes based only on counts of adults here. For our core study troops we could follow a cohort from their emergence from the den at approximately four weeks of age and we used approximate ages based on these dates of emergence and hence putative parturition to calibrate our estimates of body size. We also attempted to classify each adult mongoose in each count as male or female based on the presence or absence of descended testes, and evidence of pregnancy or lactation.

3.3.6 Site fidelity and movement metrics

We documented home range behavior in banded mongoose troops by testing for site fidelity (i.e. non-random space use) following Spencer *et al.* (1990). To measure how mongooses concentrated their movements, we calculated the mean squared distance of every location estimate from the center of activity (MSD) (Calhoun & Casby, 1958). To measure how linearly mongooses move, we used the linearity index (LI) calculated as the ratio of the 'start-to-end' distance to the total distance traveled for consecutive location estimates over a given period of time (Bell & Kramer, 1979). We tested for site fidelity in each troop in each year. For each troop*year dataset we generated 300 random permutations of their movements using the actual distances traveled between consecutive locations but with random azimuths (range: 0° to 360°). The mean and standard deviation of both the linearity index and mean squared distance for the 300 random permutations stabilized below 100 simulations. Yearly datasets exhibited site fidelity if the actual mean squared distance and linearity index were less than the mean and 95 % confidence interval for the mean squared distance and linearity index from the associated random permutations.

To estimate the time to statistical independence of location estimates (Swihart & Slade, 1985), we calculated Schoener's Ratio, calculated as the ratio of the mean squared distance between successive observations to the mean squared distance from the center of activity (Schoener, 1981). We determined the hourly interval at which Schoener's Ratio was consistently > 2 for each troop and then the interval at which the median Schoener's Ratio for the six troops was > 2.

For six mongoose troops with sufficient data, we also estimated day range, the daily distance traveled (Carbone *et al.*, 2005). We estimated this distance as the Euclidean distance between consecutive location estimates for troops for which we had ≥ 10 location estimates spanning ≥ 5 h in a day.

3.3.7 Home range estimation

To test for the validity of our home range estimates, we tested for home range asymptotes using area-observation plots (Harris *et al.*, 1990, Otis & White, 1999). We randomized (Harris *et al.*, 1990) and resampled (Hansteen *et al.*, 1997) the location data for each of 11 troops to obtain five simulations. We delineated the number of location estimates at which our home range estimates approached an asymptote as the point at which the mean and 95 % confidence interval of the simulations consistently fell within 15 % of the final home range size. We removed from our home range analysis any troops that did not approach an asymptote.

We estimated home range size for banded mongoose troops for which we had at least 30 location estimates (Seaman *et al.*, 1999). We used kernel density estimation (Worton, 1989) with a fixed, biweight kernel (Seaman & Powell, 1996), volume contouring, and unit variance standardization (Silverman, 1986). We selected our kernel bandwidth using least-squares cross-validation (LSCV) (Bowman, 1984), which did not fail for any of our analyses. We used different grid cell sizes for each home range estimate, using a resolution of 75 grid cells along the shorter of the

X or Y axis for each troop. This resolution resulted in a median grid cell size of 0.08 m (range: 0.05 m to 0.12 m) on a unit covariance matrix, which was equivalent to a median of 16.21 m (range: 12.9 m to 32.8 m) on an unstandardized scale. We used a kernel function (Eqn 3.1) (Silverman, 1986) with a kernel, K, defined by a biweight kernel, K_2 (Eqn 3.2) (Silverman, 1986), a smoothing factor (smoothing parameter, or bandwidth), h, the number of location estimates, n, and vectors of the coordinates of the evaluation point, and all other points, x and X, respectively.

$$\hat{f}(x) = \left[\frac{1}{(nh^2)}\right] \sum_{i=1}^{n} K\left\{\frac{(x - X_i)}{h}\right\} \tag{3.1}$$

The biweight kernel (Eqn 3.2) selects location estimates within a search radius (the smoothing factor, h) of an evaluation point, using the distance from the evaluation point to any other point, divided by the smoothing factor ($x'x$).

$$K_2(x) = \begin{cases} 3\pi^{-1}(1 - x'x)^2 & \text{if } x'x < 1 \\ 0 & \text{otherwise} \end{cases} \tag{3.2}$$

We applied a constant, $A(K) = 2.04$, to convert the smoothing factor for the normal kernel (used in the least-squares cross-validation) for use with a biweight kernel (Silverman, 1986). We minimized the loss function, $M_1(h)$ (Eqn 3.3) (Rodgers & Carr, 1998, Silverman, 1986, Worton, 1995), and found the global minimum using a golden section search, Routine GOLDEN (Sprott, 1991), to obtain an estimate of the least-squares cross validation smoothing factor for each dataset.

$$M_1(h) = \frac{\sum_i \sum_j \left(\left(\frac{e^{\left(\frac{-x'x}{4}\right)}}{4\pi} \right) - 2 \left(\frac{e^{\left(\frac{-x'x}{2}\right)}}{2\pi} \right) \right)}{n^2 h^2} + \frac{1}{\pi n h^2} \tag{3.3}$$

We delineated the home ranges at the commonly used, but arbitrary 95 % volume contour on the density surface. We clipped these contours to dry land in our study area, removing the Chobe River, a quarry, a wetland and sewage settling ponds from the home ranges. We retained all buildings in our home range estimates because mongooses in our study area regularly foraged and denned in or under buildings. We estimated core ranges using area-probability curves following Seaman & Powell (1990) and Powell (2000).

We subdivided each troop's location data by season for seasonal range and seasonal core range analyses. We excluded data for the transition months from our seasonal analyses to prevent spurious inferences from mis-labeling these months as wet or dry. We performed our seasonal analyses as we did for our overall home ranges and core ranges. We did not repeat the asymptote analyses or site fidelity analyses for each seasonal dataset.

3.3.8 Foraging and drinking behavior

In all observations of mongooses foraging, we recorded whether mongooses foraged in refuse. In all observations of mongooses drinking, we recorded whether mongooses drank from anthropogenic water sources, and we recorded what the water source was (e.g. lawn sprinkler, leaking faucet, greywater, sewage, kitchen drainage pipe).

3.3.9 Software and project approval

For all home range analyses, core analyses and asymptote analyses, we used ABODE (Beta v. 5) (Laver, 2005) in ArcMap 9.3.1 (Environmental Systems Resource Institute, 2009). For all other analyses we used R (R Core Team, 2012) with code that we wrote based on published algorithms and with standard algorithms in the base R distribution. We conducted our study with approval of the Virginia Tech Institutional Animal Care and Use Committee (07-146-FIW) and the Botswana government, Ministry of Environment, Wildlife, and Tourism.

3.4 Results

From direct observation, we had reliable counts of group size for 34 troops with an overall study population size of $\sim$ 597 adults and a median troop size of 13 adults (IQR: 11 to 21 adults). The distribution of troop sizes in the study population was leptokurtic and right-skewed. Banded mongooses in our study population exhibited yearly site fidelity, exhibiting more concentrated and less linear movement than we expected from simulated random permutations of their movements (Figure 3.2a, b). We used 12 mongoose troops with location data for one to four years each, resulting in 31 (troop*year) datasets and 20 524 location estimates in total (median of 301 location estimates per dataset). In only one of 31 yearly datasets did a troop not exhibit site fidelity. This dataset spanned a long-distance dispersal by a GPS-collared troop. We removed this troop from further analyses. The kernel density estimate of total home range size for one troop did not approach an asymptote (Figure 3.3a) and we excluded this troop from further analyses of total home range size. Kernel density estimates of total home range size for ten other troops approached asymptotes at a median of 335 location estimates (IQR: 135 to 478) (e.g. Figure 3.3b). For six troops with sufficient data, we used 17 357 location estimates with a median of 2134 location estimates per troop to assess time to statistical independence. Four mongoose troops exhibited times to statistical independence of 2 h, 4 h, 4 h and 6 h, respectively (e.g. Figure 3.4a), and six troops exhibited time to statistical independence of 4 h, based on their combined median Schoener's Ratio (Figure 3.4b).

For overall home range estimation, we used 7093 location estimates with a median of 589 location estimates per troop (IQR: 254 to 861). Ten troops had a median 95 % kernel density home range of 68 ha (IQR: 39 ha to 134 ha) and a median core range of 15 ha (IQR: 9 ha to 28 ha), with

the cores delineated at a median volume contour of 66 % (IQR: 58 % to 71 %) (Table 3.2). Eight troops had a median wet season range of 44 ha and median dry season range of 29 ha (Table 3.2). For estimates of day range, we used data from six troops with a total of 8993 location estimates over 197 days for a median of 27 days per troop, and a median of 38 location estimates and 8.5 h per day. Troops had a median wet season day range of 1.5 km and a median dry season day range of 0.9 km (Table 3.2).

Apoanthropic troops (i.e. those living away from humans) showed more diffuse use of their home ranges, with area-probability curves closer to random use (Figure 3.5, a and b) than synanthropic troops (i.e. those living with humans), which had more clumped home range use (e.g. Figure 3.5, c). Two troops with similar home range sizes — one apoanthropic (Figure 3.5, a) and one associated with five lodges and the town of Kasane (Figure 3.5, c) — demonstrated extreme differences in concentrating their use of their home ranges. The apoanthropic troop (Figure 3.6a and c) showed less extreme clumping in its kernel density surface, and hence, a larger core area, than did the synanthropic troop (Figure 3.6b and d), which concentrated its use in much smaller core areas around lodges and refuse sites.

Tourist lodges and safari operators dominate the economy of the study area and we had twenty lodges within our core study site, of which, banded mongooses used fourteen for foraging and denning habitat. Our study area also included a town landfill site. We had one banded mongoose troop that lived near the landfill site, but we had no evidence suggesting that this troop used the landfill, based on either our direct observations or movement data while we had the troop collared with a GPS collar. During the period over which we monitored this troop, municipal staff bulldozed the landfill almost continuously and an electric fence and a high concentration of marabou storks (*Leptoptilos crumeniferus*) also reduced the accessibility of the landfill for this troop. In Queen Elizabeth National Park, Uganda, marabou storks predated banded mongoose pups at refuse sites (Otali & Gilchrist, 2004) and we think that marabou storks may deter mongooses at the town landfill in our study area.

When available, banded mongooses in our population readily used man-made structures to den in (Table 3.3). We found mongoose dens on 525 nights from May 2008 to November 2011, for a total of 1239 den observations for 17 troops and a median of 15 nights per troop (IQR: 2 to 138). We obtained most (1223) of our observations from our ten main study troops, for which we had a median of 126 den observations per troop (IQR: 28 to 173). Mongoose troops used a median of 30 unique den sites (IQR: 27 to 36). They spent a median of two to three consecutive nights at a den and returned to the same den site after a median of 106 nights (IQR: 50 to 131). Synanthropic troops used man-made structures for den sites for 81 % of nights (Table 3.3). They favored buildings and structures (used for 38 % of nights), building material (20 % of nights), scrap (15 % of nights), and French drains (7 % of nights) (Table 3.3). Apoanthropic troops favored hollow logs (67 % of nights, compared to 1 % for synanthropic troops), termite mounds (28 % compared to 6 % for synanthropic troops), and holes in standing trees (6 % compared to 3 % for synanthropic troops) (Table 3.3).

In addition to synanthropic mongooses concentrating their home ranges and core ranges around

lodges and town refuse sites, access to anthropogenic resources also altered mongoose foraging behavior. We observed mongooses feeding from refuse in 110 of 850 (13 %) foraging observations and in 78 % of all observations of mongooses drinking, they drank from anthropogenic water sources. Of these anthropogenic water sources, mongooses drank most often from greywater and sewage (21 %) and lawn sprinklers (17 %).

3.5 Discussion

We have presented here a detailed home range study for banded mongooses. As expected from studies on banded mongooses in other ecosystems, troops in our study population exhibited site fidelity. The metrics that we used to assess site fidelity correctly identified one group of mongooses which dispersed from an established troop. Although we presented a median number of location estimates required for our kernel density estimates of home range size to approach an asymptote, we do not recommend that future studies on banded mongoose home ranges base their sampling design on these results. Researchers must consider several factors and, importantly, their research objectives, when designing their home range studies (Fieberg & Börger, 2012). Instead, we recommend that researchers use asymptote analyses, as we have shown here, during the data analysis phase to remove home range estimates for animals or social groups, when those estimates do not approach an asymptote. Further, we have provided here an objective, although arbitrary, method for delineating the point at which kernel density home range estimates approach an asymptote.

We have also presented here estimates of banded mongoose home range size, core range size, wet season and dry season range size and core size, and estimates of day range. We purposefully chose to model banded mongoose home ranges using kernel density estimation, which, in spite of its potential problems has well-known statistical properties, has a long history in the literature, requires readily-available software, and researchers can implement it relatively easily. Although newer techniques such as mechanistic models, Brownian bridges, local convex hulls, state-space models, and artificial neural networks may have improved upon some aspects of traditional kernel density estimation, we hope that using this technique will facilitate comparative studies of banded mongoose ranging behavior across ecosystems and facilitate the use of banded mongooses in macro-ecological applications such as Carbone *et al.* (2005) and Jetz *et al.* (2004).

Importantly, we present overall and seasonal core ranges based on an area-independent method. Using this method, both the median and interquartile range of the percent volume contour at which we delineated cores were above 50 % and we never delineated any cores at the commonly, but incorrectly used 50 % volume contour. The method we employed delineates cores using a statistical property of the data and reflects an ecologically meaningful aspect of home range use, namely the part of the home range where an animal (or social group) exhibits clumped or non-random and non-even space use. The 50 % volume contour reflects an arbitrary delineation without any valid statistical or ecological interpretation, and we recommend that researchers avoid

using this delineation. Further, this area-independent method for delineating cores allows researchers to make additional inferences about home range use by different animals or social groups. We show here, using this technique, that (independent of home range or core range area), synanthropic banded mongoose troops tend to have more clumped home range use than apoanthropic troops.

Banded mongooses show some plasticity in their movement ecology and spacing on a landscape and may present a useful model for home range studies. Banded mongooses in our study area used home ranges that varied 6-fold in size among different troops. The intensity with which they use different areas within their home ranges can also vary greatly, with some core ranges exhibiting 10-fold differences in size among our study troops. Researchers have published data on banded mongooses from only four ecosystems (Table 3.1). Among these ecosystems, banded mongoose population density varies by up to 55-fold (Table 3.1). Even within the same ecosystem, but in different habitat types, population density can vary by up to 3-fold. Although mean or median troop size appears to vary among ecosystems by only a small amount (0.4-fold), maximum troop sizes may vary by up to 1.6-fold. Researchers should conduct more research on populations of banded mongooses in other ecosystems in their geographic range, and should investigate what factors affect banded mongoose space use, spacing and group sizes, both within and among ecosystems.

In our study site, access to anthropogenic resources may affect where and to what degree banded mongooses concentrate their movements and may affect what resources banded mongooses use. Researchers need to investigate this potential effect of synanthropy on banded mongoose movement, denning, and foraging ecology in future research. Although we did not assess resource availability or resource selection for den sites here, our results show that banded mongooses use anthropogenic resources for denning when they are available. Synanthropic troops in our study population used these resources in the majority of den observations that we made. Further, based on observations of foraging, synanthropic banded mongooses fed frequently from refuse sites and drank readily from anthropogenic water sources. These refuse sites were isolated to only a few places within any mongoose troop's home range, and given their sparse and spatially constrained nature, the percentage of refuse-feeding observations (13 %) suggests that these sites represented important resources to synanthropic mongooses. Researchers should investigate this further with a resource selection study.

Beyond the general movement ecology of banded mongooses, the home range and foraging behavior of banded mongooses in our study area suggest that anthropogenic resources may play a role in the disease ecology of *M. mungi*. *M. mungi* infects mongooses predominantly through lesions in the *planum nasale* (nasal plane) (Alexander *et al.*, 2010). Banded mongooses use their noses extensively while foraging, both to investigate food items, and while digging in soil. The concentration of banded mongoose movement around lodges and refuse sites, and the substantial use of refuse in mongoose foraging that we present here, both suggest that banded mongooses may have considerable contact between their *plana nasale* and substrates found in highly modified parts of the landscape. While banded mongooses rest and den, they often have their *plana nasale* in contact with substrates, and we showed here that synanthropic troops used man-made

structures extensively for their den sites. Although we do not know to what degree banded mongooses shed *M. mungi* into the environment, or if this pathogen could subsequently infect other species, the concentrated use of anthropogenic resources by banded mongooses suggests that these areas in the landscape may be important in pathogen transmission and may form part of a potential environmental reservoir of *M. mungi*. In spite of this, *M. mungi* does infect apoanthropic troops and thus, access to anthropogenic resources may only be part of the puzzle. Researchers need to determine if there is a difference in the prevalence of *M. mungi* infections among these two broad groups of banded mongoose troops and researchers need to determine if access to anthropogenic resources can affect banded mongoose susceptibility to *M. mungi* infection.

3.6 Acknowledgments

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Table 3.1. Banded mongoose (*Mungos mungo*) population density, troop size, and home range size in four ecosystems. The studies reporting home range size in this table, both used kernel density estimation with 95 % volume contouring.

Study site	Habitat	Density km^{-2}	Troop size central[a] (range[b])	Home range (ha) median (IQR)
Serengeti NP[c]	Short-grass plains	0.5[d]		
Serengeti NP	Woodland	2[d]	15 (4 – 29)[d]	
Queen Elizabeth NP	Savanna grassland	17[e] – 28[f]	14 (9 – 20)[g]	76 (62 – 97)[h]
Kruger NP			(? – 75)[i]	
ne Botswana	Woodland, riparian, urban	8[j]	13 (11 – 21)[j]	68 (39 – 134)[j]

[a] Measure of central tendency. Mean for [d], median for [g,j].

[b] Total range for [d,i], interquartile range (IQR) for [g,j].

[c] National Park (NP)

[d] Waser *et al.* (1995)

[e] Rood (1975)

[f] Cant *et al.* (2002)

[g] Cant (2000)

[h] Gilchrist & Otali (2002)

[i] Pienaar (1964)

[j] This study

Table 3.2. Home ranges, overall core ranges, seasonal ranges, seasonal cores, and day ranges for ten banded mongoose (*Mungos mungo*) troops in northeastern Botswana (2008 — 2011). We delineated home ranges and seasonal ranges at 95 % volume contours, and the overall and seasonal cores by statistically clumped distributions.

	Overall home range (ha)					Seasonal range (ha)				Day[a] (km)	
						Wet (ha)		Dry (ha)		Wet	Dry
Troop	n_{locs}[b]	Asym.[c]	95 %	Core	%[d]	95 %	Core	95 %	Core		
cch	843	500	50	9	71	38	11	31	11	1.7	0.6
cgl	1159	900	33	9	70	29	9	12	6	1.3	0.9
csl	2284	1250	134	17	65	164	30	68	22	2.1	0.9
dum	80	70	194	50	61						
evl	79	66	175	31	55						
hip	206	62	131	74	77	94	43	124	50		
kub	397	330	41	12	67	47	21	27	12	2.2	0.9
mow	585	340	38	7	55	40	13	26	7		
sef	867	330	86	18	57	73	16	38	7	0.4	0.2
trs	593	410	26	7	75	23	6	22	7	0.4	1.2

[a] Day range (daily distance traveled)

[b] Number of location estimates

[c] Asymptote: Number of location estimates at which an asymptote was approached

[d] Percent volume contour for statistical core

Table 3.3. Percentage of nights spent in various den types by banded mongooses (*Mungos mungo*) in northeastern Botswana (2008 – 2011) (this study) and Queen Elizabeth National Park, Uganda (Rood, 1975). We weighted percentages by the number of observations for each of 11 synanthropic and 6 apoanthropic troops observed on 525 nights for 1239 (troop*night) observations. We obtained most (1193) of our observations for synanthropic troops from eight of our ten main study troops, for which we had a median of 147 den observations each. We obtained most (27) of our observations for apoanthropic troops from one of our ten main study troops.

	northeastern Botswana[a]		Queen Elizabeth NP[b]
Den type	Synanthropic[c]	Apoanthropic[d]	
Man-made structures			
Buildings and structures	38		3
Building material	20		
Scrap	15		
French drains	7		
Overturned boat	1		
Slash pile	1		
Man-made total	81	0	3
Natural structures			
Hollow logs	1	67	
Termite mounds	6	28	65
Holes in trees	3	6	
Hole in ground	2		11
Rocks	6		
Erosion gullies			21
Natural total	19	100	97
Number of observations	1203	36	144
Number of troops	11	6	6

[a] Northeastern Botswana (Chobe National Park, Kasane Forest Reserve, towns of Kasane and Kazungula) (this study).

[b] Queen Elizabeth National Park, Uganda (Rood, 1975)

[c] Synanthropic (associated with humans)

[d] Apoanthropic (not associated with humans)

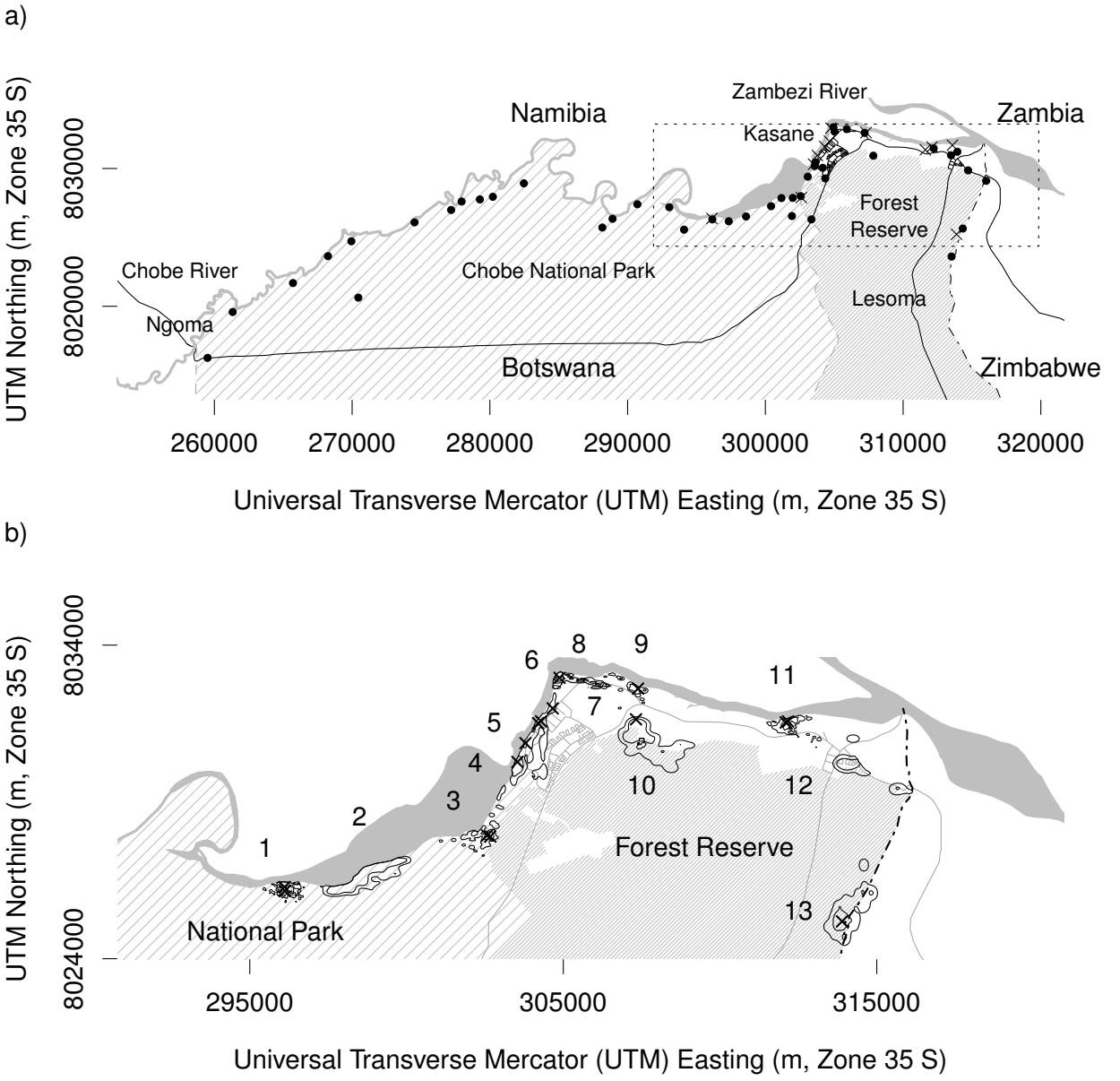

Figure 3.1. (a) Banded mongooses (*Mungos mungo*) in 41 troops (black dots) lived along the Chobe River (solid grey polygon) in northeastern Botswana (2008 – 2011). (b) Our core study troops (polygons of 95 % kernel density home ranges, 1 to 13) lived in the Chobe National Park (troops 1, 2, 3 and 4), Kasane Forest Reserve (troops 3, 10, 12 and 13), and in the towns of Kasane and Kazungula (troops 5, 6, 7, 8, 9, 11 and 12). Synanthropic troops (i.e. those living with humans) lived at lodges (troops 1, 3, 5, 6, 7, 8, 9, 11 and 13) or in towns (troop 12) or in close association with a military camp (troop 4). Two (apoanthropic) troops had no access to anthropogenic resources (troop 2 and 10). Troop 10 lived near the town refuse site but did not forage in the site. Black crosses indicate substantial lodge or town refuse sites.

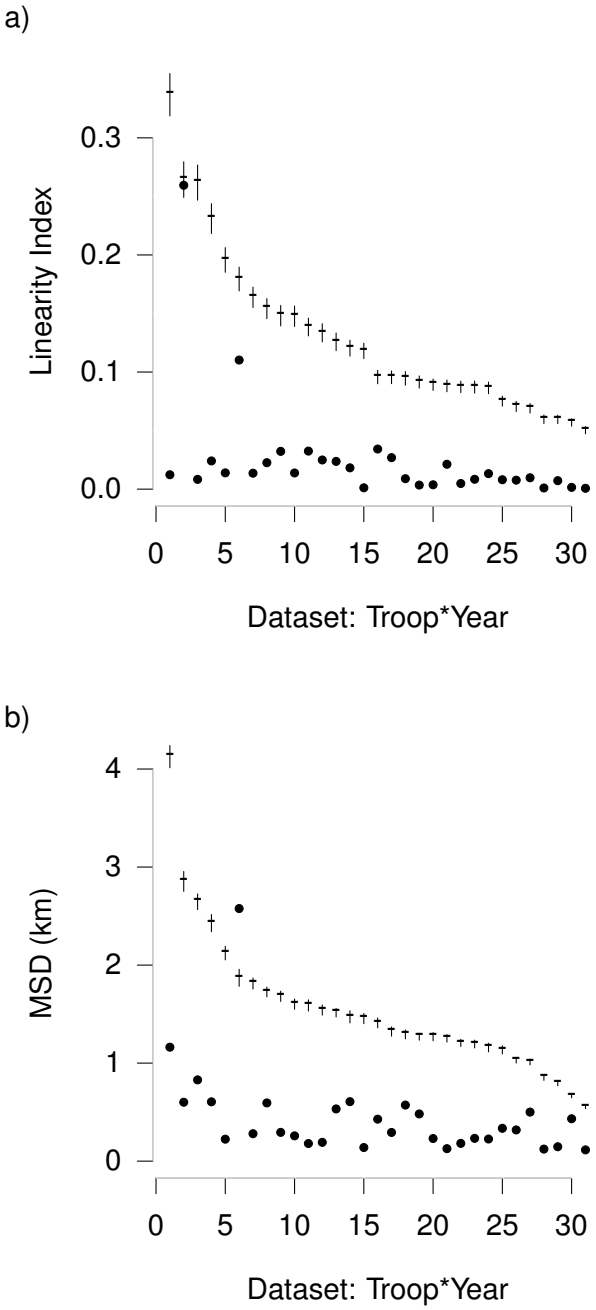

Figure 3.2. Yearly movements (black dots) of banded mongoose (*Mungos mungo*) troops in northeastern Botswana (2008 – 2011) had (a) lower linearity indices (i.e. less linear movement) and (b) lower mean squared distances (i.e. more concentrated movement) than the mean and 95 % confidence interval (dash with error bar) of 300 simulated random walks per troop per year. We excluded from further analysis one yearly dataset that did not display site fidelity (dataset 2 and dataset 6 in (a) and (b), respectively). This dataset spanned a dispersal by group of mongooses (including one animal with a GPS collar) evicted from a larger troop.

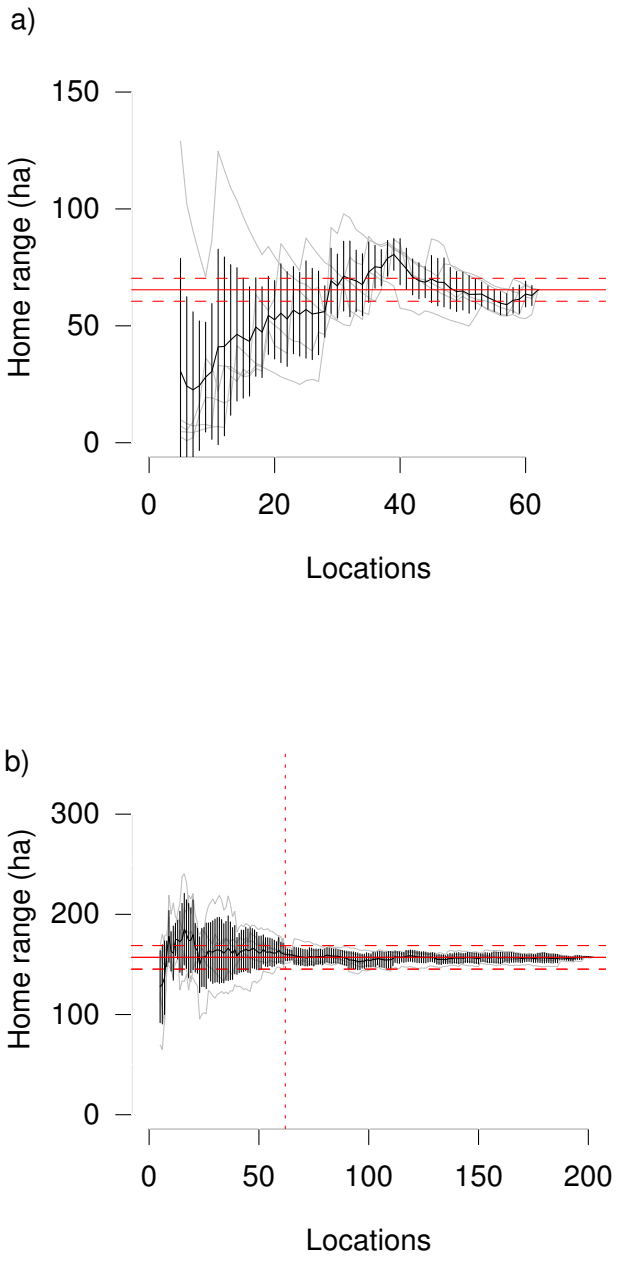

Figure 3.3. (a) The kernel density estimate of total home range size for one banded mongoose (*Mungos mungo*) troop in northeastern Botswana (2008 – 2011) did not approach an asymptote. (b) For other troops (as with this example), home range sizes approached an asymptote with a median of 335 location estimates (this example, 62 location estimates). Home range size approached an asymptote when the mean and 95 % confidence interval (black lines with error bars) of five randomizations (grey lines) fell and then remained within 15 % of the final home range size.

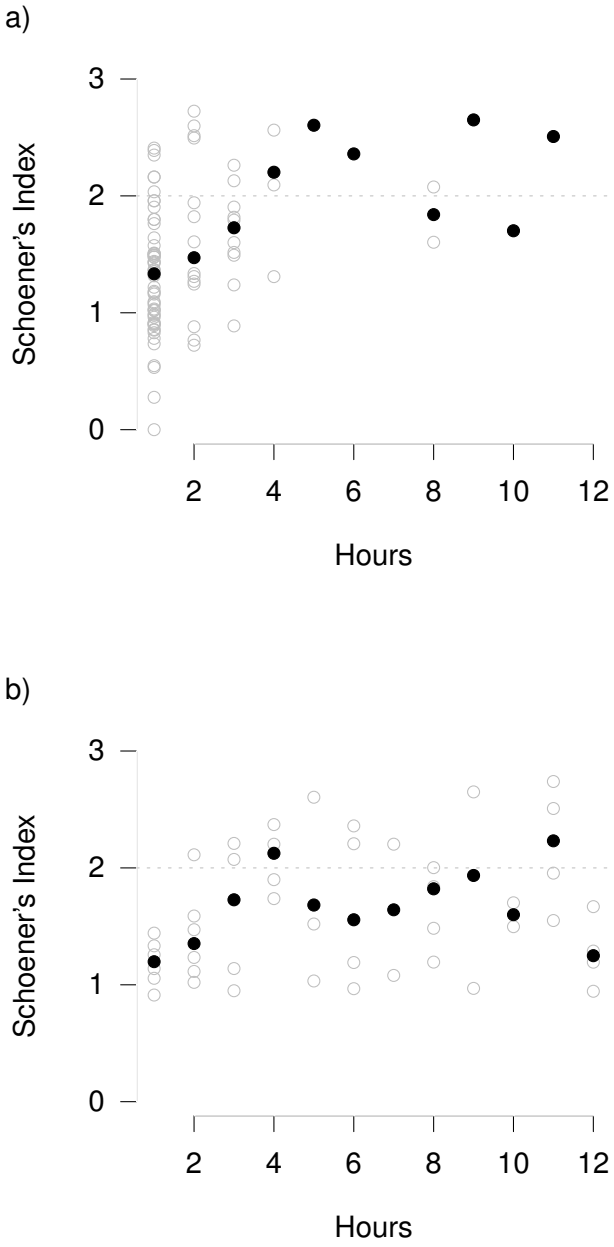

Figure 3.4. Schoener's Ratio (t^2/r^2) (grey dots) (a) for one typical troop, and (b) for six banded mongoose (*Mungos mungo*) troops in northeastern Botswana (2008 – 2011). Schoener's Ratio is approximately normally distributed with a mean of 2 (horizontal dotted line) for a dataset with more than 30 independent points from a non-shifting and non-expanding home range. The median Schoener's Ratio (black dots) suggested statistically independent movements at an interval of 4 h.

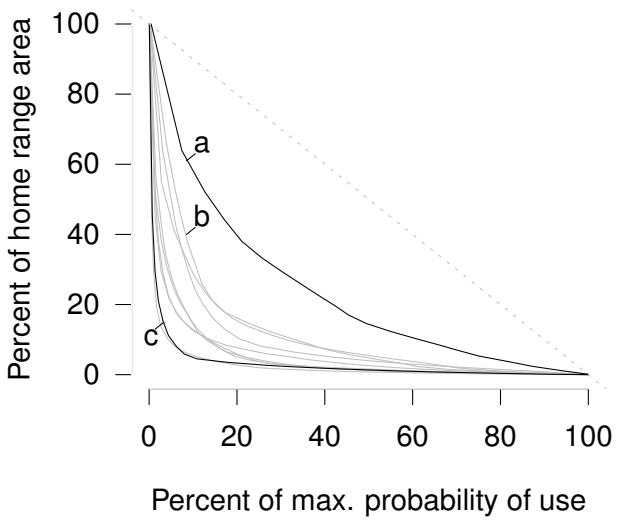

Figure 3.5. Area-probability curves for eight synanthropic and two apoanthropic (lines a and b) banded mongoose (*Mungos mungo*) troops in northeastern Botswana (2008 – 2011). Lines lying farther from the diagonal (dotted line) suggest more clumped use than curves closer to the diagonal. Troops can have differences in the concentration of their use of a home range (e.g. black lines, a and c). These two troops had similar home range sizes (131 ha and 134 ha, respectively) but dramatically different core range sizes (74 ha and 17 ha, respectively).

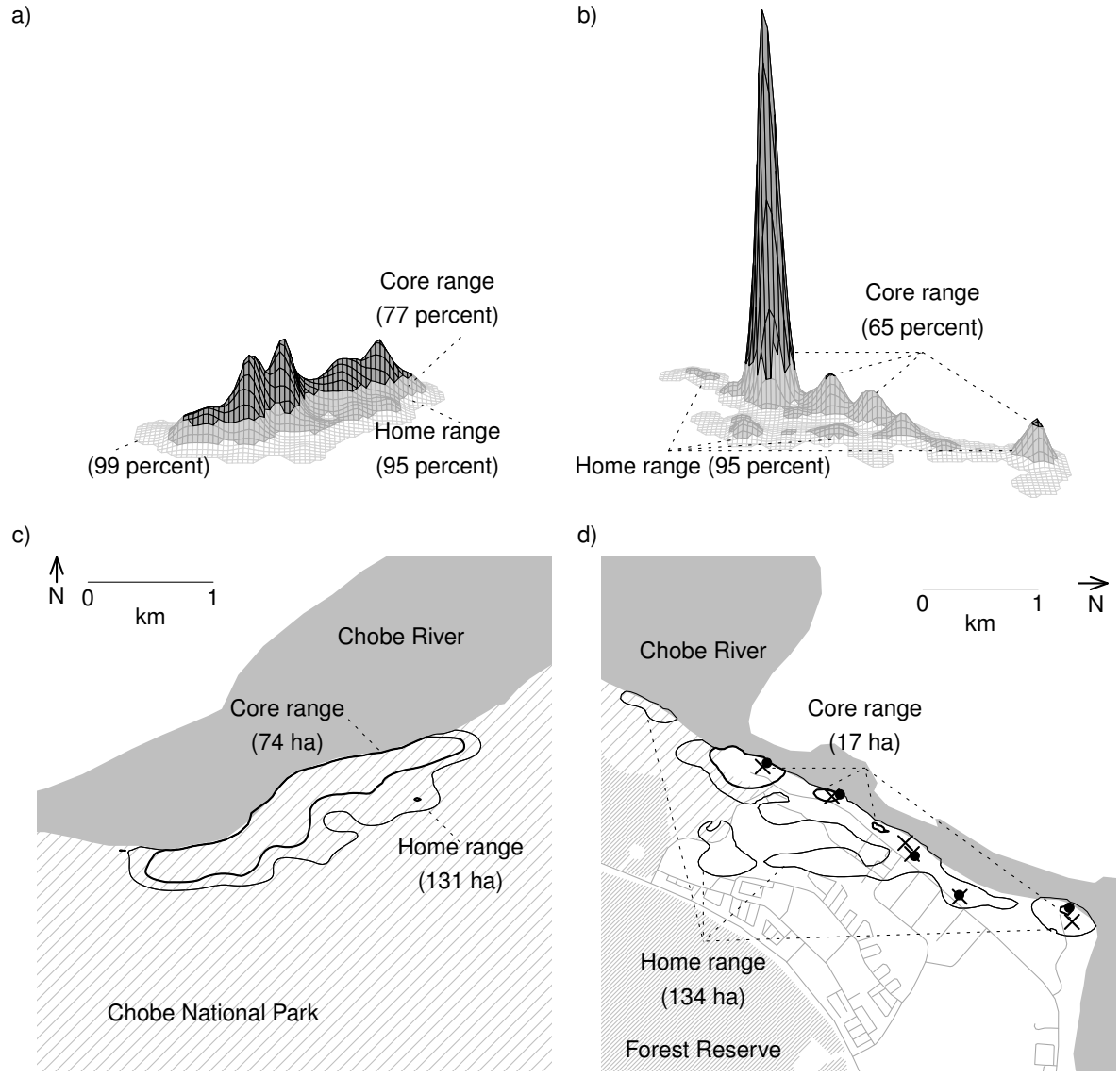

Figure 3.6. Utilization distributions (a and b) and home and core ranges (c and d) for two banded mongoose (*Mungos mungo*) troops in northeastern Botswana (2008 – 2011). An apoanthropic troop (a and c) lived in the Chobe National Park. A synanthropic troop (b and d) lived in the town of Kasane (road network in grey lines). This troop used six substantial refuse sites (crosses), five tourist lodges (black dots), a central business district, and residential areas. We scaled the two kernel density surfaces (a and b) to the same x, y, and z dimensions and rotated plots (b) and (d) by 90° for comparison. The z-axis (a and b) represents density of use. The limits of the light-grey, dark-grey, and black gridlines (a and b) represent approximate 99 % and 95 % volume contours and statistical cores, respectively.

Chapter 4

Non-invasive monitoring of glucocorticoid metabolites in banded mongooses (*Mungos mungo*) in response to physiological and biological challenges

Non-invasive monitoring of glucocorticoid metabolites in banded mongooses (*Mungos mungo*) in response to physiological and biological challenges

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The original published article appears in Appendix A

4.1 Abstract

Free-ranging banded mongooses are infected by the novel pathogen, *Mycobacterium mungi* in northern Botswana. A reliable method for determining stress-related physiological responses in banded mongooses will increase our understanding of the stress response in *M. mungi* infection. Therefore, our aim was to examine the suitability of four enzyme immunoassays (EIAs) for monitoring adrenocortical endocrine function in captive and free-ranging banded mongooses based on fecal glucocorticoid metabolite (FGM) analysis. A conducted adrenocorticotropic hormone challenge revealed suitability of a valid measurement of FGM levels in banded mongoose feces for all four tested EIAs, with an 11-oxoetiocholanolone assay detecting 11,17-dioxoandrostanes (11,17-DOA) performing best. Subsequent analyses using only this EIA showed the expected decrease in FGM concentrations 48 h after administering dexamethasone sodium phosphate. Furthermore, captive mongooses showed higher FGM concentrations during reproductive activity, agonistic encounters and depredation events. Finally, a late-stage, tuberculosis-infected moribund mongoose in a free-ranging troop had a 54-fold elevation in FGM levels relative to the rest of the troop. Measurements of gastrointestinal transit times and FGM metabolism post-defecation indicate that the time delay of FGM excretion approximately corresponded with food transit time and that FGM metabolism is minimal up to 8 h post-defecation. The ability to reliably assess adrenocortical endocrine function in banded mongoose now provides a solid basis for advancing our understanding of infectious disease and endocrinology in this species.

4.2 Introduction

Probably still the most widely used approach to monitoring endocrine function is the measurement of circulating hormones, but obtaining blood samples from wild, free-ranging animals is often difficult or impossible and may introduce confounding effects of capture- or handling-induced stress (Sheriff *et al.*, 2011). Non-invasive hormone monitoring, especially through excretions such as feces, urine and saliva, is already a well-established approach (Ganswindt *et al.*, 2012a, Keay *et al.*, 2006, Millspaugh & Washburn, 2004, Möstl *et al.*, 2005, Palme, 2005, Schwarzenberger, 2007, Sheriff *et al.*, 2011, Wielebnowski & Watters, 2007). It is not, however, without problems (Wielebnowski & Watters, 2007) and validation of non-invasive assays is vital (Touma & Palme, 2005). This is especially true when applied to a species for the first time, since the particular circulating glucocorticoids (e.g. cortisol versus corticosterone), and their metabolism and routes of excretion are species- (Palme *et al.*, 2005) and sex-specific (Palme *et al.*, 2005, Touma *et al.*, 2003).

Banded mongooses (*Mungos mungo*) are small, diurnal, cooperatively breeding mammals in the order Carnivora and family Herpestidae, distributed widely in sub-Saharan Africa. Free-ranging banded mongooses in our study population in northern Botswana are infected with the novel *Mycobacterium mungi* which has caused seven outbreaks in the population since 2000 (Alexander *et al.*, 2010). A viable non-invasive approach to monitor glucocorticoid output in banded mon-

gooses would facilitate research on the possible links between the stress response, allostatic overload and *M. mungi* infection dynamics. Unfortunately, no test system to monitor adrenocortical endocrine function based on fecal glucocorticoid metabolite (FGM) analysis has been validated to date for banded mongooses.

Therefore, our objectives were to a) validate an enzyme immunoassay for measuring FGMs in banded mongooses using physiological/pharmacological challenges and b) biological challenges associated with the reproductive cycle and chronic disease. Furthermore, we c) determined the gastrointestinal transit time and the rate of metabolism of FGMs post-defecation to further evaluate the approach of non-invasive hormone measurements as a tool to provide information on the level of stress experienced in this species.

4.3 Material and Methods

4.3.1 Study area and animals

We conducted this study on 13 troops (troop size range 5 to 64) of free-ranging banded mongooses and four (one female and three males) captive animals for almost four years from October 2007 to June 2011. We monitored free-ranging mongoose troops over an area of approximately 120 km^2 in the towns of Kasane and Kazungula (approx. 17 km^2 of developed land) and surrounding areas of the Chobe National Park (approx. 30 km^2) and Kasane Forest Reserve (approx. 73 km^2) in northeastern Botswana, with our study site centered at approximately 25.163° E and 17.828° S.

This area has rainfall seasonal, with a wet season from November to March and annual mean (SE) rainfall in Kasane (1975 – 2005) of 574 (30) mm (Batisani & Yarnal, 2010). Soil groups in the study included gleysols, fluvisols, luvisols and arenosols (Aarrestad *et al.*, 2011) and the altitude was between 927 and 1012 m above mean sea level.

The captive troop was housed together in an outdoor enclosure (approx. 95 m^2) at the Center for African Resources: Animals, Communities and Land use (CARACAL) research facility in Kasane. The four animals were raised in captivity from approximately two weeks of age and were adults of approximately three years of age at the time of the pharmacological challenges.

We conducted this study under approval and in accordance with the guidelines of the Virginia Tech Institutional Animal Care and Use Committee (07-146-FIW).

4.3.2 Observations and sample collection

For free-ranging troops we made approximately 3600 troop observations between October 2007 and June 2011, recording spatially-referenced weather, habitat, behavioral, clinical and other rel-

evant ecological data. For troops marked with radio collars, we found their evening dens in the morning before sunrise and returned to collect fresh feces later in the morning. We collected feces from unmarked troops opportunistically during observations, or after following troops to their dens the evening prior to fecal collection. To minimize disturbance (Wielebnowski & Watters, 2007) we waited until after any over-marking had been completed (Jordan *et al.*, 2011a,b,c, Müller & Manser, 2008) and the mongooses had left the den site on foraging bouts before collecting feces. We avoided additional diurnal variation by collecting feces only in the morning (Wielebnowski & Watters, 2007) or by using time of day as a factor in analyses. We collected samples using transparent plastic bags and estimated by touch, the temperature, consistency and relative water content of the collected material to estimate the freshness of the sample. Fresh feces were warm (within a few minutes of defecation), wet and pliable. We excluded old feces that were dry and friable. We placed collected material on ice for a maximum of four h prior to storage at -20° C. We collected 25 samples from a free-ranging troop at two sampling events in conjunction with observed putative stressful events which we used for the biological validation of our enzyme immunoassay.

We collected a total of 304 fecal samples from the four captive mongooses over 97 sampling events. Collection took place daily at 8 AM between April 2010 and May 2011. We collected fecal samples following the protocol for sample collection for free-ranging troops (above) but froze feces within approximately one h of defection at -20° C until further processing.

4.3.3 Determination of Gastrointestinal transit (GIT) time

To estimate GIT time for banded mongooses, we conducted an experiment using food-grade dyes and uncooked rice, adding these ingredients to the regular food of the four captive mongooses. We fed them daily at 8 AM as per their normal regimen and measured the time lag between consumption of the marked meal and the excretion of marked feces.

4.3.4 Post-defecation metabolism

Especially for the free-ranging troops, fecal sample collection was often impossible directly after defecation, therefore we assessed the metabolism rate of FGMs post defecation to ensure reliable data interpretation. We performed a post-defecation metabolism experiment according to the procedure described by Möstl *et al.* (1999). Therefore, we collected fecal samples from four captive mongooses and divided each sample into five subsamples which we placed under environmental conditions (full sunlight) for 0, 2, 4, 6, or 8 h prior to storage at-20° C. Maximum (27° to 33° C) ambient temperatures varied slightly during the experiment and we recorded no rainfall.

4.3.5 Steroid extraction and analysis

We lyophilized, pulverized and sifted fecal samples using a mesh strainer to remove fibrous material as described by Ganswindt *et al.* (2010). We then extracted approximately 0.05 g of the fecal powder with 80 % ethanol in water (1.5 ml) according to the procedure described by Ganswindt *et al.* (2010) and additionally determined the organic content of each sample according to the procedure described by Ganswindt *et al.* (2012b). All steroid concentrations are expressed henceforth per mass of dry organic fecal matter. We measured the resulting extracts for immunoreactive FGMs using cortisol, corticosterone and two different 11-oxoetiocholanolone EIAs. Details of the EIAs including cross-reactivities of the antibody are described by Palme & Möstl (1997) and Möstl *et al.* (2002). The 11-oxoetiocholanolone EIA detecting 11,17-DOA that we used for the majority of our analyses, had an intra-assay CV of 2.8 % to 4.0 % and an inter-assay CV of 12.1 % to 16.8 %. The sensitivity of the assay at 90 % binding was 3 pg/well. We performed assays on microtiter plates according to the procedure described by Ganswindt *et al.* (2002).

4.3.6 ACTH challenge test

We intramuscularly injected long-acting ACTH preparation (Tetracosactide 1 mg ml^{-1}: Synacthen Depot; Novartis, Basel, Switzerland, Lot S0892) into three captive banded mongooses using doses of 200 µg kg^{-1} for one adult female and one adult male and 133 µg kg^{-1} for a second adult male. We additionally injected (*i.m.*) an equivalent volume (0.3 ml) of sterile isotonic saline solution as a control into an additional adult male. We collected samples on the day of and for three days after the ACTH injection, checking for feces in the enclosure hourly during daylight hours when mongooses are typically active.

4.3.7 Dexamethasone suppression test

Using the same experimental setup as for the ACTH challenge test (see 2.6), we injected (*i.m.*) 105.6 µg kg^{-1} dexamethasone sodium phosphate (DEX; 2.64 mg ml^{-1}: Dexa 0,2 Phenix; Virbac RSA, Centurion, South Africa, Lot BJ77) into the three animals of the experimental group 21 days after the ACTH challenge test. We administered an equivalent volume (0.06 ml) of saline into our control male.

4.3.8 Suppression of reproductive activity

To suppress estrous in the captive female mongoose, we administered an orally-delivered progestin contraceptive, megestrol acetate (Ovarid; Schering-Plough Corporation, Kenilworth, USA) between September 2008 and May 2010. During this time, neither the males nor the female in the captive group engaged in any reproductive behavior and the animals were not visited or in-

vaded by any free-ranging mongooses. After May 2010, we observed estrous behavior and parturition as well as the troop invasions and depredation events that coincided with these reproductive events.

4.3.9 Data analysis

We standardized FGM concentrations relative to a predefined baseline to give relative differentials. Baseline FGM concentrations were determined as the median of the four captive animals a) on the day of ACTH injection (day 0 ACTH), b) on the day of DEX injection (day 0 DEX) and c) during their pre-reproductive period from 29 January 2010 to 8 October 2010. Furthermore, we used as baselines the starting FGM concentration for each captive mongoose (hour 0) in the post-defecation metabolism study and the median FGM concentration for the clinically healthy troop members of the sick animal in the disease comparison. For the ACTH and DEX analyses, we used the median FGM concentration for each animal on each day (when a mongoose defecated multiple times on a given morning). We then report medians with interquartile range for the relative differentials for the experimental mongooses. The sample sizes for the post-defecation metabolism study were small (four mongooses with each feces sampled at five sampling events) and hence we could not reliably assess normality for the data. Thus, we tested for differences in the distributions of FGM concentrations among sampling events using Friedman's rank sum test using R (R Core Team, 2012).

4.4 Results

4.4.1 Gastrointestinal transit (GIT) time

Gastrointestinal transit time for the four captive mongooses based on the use of food-grade dyes and uncooked rice was a minimum of 24 h (first clearance of marked food items), although residual marking of the feces continued for up to 72 h (final clearance of marked food items).

4.4.2 ACTH challenge test

Using the 11-oxoetiocholanolone EIA detecting 11,17-DOA, median FGM concentrations increased more than 2-fold above pre-injection levels 24 h post ACTH administration (i.e. 300 % of the starting values). The median baseline, peak and nadir values were 0.81 $\mu g\, g^{-1}$ org, 2.89 $\mu g\, g^{-1}$ org and 0.63 $\mu g\, g^{-1}$ org, respectively. In comparison, the remaining three assays tested revealed a 1-fold elevation (i.e. 200 % of starting values) in median FGM concentrations 24 h post ACTH injection (Figure 4.1). Their median baseline, peak and nadir values were 1.5 $\mu g\, g^{-1}$ org, 2.73 $\mu g\, g^{-1}$ org, 0.77 $\mu g\, g^{-1}$ org (11-oxoetiocholanolone EIA detecting 5β-3α-ol-11-one), 58.4 ng g org,

104.2 ng g org, 62.3 ng g org (cortisol) and 1.03 µg g^{-1} org, 2.12 µg g^{-1} org, 0.67 µg g^{-1} org (corticosterone). In all cases, median FGM levels returned to pre-injection baseline levels at 48 h (Figure 4.1). Therefore, only the 11-oxoetiocholanolone EIA measuring 11,17-DOA was used for any further analysis.

Interestingly, the saline-injected control animal showed a similar response to that of the experimental animals (Figure 4.1), indicating that handling was presumably the prominent stressor in this experiment.

4.4.3 Dexamethasone suppression test

The administration of dexamethasone revealed an almost 1-fold decrease (15 % of starting values) in median FGM levels in the experimental group 48 h post-injection, before FGM concentrations returned approximately to their pre-injection baseline at 72 h (Figure 4.2). In contrast to the conducted ACTH challenge, no handling-related increase in FGM concentrations could be detected in the control animal 24 h post-injection. FGM levels of that mongoose, however, increased substantially 72 h after handling (Figure 4.2).

4.4.4 Biological validation

Suppression of reproductive activity

We observed several, partly reproduction-related, potentially stressful events in the captive mongooses after administration of contraceptives was stopped. These events were associated with increases in FGM levels relative to the baseline FGM concentration while the progestin contraceptive was administered (2010/01/29 – 2010/10/08). We observed the following range of FGM concentrations for the three different observation periods with the captive troop: contraceptive period, n = 98, 0.03 µg g^{-1} to 6.91 µg g^{-1} org. content; reproductive period, n = 104, 0.08 µg g^{-1} to 18.37 µg g^{-1} org. content; pharmacological validation period (ACTH and DEX), n = 99, 0.11 µg g^{-1} to 43.01 µg g^{-1} org. content.

Case A After contraception was stopped, troop median FGM concentration was 2.98 µg g^{-1} org. content (2010/12/15 – 2011/04/20, n = 104). This was 10-fold higher compared to the median FGM level (0.28 µg g^{-1} org. content) in 2010 while contraception was administered (i.e. 1100 % of the baseline). Troop FGM concentrations increased leading up to and after the birth of the first litter. Three days prior to parturition, the median male FGM concentration increased to 3.55 µg g^{-1} org. content (a 12-fold increase relative to the pre-reproductive period, or 1300 % of baseline). One week prior to parturition and on the day of parturition, the female's median FGM concentrations were 4.32 µg g^{-1} org. content and 2.07 µg g^{-1} org. content, respectively, which reflect 15-fold and 7-fold increases relative to the pre-reproductive period (i.e 1600 % and 800 % of

baseline). For the month shortly after parturition and the loss of the first litter, troop median FGM concentration was 0.45 µg g^{-1} org. content (2011/01/03 – 2011/01/28, n = 14, range: 0.08 µg g^{-1} to 1.11 µg g^{-1} org. content) before FGM concentrations again increased towards the second parturition. Of these 14 samples, 12 had FGM concentrations below the upper 95 % confidence limit of the pre-reproductive baseline and four samples were below the baseline median of 0.28 µg g^{-1} org. content.

Case B On 24 December 2010 an African rock python (*Python sebae natalensis*) entered the enclosure and predated at least two pups before it was discovered and removed a day later. On the 26th of December, three free-ranging male mongooses entered the enclosure, at which time no more pups were observed. All males, including the resident ones, fought over access to the female, before the invading males were trapped and removed one day later. We observed guarding and mating behavior, as well as putative estrus of the female, which ended on the 30th of December. During this time span, the female's FGM concentrations were elevated up to 18.37 µg g^{-1} org. content (28th of December) which was a 66-fold increase relative to the 2010 troop pre-reproductive baseline levels (6700 % of baseline) and an 8-fold increase relative to the female's FGM concentrations detected on the day of putative parturition (median FGM 2.07 µg g^{-1} org. content). By the 31st of December her FGM concentrations had decreased to the level seen at parturition (1.38 µg g^{-1} to 1.62 µg g^{-1} org. content) which were still 4 – 5-fold higher than the troop pre-reproductive baseline FGM concentrations (i.e. 500 % to 600 % of baseline).

Case C In the ten days after the second parturition, the female's median FGM concentration was 7.98 µg g^{-1} org. content, which was a 28-fold increase relative to pre-reproductive baseline (i.e. 2900 % of baseline). During this time, three of the four pups died. For the month after this period, her median FGM concentration dropped to 3.56 µg g^{-1} org. content, a 12-fold increase relative to the pre-reproductive baseline (i.e. 1300 % of baseline) and a 0.6-fold decrease relative to the post-parturition period (i.e. 44 % of the post-parturition period).

FGM levels in relation to disease

The FGM concentration of a free-ranging mongoose diagnosed (after necropsy) with a late stage *Mycobacterium mungi* infection was 188.16 µg g^{-1} org. content. This was a 54-fold increase (i.e. 5500 % of baseline) relative to the median FGM concentration for the remaining 24 putatively healthy troop members of the infected animal (3.4 µg g^{-1} org. content, range: 0.5 to 15.5).

4.4.5 Post-defecation metabolism

FGM concentrations in four feces decreased 0.38-fold over the first two h after defecation (i.e. to 62 % of the starting FGM concentration), but remained at approximately the same level up to six

h thereafter (Figure 4.3). Using the Friedman rank sum test, we failed to reject the null hypothesis that the FGM distributions were the same across the repeated measures (sampling times), $\chi^2 = 3$, df$= 4, p = 0.5$.

4.5 Discussion

We have shown that an 11-oxoetiocholanolone EIA can be reliably used to measure biologically relevant changes in glucocorticoid metabolite concentrations in banded mongoose feces. We validated this assay by conducting an ACTH challenge and dexamethasone suppression test as well as by monitoring putative stressful events in captive and free-ranging animals as a form of biological validation.

Although our chosen assay detects the expected stimulation and suppression of the HPA axis by ACTH and dexamethasone (respectively) in terms of relative changes in fecal glucocorticoid metabolite output, the revealed signal might be underestimated due to the suboptimal sampling regimen in our experiments, which was unavoidable due to the logistical setup at the research facility in Kasane and might have resulted in missing the peak samples during the partly one-off collection per day. Also the use of suboptimal doses for the pharmacological challenges is conceivable, especially if individual susceptibility has to be taken into account. Differing exercise regimens (Campbell *et al.*, 2009) and differing exposure to chronic stress (Rich & Romero, 2005) can lead to individual differences in adrenal sensitivity to exogenous ACTH. Further, there are numerous pathways in addition to ACTH that could play a role in regulating glucocorticoids (Bornstein *et al.*, 2008). Individual variation could occur in any of these, leading to individual variation in glucocorticoid production in response to exogenous ACTH.

During the ACTH challenge, the control male showed a similar response to that of the experimental animals suggesting that the signal was induced through handling — not through a pharmacological response to the ACTH. Similarly, in African buffalo (*Syncerus caffer*), anesthesia alone induced a glucocorticoid response equal to that of ACTH (Brown *et al.*, 1991). This response was not induced 21 days later, however, when we performed the DEX suppression test although we used the same handling and injection protocols. It is possible that in our study stress responses may be socially transmitted (i.e. a social contagion), perhaps via a chemosignal from the experimental mongooses in response to ACTH and DEX which in turn induces similar behavioral and glucocorticoid responses in the control male. This was first demonstrated in Sprague-Dawley rats exposed to a stressor in which a chemosignal induced a behavioral response in control subjects (Abel, 1994). This chemosignal was not produced by hypophysectomized rats but was produced by sham-operated rats and by hypophysectomized rats treated with ACTH, suggesting that the pituitary and ACTH may play a key role in such chemosignals (Abel, 1994). In addition, chemosignals from stressed rats have been shown to increase glucocorticoids in conspecifics housed in the same facility but not subjected to the same stressor (Fuchs *et al.*, 1987).

Reproductive behavior in banded mongooses appears to be associated with increased production

of glucocorticoids. Our captive troop was fed a constant diet throughout the study and always had access to drinking water and dens. Further, there was no clinical indication of disease in the troop. Aside from seasonal temperature changes, these mongooses were not exposed to any seasonal dietary, denning or reproductive constraints while the adult female's estrus was suppressed. As a result, their 2010 baseline FGM concentrations were low, with low variability. Once the female came into estrus, after ceasing the birth control treatment, FGM concentrations in the entire captive troop increased dramatically. These increases were possibly related to several correlated factors, including 1) increases in the female's glucocorticoid production associated with estrus, pro-estrus, parturition and lactation, 2) increases in male glucocorticoid production associated with guarding and mating the female, 3) increases in the troop's glucocorticoid production in response to other stressors brought about by the reproductive cycle: a) attraction of predators to and depredation of juveniles, b) post parturition attraction of invading male mongooses to the estrous female and c) non-depredation-related juvenile mortality. The pattern shown here by banded mongooses was approximately similar to that shown in meerkats (*Suricata suricatta*) where female FGMs were low at conception and increased to parturition in females that did not have postpartum conception (Barrette *et al.*, 2012). Female meerkats with postpartum conception, however, had lowered FGMs in the last two weeks of gestation (Barrette *et al.*, 2012). In contrast, the captive female in our study did conceive after her first litter but her FGMs appeared to remain high until parturition.

It is interesting to note that the female only became estrous in late October, nearly five months after stopping birth control treatment and had only two litters, with the second one in late February. Free-ranging females in our study area generally display estrus in the late dry season each year (approximately mid-September). Pregnancy can be identified within about 30 days of conception from swelling of the female's abdomen and nipples (approximately mid-October) with the first parturition of the season roughly coinciding with the first significant rainfall event and associated termite eruption (approximately mid-November). Parturition of the final litter of the season is early to mid-March. The captive mongooses were fed a consistent diet (volume and nutrient content) throughout this time period. We suggest that cues other than seasonal dietary changes are responsible for triggering estrus and reproductive activity in banded mongooses in this population.

In addition to physiological and biological challenges monitored in the captive troop, we were also able to detect biologically relevant differences in FGM concentrations in a monitored free-ranging troop through the assessment of a late stage TB case — confirmed on antemortem clinical signs, gross pathology and histopathology (Alexander *et al.*, 2010). An animal exposed to a severe and chronic biological challenge such as a chronic disease is expected to have elevated FGM concentrations. The adrenal response to infection by ectoparasites and nematodes has been equivocal in wildlife species with decreased glucocorticoids in response to parasite reduction in female blue tits (*Cyanistes caeruleus*) (Lobato *et al.*, 2008), *Peromyscus* spp. (Pedersen & Greives, 2008) and cliff swallows (*Petrochelidon pyrrhonota*) (Raouf *et al.*, 2006), but no effect on glucocorticoids after parasite reduction in raccoons (*Procyon lotor*) (Monello *et al.*, 2010) and Rocky Mountain big horn sheep (*Ovis canadensis canadensis*) (Goldstein *et al.*, 2005). Chronic

infection by *M. tuberculosis* in humans, however, leads to increased glucocorticoids (Sarma *et al.*, 1990), possibly mediated by cytokine activation of the HPA axis during the immune response to infection (Bottasso *et al.*, 2007, Bozza *et al.*, 2007, del Rey *et al.*, 2007). Futher, increases in in FGMs have been detected in mice inoculated with mouse scrapie as the disease approaches late stage (Voigtländer *et al.*, 2006). Thus, although glucocorticoids are often implicated in immunosuppression, they may also be altered in response to parasitism (Klein, 2004) and this positive feedback may result in "vicious circles" of susceptibility, infection and transmission within an individual and within a social group or population (Beldomenico & Begon, 2010).

FGM excretion time approximately matched the GIT time in banded mongooses, a finding similarly shown in other species (Dehnhard *et al.*, 2001, Harper & Austad, 2000, Hulsman *et al.*, 2011, Martínez-Mota *et al.*, 2008, Palme *et al.*, 1996, Schatz & Palme, 2001, Touma *et al.*, 2003, Wasser *et al.*, 2000).

We found FGM levels to be stable over time since defecation, indicating that samples collected within eight h post-defecation should give a reliable reflection of FGM concentrations in the collected feces.

4.6 Conclusions

Banded mongoose stress response can be reliably assessed non-invasively using an 11-oxoetiocholanolone enzyme immunoassay which specifically detects 11-17-dioxoandrostanes. Gastrointestinal transit time in captive banded mongooses is at least 24 h. FGM concentrations in banded mongoose remain stable up to eight h after defecation. In spite of having a consistent diet, captive banded mongooses appear to become reproductively active at the same time of the year as free-ranging banded mongooses. FGMs appear to increase in both male and female captive banded mongooses as they approach parturition. Parturition may be associated with other putatively stressful events such as predator attraction, predation and agonistic troop encounters. Late stage tuberculosis-infected banded mongooses may show an over 50-fold elevation in FGM levels relative to putatively healthy troop members.

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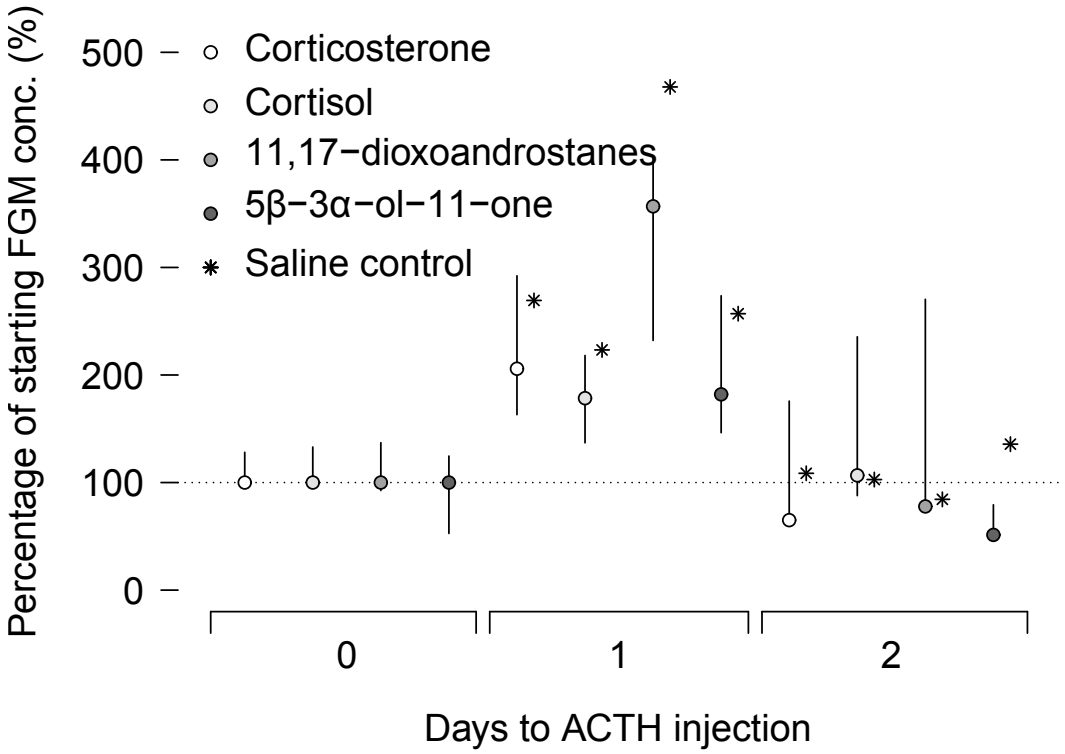

Figure 4.1. FGM levels (median and interquartile range) for three captive banded mongooses (one female, two males) in response to ACTH administration. Levels are expressed relative to the pre-ACTH levels (day of injection, 100 %). The saline control male's FGM response to the handling and saline injection is indicated by the asterisks.

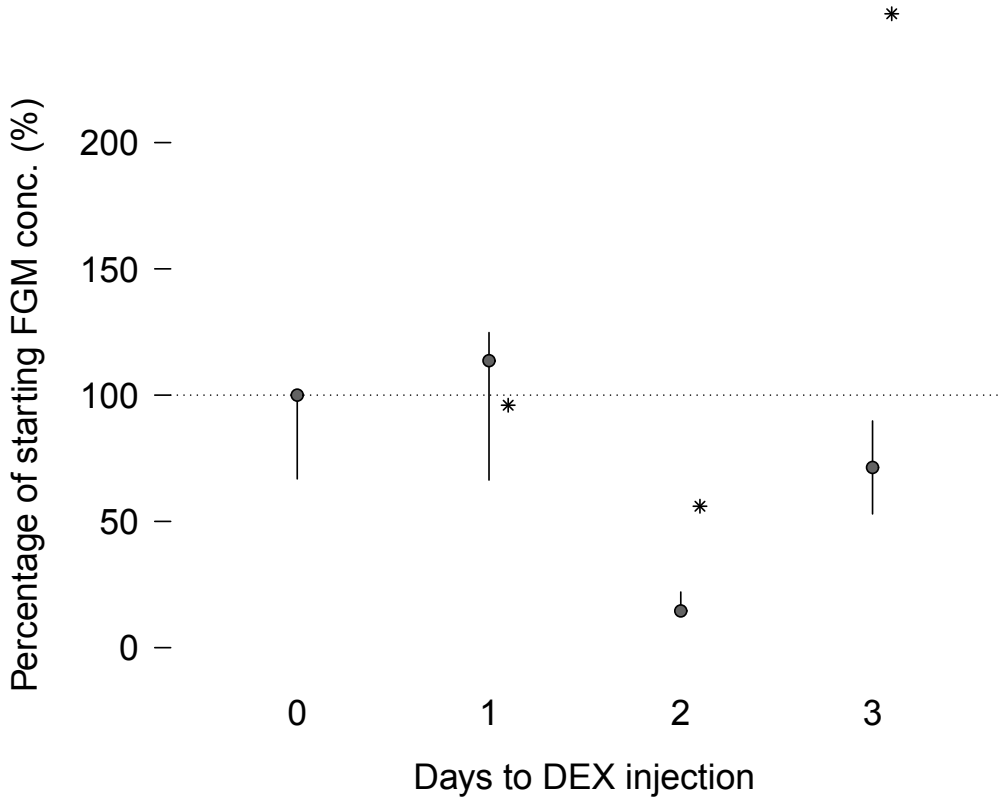

Figure 4.2. FGM (11,17-DOA) levels (median and interquartile range) determined with an 11-oxoetiocholanolone EIA for three captive banded mongooses (one female, two males) in response to a dexamethasone (DEX) suppression test. Levels are expressed relative to the pre-DEX levels (day of injection, 100 %). The saline control male's FGM response to the handling and saline injection is indicated by the asterisks.

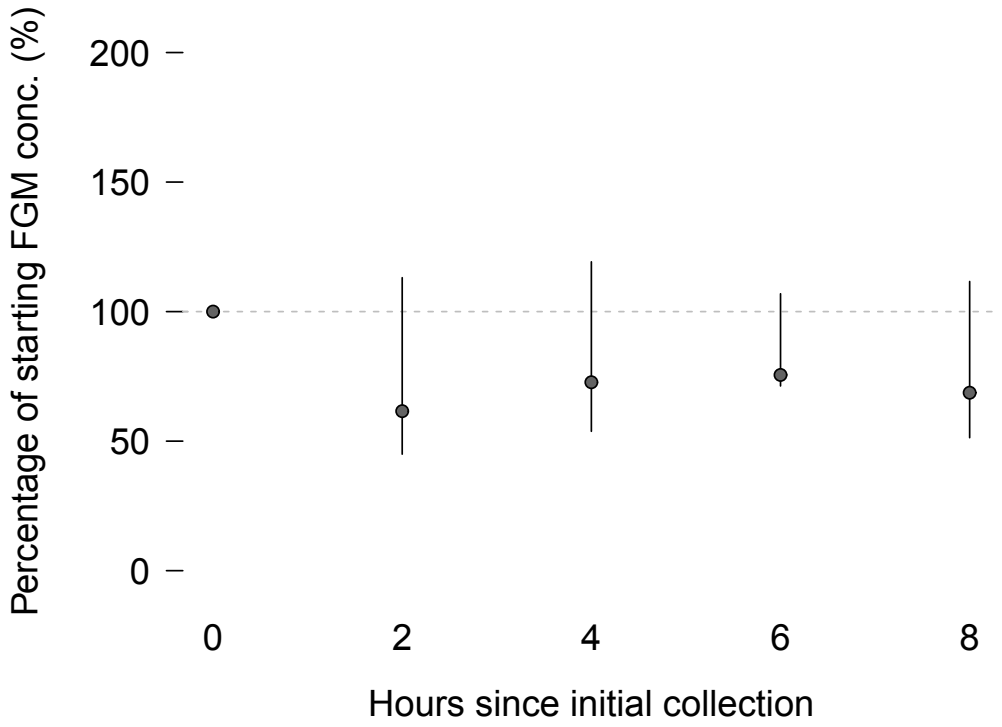

Figure 4.3. Median and interquartile range of FGM (11,17-DOA) levels determined with an 11-oxoetiocholanolone EIA in four banded mongoose fecal samples from one female, three males over time since defecation. Levels are expressed relative to the starting concentration (100 % at t = 0 h). Using the Friedman rank sum test, we failed to reject the null hypothesis that the FGM distributions were the same across the repeated measures (sampling times), $\chi^2 = 3$, df$= 4, p = 0.5$.

Chapter 5

Field and experimental glucocorticoid responses to reproduction and food limitation in banded mongooses (*Mungos mungo*)

Field and experimental glucocorticoid responses to reproduction and food limitation in banded mongooses (*Mungos mungo*)

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5.1 Abstract

Glucocorticoids help to mediate an animal's response to physiological challenges. Prolonged elevation of glucocorticoids may lead to homeostatic overload and may impair immune function. By measuring glucocorticoid responses to a variety of ecological factors, researchers might determine which factors are associated with glucocorticoid elevations and whether any of these factors may then increase the potential for pathologies in animals. We combined an extensive field study with experimental manipulations to investigate the roles of reproduction, predation risk, and food limitation in eliciting apparent homeostatic overload in a population of banded mongooses (*Mungos mungo*) that suffers from yearly outbreaks of a novel *Mycobacterium tuberculosis* complex pathogen, *M. mungi*. We manipulated reproduction and food supply in a captive troop of mongooses and compared their glucocorticoid responses to those of 13 free-ranging troops in the same study area. We aimed to determine whether banded mongooses in this population suffer from chronic elevations of glucocorticoids, and we aimed to assess the relationship of glucocorticoid elevations to factors associated with nutrition, reproduction, and predation. Using collected feces, fecal glucocorticoid analysis, and direct observation of mongoose behavior, we found that reproduction and its associated challenges dramatically increased glucocorticoid production, which otherwise remained low and stable in a captive troop with a constant food supply and lowered predation risk. Variation in glucocorticoid production in free-ranging banded mongooses was best explained by food limitation as described by current nutritional limitation (proportion of fecal organic matter), recent rainfall (which increases soil macrofauna availability), and access to concentrated anthropogenic food resources. Habitat differences in soil macrofauna density and reproductive events also explained variation in glucocorticoid production, but to a lesser degree. Predation risk, as measured by canopy cover (escape cover from aerial predators) and group size (decreased per capita vigilance), explained very little of the variation in glucocorticoid production. In the late dry season, banded mongooses in our population may face a "perfect storm" of nutritional limitation, agonistic encounters at concentrated food resources, aggressive evictions, estrus, competition for mates, parturition, and predation pressure on pups. We suspect that this perfect storm may push glucocorticoid responses into homeostatic overload and may impair cell-mediated immunity in banded mongooses. Future research needs to determine if this homeostatic overload does occur and whether it is a contributing factor in the yearly *M. mungi* outbreaks seen in this population.

5.2 Introduction

Animal physiology plays an important role in many current conservation issues (Tracy *et al.*, 2006, Wikelski & Cooke, 2006), and glucocorticoids, specifically, are important physiological mediators of an animal's response to a variety of challenges (Sapolsky *et al.*, 2000). Researchers have highlighted glucocorticoids as mediators of this response using the concepts of stress and homeostasis (Chrousos & Gold, 1992, Selye, 1936, 1951, 1973), allostasis (McEwen & Wing-

field, 2003, 2010, Wingfield, 2005), and predictive and reactive homeostasis within the reactive scope model (Romero *et al.*, 2009). Glucocorticoids have a complex role in determining performance and reproductive success and their role involves trade-offs with energetics and immune function (Moore & Hopkins, 2009). Glucocorticoids play a particularly complex role in immune function (Elenkov & Chrousos, 1999, Sapolsky *et al.*, 2000), whereby chronically-elevated levels of glucocorticoids may result in allostatic load or homeostatic overload (McEwen & Seeman, 1999). This homeostatic overload may then suppress cell-mediated immunity (Dhabhar, 2000, Dhabhar & McEwen, 1999). Cell-mediated immunity may confer resistance to intracellular pathogens such as mycobacteria in general, and *Mycobacterium tuberculosis* in particular (Cooper, 2009, Flynn & Chan, 2001).

We recently discovered a novel *Mycobacterium tuberculosis* complex pathogen, *M. mungi*, which infects banded mongooses *Mungos mungo* in northeastern Botswana (Alexander *et al.*, 2010). Banded mongoose are small-bodied (< 2 kg), diurnal (predominantly insectivorous) carnivorans in the family Herpestidae that breed co-operatively (Gilchrist, 2004, Hodge, 2005, Rood, 1974) but exhibit limited social dominance (Cant, 2000, Gilchrist, 2006) and exhibit low reproductive skew (De Luca & Ginsberg, 2001). Outbreaks of *M. mungi* occur in multiple mongoose troops, with up to 17 % of troop members becoming infected (case fatality rate of 100 %, P. Laver and K. Alexander, *unpublished data*). This population of banded mongooses lives in close contact with humans in the town of Kasane, Botswana, and at tourist lodges in and around the Chobe National Park. We need to understand the epidemiology of *M. mungi* outbreaks for the conservation of this population of banded mongooses and because we do not know if other species, including humans, are susceptible to *M. mungi* infection.

Researchers have determined that many ecological covariates can affect glucocorticoid production in wildlife. Some of these covariates include direct anthropogenic disturbance (Mullner *et al.*, 2004), anthropogenic habitat change (Bonier *et al.*, 2007, Wasser *et al.*, 1997), anthropogenic food provisioning (Foerster & Monfort, 2010), climatic events (Quillfeldt *et al.*, 2004, Romero & Wikelski, 2001), physical injury (Ganswindt *et al.*, 2010b), parasitism (Raouf *et al.*, 2006), sociality and group size (Foley *et al.*, 2001, Saino *et al.*, 2003), dominance hierarchies (Creel, 2005, Foley *et al.*, 2001), predation risk (Monclús *et al.*, 2009), pregnancy (Barrette *et al.*, 2012, Behringer *et al.*, 2009) and food limitation.

Many researchers have focused on energetic limitation as a covariate because glucocorticoids may control important aspects of mobilizing energetic resources in animals (Dallman *et al.*, 1993). As animals face progressive nutritional limitation, glucocorticoid production initially facilitates some gluconeogenesis but then glucocorticoid production decreases as glucagon production increases. Finally, the animal reaches a condition threshold at which glucocorticoid production again increases to facilitate protein catabolism (Romero *et al.*, 2009), a strategy exemplified by Galápagos marine iguanas (*Amblyrhynchus cristatus*) enduring starvation conditions during El Niño events (Romero & Wikelski, 2001). Researchers have shown this general pattern of increased glucocorticoid production in response to food limitation in a variety of species in field studies (Champoux *et al.*, 1993, Dunn *et al.*, 2013, Foley *et al.*, 2001, Ganswindt *et al.*, 2010a, Kitaysky *et al.*, 1999b, Rasmussen *et al.*, 2008) and through experimental manipulation (Ki-

taysky *et al.*, 1999a, Pravosudov & Kitaysky, 2006, Pravosudov *et al.*, 2001, Saino *et al.*, 2003). We know of only one study, on white-tailed deer (*Odocoileus virginianus*) fawns, that showed a decline in glucocorticoid production with increasing food limitation over the course of a season (i.e. not a fast during which we might expect reduced glucocorticoids and increased production of glucagon) (Taillon & Côté, 2008). Taillon & Côté (2008) suggested that food-limited fawns suppressed glucocorticoid production to prevent excessive depletion of protein and fat reserves as the season progressed.

Energetic limitation may serve as a proximate factor in many other ecological covariates, thus introducing into statistical models of glucocorticoid variation, multicolinearity inherent in the physiology of the animals. For instance, reproductive activity is an energetically costly behavior for mammals (Gittleman & Thompson, 1988, Speakman, 2008) and researchers may find it difficult to tease apart glucocorticoid responses to reproductive challenges related to energetics from those related to other aspects of reproduction. Thus, dominance or submission within a dominance hierarchy may affect glucocorticoid production due to energetic costs of controlling or being excluded from resources, or from direct agonistic encounters. Yet, other hormonal mediators of reproductive behavior may affect glucocorticoid production, as with musth in African elephants (Ganswindt *et al.*, 2010a). Although researchers have shown increases in glucocorticoid production in female bonobo *Pan paniscus* (Behringer *et al.*, 2009) before and after parturition, and increases during pregnancy leading up to parturition in meerkat (*Suricata suricatta*) (Barrette *et al.*, 2012), it is not clear from these studies what proximate factors elicited these responses. In some non-mammalian vertebrate taxa, increased glucocorticoid production and deposition in eggs by females in poor body condition may even result in sex-biased changes to offspring quality (Love *et al.*, 2005) and may thus have adaptive significance in unpredictable environments.

If cell-mediated immunity confers resistance to *M. mungi*, as it does for other mycobacteria, then suppression of this immunity by chronically-elevated glucocorticoids may play an important role in the epidemiology of *M. mungi* outbreaks in banded mongooses. We know very little about endocrinology in banded mongooses. With our recent validation of a technique for non-invasive monitoring of their fecal glucocorticoid metabolites (Laver *et al.*, 2012) (Chapter 4), this work is now possible. Using that technique, we wanted to determine in this study (1) if banded mongooses in northeastern Botswana experience chronic elevations of glucocorticoids, (2) when these periods of elevated glucocorticoids occur (if they do), and (3) which ecological covariates best explain variability in glucocorticoid production in banded mongooses.

Given the multitude of ecological covariates that could affect glucocorticoid production, and given the potential complexity of interactions among covariates, we adopted a combined observational and experimental approach to address our three objectives. To determine baseline glucocorticoid levels in banded mongooses, we experimentally suppressed reproductive activity and maintained a constant food supply in a captive troop kept in an outdoor enclosure in the same study site as our free-ranging study animals. To determine the effect of reproduction on glucocorticoid response under conditions of constant food supply, we stopped the experimental contraception and allowed reproduction in the captive troop. To model the ecological covariates of variability in banded mongoose glucocorticoid production, we compared several free-ranging troops along

a gradient of food limitation using fecal collection and behavioral observation across multiple years.

5.3 Methods

5.3.1 Study area and animals

We conducted our study on 13 troops (troop size range 5 to 64) of free-ranging banded mongooses and four (one female and three males) captive animals in a dystrophic, or nutrient-poor, savanna woodland ecosystem in northeastern Botswana from October 2007 to November 2011. We monitored free-ranging mongoose troops over an area of $\sim 120\,\mathrm{km}^2$ in the northeastern corner of the Chobe National Park ($\sim 30\,\mathrm{km}^2$), in the northern Kasane Forest Reserve ($\sim 73\,\mathrm{km}^2$), and in the towns of Kasane and Kazungula ($\sim 17\,\mathrm{km}^2$) at 25.163° E, 17.828° S. In Chapter 3 we provide extensive details on the study site and study animals.

The captive troop was housed together in an outdoor enclosure ($\sim 95\,\mathrm{m}^2$) at the Center for African Resources: Animals, Communities and Land use (CARACAL) research facility in Kasane. The four animals were raised in captivity from approximately two weeks of age and were adults of two to three years of age at the time of our sampling. The captive troop was fed 820 g of canned wet pet food at 8 AM each morning and this diet was supplemented sporadically by facility staff with natural food items such as a variety of coleopterans, spirostreptid millipedes, and occasional bushveld rain frogs (*Breviceps adspersus*). The four animals fed together and individual intake of supplemented food may have varied although no animal ever dominated the daily provision of pet food. These mongooses also foraged within their enclosure which consisted only of a $\sim 1.5\,\mathrm{m}$ wall and which had the same substrate and vegetation as the surrounding landscape.

We conducted our study with approval of the Virginia Tech Institutional Animal Care and Use Committee (07-146-FIW) and the Botswana government, Ministry of Environment, Wildlife, and Tourism.

5.3.2 Observations and sample collection

Laver *et al.* (2012) (Chapter 4) provide details on our methods for fecal sample collection, transportation and storage. Briefly, for this study we collected and analyzed 1542 feces from 13 free-ranging banded mongoose troops marked with radio collars, during 138 sampling events (by troop by date) from 2 June 2008 to 16 December 2010. We also collected and analyzed 202 feces from a captive troop during 68 sampling events (by Julian date) from 29 October 2008 to 20 April 2011. We collected all feces within 4 h of defecation, during which time fecal glucocorticoid metabolite levels remained stable (Laver *et al.*, 2012) (Chapter 4). In Chapter 3 we provide details on our methods for troop behavioral observations.

5.3.3 Steroid extraction and analysis

Laver *et al.* (2012) (Chapter 4) provide details on our steroid extraction and analysis. Briefly, we lyophilized, pulverized and sifted fecal samples using a mesh strainer to remove fibrous material as described by Ganswindt *et al.* (2010b). We then extracted ~ 0.05 g of the fecal powder with 80 % ethanol in water (1.5 ml) following Ganswindt *et al.* (2010b) and determined the organic content of each sample following Ganswindt *et al.* (2012). We express all steroid concentrations per mass of dry organic fecal matter. We measured the resulting extracts for immunoreactive fecal glucocorticoid metabolites using an 11-oxoetiocholanolone enzyme immunoassay (EIA) detecting 11,17-dioxoandrostanes (11,17-DOA). During validation of this assay (Laver *et al.*, 2012) (Chapter 4), this EIA had an intra-assay CV of 2.8 % to 4.0 % and an inter-assay CV of 12.1 % to 16.8 %. The sensitivity of the assay at 90 % binding was 3 pg/well. We performed assays on microtiter plates according to the procedure described by Ganswindt *et al.* (2002).

5.3.4 Suppression of reproductive activity

We administered an orally-delivered progestin contraceptive, megestrol acetate (Ovarid; Schering-Plough Corporation, Kenilworth, USA) between September 2008 and May 2010 to suppress estrus in the captive female mongoose. During this non-reproductive period, neither the males nor the female in the captive group engaged in any reproductive behavior. During this period, no free-ranging banded mongooses visited or invaded this captive troop. After ending contraception in May 2010, we observed estrous behavior and parturition in the captive female. Further, free-ranging mongoose troops invaded the captive troop and pup depredation events also coincided with this reproductive period.

5.3.5 Ecological covariates

A priori we narrowed the ecological covariates down to three plausible categories. We chose nutritional limitation as the first category because (1) our population of banded mongooses lives in a dystrophic ecosystem with dramatic seasonal differences in rainfall and primary production, (2) the troops in our population have variable access to anthropogenic food provisioning, (3) we have observed behavioral indicators of responses to food limitation, in the form of movement and home range dynamics (Chapter 3), and (4) food limitation explains variability in glucocorticoid production across a broad range of vertebrate taxa. We chose reproduction as the second category, broadly including estrus, mate guarding, mating, pregnancy, parturition, lactation and parental care. We chose reproduction because (1) evictions of adults from troops and the associated agonistic encounters occurred during estrus in banded mongoose troops in Uganda (Cant *et al.*, 2001, Gilchrist, 2006) and approximately around the time of first estrus of the season in our population (Fairbanks *et al.*, In prep), (2) banded mongoose troops share the costs of parental care, whereby escorts provision the pups nearest to them regardless of relatedness

(Gilchrist, 2004), males share guarding duties of altricial young (Rood, 1974), and females allosuckle (Neal, 1970). We chose predation risk as the third category. Although Gilchrist *et al.* (2004) suggested that banded mongooses breed communally because of the benefit of rearing young cooperatively and because of a lack of inbreeding costs, other herpestids such as meerkats may benefit from group-mediated anti-predator behavior, with benefits increasing with increasing group size (Clutton-Brock *et al.*, 1999). Banded mongooses exhibit recruitment calls in response to potential predators and rival troops, which elicit a mobbing response from troop mates (Furrer & Manser, 2009). This benefit of group-living in herpestids may be limited to species that travel together (Macdonald, 1983), and a group-living but solitary-foraging herpestid, the yellow mongoose (*Cynictis penicillata*), does not exhibit this group-size effect (le Roux *et al.*, 2009). Thus, we chose predation risk as a covariate because group-mediated anti-predator behavior may facilitate group-living in banded mongooses. To model these covariates we subdivided each into specific components.

Nutritional limitation

For nutritional limitation, we predicted that fecal organic matter would best describe an animal's current nutritional status, which rainfall could influence indirectly by affecting the availability of soil macrofauna over short temporal and spatial scales. Rainfall may cause soil macrofauna to migrate upwards in the soil column (Dangerfield, 1997) and generally leads to increased soil macrofauna availability in the wet season, relative to the dry season in our study area (Dangerfield, 1997). Further, two important natural food sources for banded mongooses in our study area respond behaviorally to rainfall events and increase their availability to mongooses. Termite alates erupt for their 'nuptial flight' at the time of the first substantial rainfall event of the season (Schuurman, 2006). Secondly, spirostreptid millipedes in this region forage on the ground surface after rainfall events (Dangerfield *et al.*, 1992). We summed the rainfall measured at a centrally-located meteorological station for the seven days prior to each "covariate day," which we explain below.

At coarse spatio-temporal scales the availability of soil macrofauna in general (the predominant natural food resource of banded mongooses in our study area), and access to anthropogenic food resources could also affect a troop's nutritional status. In banded mongooses, fecal glucocorticoid metabolite excretion approximately matches the gastrointestinal transit time (which is a minimum of 24 h) (Laver *et al.*, 2012) (Chapter 4). Because we collected all feces used in this analysis in the morning of each sampling day, we used ecological covariates from two days prior to each fecal sampling event (henceforth, "covariate days") in our analysis. For each covariate day we plotted a day range for each troop based on their movement data (Chapter 3). When we had multiple location estimates spanning multiple hours for a troop on a covariate day, we plotted a concave hull around the location estimates. When we had only sparse movement data or only a single location estimate for a troop on a covariate day, then we centered a circle on the location estimates with an area equivalent to the median daily minimum convex polygon for that troop in a given season. We calculated the median wet season and dry season daily minimum convex polygons for

six troops and used the median value for these six troops within a season for the remaining troops in our analysis. These concave hulls and circles (henceforth "covariate ranges") approximately represented the portion of a troop's home range containing the ecological covariates that should affect fecal glucocorticoid metabolite concentrations in feces that we collected two days later.

From approximately 3600 troop observations throughout the study site in which we recorded spatially-referenced habitat, we digitized broad habitat zones to match the habitat classifications of Dangerfield (1997), who sampled soil macrofauna in our study area and provided estimates of macrofauna density (m^{-2}) by season and by habitat type. We multiplied the area of each habitat within a covariate range by the appropriate seasonal soil macrofauna density from Dangerfield (1997) and then estimated the mean macrofauna density across the covariate range. We modeled access to concentrated anthropogenic food resources as a binary factor based on whether a troop's covariate range overlapped with a tourist lodge or with one of the substantial refuse sites in the towns of Kasane and Kazungula.

Reproductive activity

For reproductive activity, we used direct behavioral observations of mate guarding and mating and direct observations of pups to delineate putative dates for estrus, mating, and parturition for each troop. Because pups remain hidden in dens for the first four weeks after parturition, we estimated pup ages at emergence and from observations of adults carrying pups to new dens. We used these ages to estimate parturition and hence conception (when we lacked direct observation of reproductive behavior).

Predation risk

For predation risk, we predicted that canopy cover would confer protection from aerial predators (the predominant natural predator of adult banded mongooses in our study area), and that larger group sizes would lead to lower per capita vigilance (Fairbanks & Dobson, 2007, Lima & Dill, 1990, Macdonald, 1983). We used monthly estimates of troop size (adults) for each troop throughout the study period. To obtain troop size, we counted adults during direct behavioral observations on multiple days in each month. Mongoose troops typically forage as a group but some animals may guard pups in a den or forage separately from the rest of the group. Thus, we used the maximum number of adults that we counted consistently within a month to estimate troop size. Many of our study troops were small enough that we could estimate troop size with high precision. For two large troops in our study area, our estimates may have less precision, but these troops were an order of magnitude larger than some of our smaller troops and we suspect that the coefficient of variation on these estimates would be suitably low for our analysis.

Across the entire study site we digitized the canopies of 62 000 individual trees and bushes from satellite imagery from Google Earth (Google Inc., Mountain View, CA, USA). From these digi-

tized tree and bush canopies, we obtained estimates of percentage canopy cover for each covariate range.

5.3.6 Model building and selection

To explore ecological covariates that may describe glucocorticoid production at the population level, we used seven fixed effects and two random effects to build our *a priori* models of fecal glucocorticoid metabolite concentration in free-ranging banded mongooses. To ensure a balanced design for model averaging, we used all subsets of our global model. All of these subsets were plausible models and we did not include any interaction terms. Our global model had as fixed effects the proportion of fecal organic matter in a bolus (org), access to concentrated anthropogenic food sources (anth), amount of rainfall over the previous 7 d (rain), percentage canopy cover (cc), troop size (size), troop breeding status (breed), and density of soil macrofauna (macro). In all models, we modeled as random effects the troop identity (1|troop) and sampling event (1|event) for which we had repeated measures.

To test for an effect of anthropogenic food provisioning at the troop level we developed simplified *a priori* models for fecal glucocorticoid metabolite concentration in a single free-ranging troop and the captive troop. Only the fixed effect of fecal organic matter in a bolus (org) from our global model (above) varied in the captive troop and only the random effect of sampling event (1|event) applied to models within a single troop. Although rainfall varied for the captive troop as it did for the free-ranging troops, *a priori* we considered this covariate unimportant for the captive mongooses, which we assumed derived relatively little of their diet from foraging in the enclosure. Thus, we used two candidate models for the model fitting in the two troops: a mixed effect model with fecal organic matter as fixed effect and sampling event as random effect and a random effect model with only sampling event. We predicted that fecal organic matter would describe fecal glucocorticoid metabolites in the free-ranging troop but not in the captive troop.

In our three analyses we modeled fecal glucocorticoid metabolite concentrations (natural log transformed) as the response variable in linear mixed models which we fitted with the 'identity' link function using *glmer* in Package 'lme4' (Bates *et al.*, 2012) in R (R Core Team, 2012). To improve interpretability of parameter estimates, we standardized numeric variables to $\bar{x} = 0, \sigma = 0.5$ and binary variables to $\bar{x} = 0$ with a difference of 1 between categories (Gelman, 2008), using Package 'arm' (Gelman *et al.*, 2012). We investigated multicolinearity by assessing variance inflation factors (VIFs) (Anderson *et al.*, 2001). We evaluated candidate models for all of our analyses using an information-theoretic approach (Anderson, 2008) using Akaike's Information Criterion (Akaike, 1974) with small sample size correction (AIC_c) (Anderson, 2008), based on marginal likelihood (Vaida & Blanchard, 2005). We used multimodel inference and model averaging (Burnham & Anderson, 2002) with Akaike weights (w_i) of all candidate models. After model selection, we used 85 % confidence intervals (Anderson, 2008, Anderson *et al.*, 2001, Arnold, 2010) to assess goodness of fit of parameter estimates and Ω_0^2 to assess variation explained by the global model (Xu, 2003).

5.4 Results

While we suppressed reproduction in a troop of captive banded mongooses and fed them a constant diet, they showed no discernible fecal glucocorticoid metabolite response to season (Figure 5.1(a)). They had low fecal glucocorticoid metabolite concentrations with low variability (n = 98, median = 0.28 μg g^{-1} org. content, interquartile range = 0.37 μg g^{-1} org. content). During their reproductive period the same mongooses had 10-fold higher fecal glucocorticoid metabolite concentrations with considerably higher variability (n = 104, median = 2.98 μg g^{-1} org. content, interquartile range = 4.37 μg g^{-1} org. content). Peak fecal glucocorticoid responses during this reproductive period occurred shortly after each parturition event and coincided with the captive female's behavioral estrus and mating, depredation of pups by an African rock python (*Python sebae natalensis*), a troop invasion by foreign males, and the loss of three out of four pups from a second litter (Figure 5.1(b)). Aside from these physiological challenges which were directly related to reproduction, fecal glucocorticoid metabolites appeared to increase leading up to parturition. After the loss of the litter and the removal of the foreign males, fecal glucocorticoid metabolites increased 5-fold from January 2011 (n = 14, median = 0.45 μg g^{-1} org. content, interquartile range = 0.28 μg g^{-1} org. content) to February 2011 (n = 21, median = 2.64 μg g^{-1} org. content, interquartile range = 3.68 μg g^{-1} org. content), unrelated to any discernible external physiological challenges.

For free-ranging banded mongooses, the proportion of fecal organic matter in a bolus, amount of rainfall in the week prior to fecal collection, and access to concentrated anthropogenic food resources were important in explaining the variation in fecal glucocorticoid metabolites (Table 5.1, Figure 5.2). We selected fecal organic matter and rainfall in all of our best candidate models ($\Delta\text{AIC}_c < 2$, Table 5.1) and these two variables had the largest standardized effect sizes after model averaging across all candidate models (Figure 5.2). Across all candidate models, fecal organic matter was the most important ecological covariate with a summed Akaike weight (Σw_i) of 1 (Figure 5.2). The only covariates we considered unimportant were group size and canopy cover which had relatively low importance, high variability in their parameter estimates, or had a small effect size (for canopy cover) (Figure 5.2). As a measure of goodness of fit, our global model explained 55 % of the variation in fecal glucocorticoid metabolites, with $\Omega_0^2 = 0.55$ (Xu, 2003). Variance inflation factors for all covariates in the global model were below 2.

When we modeled only a mixed effect model with a fixed effect of fecal organic matter and a random effect of sampling event, for a single free-ranging ("urban + lodge") troop of banded mongooses, we selected the mixed effect model with fecal organic matter outright ($w_i = 1$) (Table 5.2) and this model explained 54 % of the variation in fecal glucocorticoid metabolites ($\Omega_0^2 = 0.54$). In the same analysis for the non-reproductive period of the captive troop, both the mixed effect model and the random effect model had some Akaike weight (Table 5.3). The mixed effect model had an evidence ratio (w_i/w_j) of 4.9 (Table 5.3) and explained 77 % of the variation in the captive troop's fecal glucocorticoid metabolites ($\Omega_0^2 = 0.77$), however, the random effect model without fecal organic matter was within 4 ΔAIC_c units of the mixed effect model and explained 76 % of the variation ($\Omega_0^2 = 0.76$).

A broad-scale, *post hoc* comparison of fecal glucocorticoid metabolite concentration and fecal organic matter illustrated the relationships between fecal glucocorticoid metabolites and the three most important covariates that we selected from our models: fecal organic matter, rainfall, and access to concentrated anthropogenic resources (Figure 5.3).

An additional *post hoc* comparison at a finer temporal scale suggested that within a group of banded mongoose troops (grouped by levels of access to anthropogenic resources), monthly levels of fecal organic matter exhibited high variability but no clear seasonal pattern (Figure 5.4(a – c)). When a troop had a constant food supply, then reproduction and its associated physiological challenges appeared to drive fecal glucocorticoid metabolite concentrations (Figure 5.1(a and b), Figure 5.4(d)). Reproductive activity, and hence fecal glucocorticoid metabolite concentrations followed a broadly seasonal pattern (Figure 5.4(d)). In free-ranging troops, fecal glucocorticoid metabolite concentrations appeared to increase towards the end of the dry season and did not exhibit the seasonal pattern related to reproduction seen in the captive troop (i.e. the continued elevation of fecal glucocorticoid metabolites through the wet season) (Figure 5.4(e and f)). The peak fecal glucocorticoid metabolite response in urban troops with access to tourist lodges occurred in November, the approximate start of the wet season and approximate time of first parturition each wet season (Figure 5.4(e)). For troops in the Chobe National Park with access to a lodge, the peak fecal glucocorticoid metabolite response occurred in September at the approximate time of first estrus, eviction and dispersal (Figure 5.4(f)). The fecal glucocorticoid metabolites in the peak month represented 40-fold, 12-fold, and 16-fold increases relative to the nadir month in the captive, "urban + lodge", and "park + lodge" troops, respectively (Table 5.4). The fecal glucocorticoid metabolites in the nadir months for the "urban + lodge" and "park + lodge" troops represented 2-fold and 3-fold increases (respectively) relative to the nadir month for the captive troop (Table 5.4) and 0.5-fold and 0.9-fold increases (respectively) relative to the captive troop's long-term non-reproductive baseline. In contrast, the fecal glucocorticoid metabolites in the peak month for the "urban + lodge" troops represented only a 0.04-fold decrease relative to the captive troop's peak month, but the fecal glucocorticoid metabolites in the peak month for the "park + lodge" troops represented a 0.7-fold increase relative to the captive troop's peak month (Table 5.4).

5.5 Discussion

Our study identifies (in decreasing order of importance) nutritional limitation, reproduction, and apparent predation risk as potential covariates that explain the variability in banded mongoose glucocorticoid production at the population level. Here we showed experimentally in captive banded mongooses that in the absence of nutritional limitation, reproduction, along with its associated predation risks and intraspecific agonistic interactions, increased banded mongoose glucocorticoid production dramatically. We also showed experimentally that a constant provision of anthropogenic food to captive mongooses may result in substantially lowered baseline glucocorticoid levels. Further, we showed from an extensive correlative field study of free-ranging

banded mongoose troops that an animal's current nutritional status, as measured by fecal organic matter, best explains variability in fecal glucocorticoid metabolite concentrations in banded mongoose feces. This effect was overwhelming in free-ranging banded mongooses, but it was only marginally better than a random intercept model (controlling for repeated measures) in a captive troop fed a constant supply of food. One major caveat for our results and interpretation is the potential effect of dietary differences (independent of food limitation) among banded mongoose troops on fecal glucocorticoid metabolism and excretion (Wielebnowski & Watters, 2007). We validated our fecal glucocorticoid metabolite assay using captive mongooses fed on a diet of processed pet food (Laver *et al.*, 2012) (Chapter 4). For a more rigorous approach, future studies should validate assays using mongooses fed *ad libitum* on a variety of diets to determine whether diet composition affects fecal glucocorticoid metabolite concentrations.

We do not know why captive mongooses should exhibit this residual effect of nutritional status or why they should exhibit such considerable variation in proportion of fecal organic content longitudinally or within a month (Figure 5.4(a)). *Post hoc* consideration suggests that although workers at the captive facility fed the captive troop a constant supply of food, they did not feed the mongooses *ad libitum*. Further, when we measured organic content of their pet food, following the same method as for their feces, we found a proportion of organic matter of approximately 0.92 which would account for some of the inorganic fraction in the captive mongoose feces. Because of this inorganic fraction and the lack of *ad libitum* food provisioning, the animals may have had to supplement their diet with foraging in the enclosure. This foraging may account for the remaining inorganic fraction in their feces. Although the captive mongooses fed from two open bowls at which all four animals could gain access, we did observe some aggression but no outright dominance at the feeding bowls. In situations where a single animal can control access to a food item, dominant banded mongooses do exclude subordinates from the food source (De Luca & Ginsberg, 2001) but in free-ranging mongooses this dominance accounts for only minor effects on nutritional status (De Luca & Ginsberg, 2001). Dominance during communal feeding in our captive mongooses may have resulted in within-group differences in food intake that we could not perceive from our behavioral observations.

In our extensive field study, we expected to find reproduction as an important explanatory variable for the variability in fecal glucocorticoid metabolite concentrations in free-ranging mongooses. Unfortunately our modeling framework could detect only broad patterns and the effect of nutritional limitation swamped the effect of other explanatory variables at the population level. We suspect, *post hoc*, that access to anthropogenic food resources (Figure 5.3(b)) and broad soil substrate differences (not shown) may explain differences in fecal organic matter at the troop level. We need future research to focus on analysis within mongoose troops with a modeling framework capable of detecting what appears to be a complex combination of late dry season food limitation and first reproduction in a season.

Based on longitudinal fecal glucocorticoid metabolite profiles (Figure 5.4(e and f)) and the results of our analysis, it appeared *post hoc* as though free-ranging banded mongooses increased glucocorticoid production as they became nutritionally limited, as the food-limiting dry season progressed. Peak glucocorticoid production appeared to coincide with reproductive events but

the amount and nature of anthropogenic food provisioning appeared to moderate when this peak occurred. In troops with more dispersed and potentially greater total anthropogenic provisioning, the peak was delayed until right at the end of the dry season and coincided approximately with the first parturition of the season. In the putatively more food-limited troops in the Chobe National Park with access to only a single lodge each, peak glucocorticoid production appeared to occur earlier in the dry season and coincided approximately with the first mating opportunities of the season. This time period also happened to be when most troop evictions and fissions occurred. Further, these "park + lodge" troops concentrated their movements and their foraging around lodge refuse sites (Chapter 3) and these concentrated food sources increased aggression and agonistic encounters within a troop (Fairbanks *et al.*, In prep.). Thus, free-ranging banded mongooses may face a confluence of factors that cause extreme glucocorticoid production in the late dry season — a perfect storm of nutritional limitation, agonistic encounters at concentrated food sources, aggressive evictions of subordinates, estrus and competition for mating opportunities, parturition and subsequent predation of pups. Increased access to more dispersed anthropogenic food sources may mitigate and delay the effect of this combination of factors, and then, increased availability of food during the wet season may mitigate the effect of subsequent reproductive activity as the wet season progresses.

Although we need further research to elucidate the nuances of this seasonal glucocorticoid response, free-ranging mongooses clearly had a chronic exposure to elevated glucocorticoids during part of the year and this chronic exposure may have important epidemiological consequences. Glucocorticoids have a varied effect on immune function. Under different conditions, glucocorticoids may enhance, permit or suppress immune function (Sapolsky *et al.*, 2000). Glucocorticoids play an important role in allostasis and immune function (McEwen & Seeman, 1999) and are implicated in the redistribution of immune cells, specifically leukocytes and cytokines during the immune response (Dhabhar, 2000, 2002, 2003). However, chronic hypothalamic-pituitary-adrenal axis activation may lead to allostatic load (McEwen & Seeman, 1999) and immune suppression — especially lowered skin immunity (Dhabhar, 2000, Dhabhar & McEwen, 1999). The effect of glucocorticoids and catecholamines on immune function is complex and involves the suppression of cellular immunity and activation of humoral immunity, mediated by a glucocorticoid-induced change in the Th1/Th2 balance via suppression of Interleukin-12 (IL-12) and Interferon-gamma (IFN-γ) (Elenkov & Chrousos, 1999). Further, IL-12-dependent IFN-γ secretion is important in mycobacterial immunity in humans (Altare *et al.*, 1998) and the IL-12 cytokine pathway is important in responses to intracellular bacteria in general (Jong *et al.*, 1998). Thus, glucocorticoid-mediated immune suppression may play a particularly important role in mycobacterial infections or disease progression.

In our study population of banded mongooses, one likely portal of entry for *M. mungi* is through lesions on the *planum nasale* or skin (Alexander *et al.*, 2010). We have found tuberculous lesions in the skin of 13 of 18 (72 %) infected mongooses for which we examined skin lesions or the *planum nasale* histologically (M. Williams and K. Alexander, unpublished data) and 75 % of injured mongooses developed clinical signs of *M. mungi* infection (Fairbanks *et al.*, In prep.). Thus, the role of chronic glucocorticoid production in lowered skin immunity (Dhabhar, 2000, Dhabhar

& McEwen, 1999), suppressed cellular immunity in general (Elenkov & Chrousos, 1999), and suppressed mycobacterial immunity in particular (Altare *et al.*, 1998, Jong *et al.*, 1998) suggests that chronic stress responses may be important in *M. mungi* infections in banded mongooses. A possible next step in research on *M. mungi* infection in banded mongooses may include challenging mongooses with a phytohemagglutinin (PHA) skin test (Bonforte *et al.*, 1972, Lawlor Jr *et al.*, 1973) during periods of low and high baseline glucocorticoids to determine if the chronic elevation of glucocorticoids we found does induce an epidemiologically-relevant change in cellular immunity. Researchers should combine this test with flow cytometry (Tella *et al.*, 2008) or histological examination (Turmelle *et al.*, 2010) to determine which leukocytes mediate the inflammation typically measured in a phytohemagglutinin skin test (Turmelle *et al.*, 2010).

By testing for a epidemiologically-relevant change in cellular immunity using the phytohemagglutinin skin test, researchers could determine whether the chronic glucocorticoid elevation we found in banded mongooses does lead to a pathology from homeostatic overload. In the context of the reactive scope model (Romero *et al.*, 2009), we suspect that the "urban + lodge" troops show glucocorticoid responses to the combined effect of late dry season food limitation and first parturition which fall within the range of predictive homeostasis (because the median values in peak months differ only marginally from the captive troop's median values in peak months). Some animals in these troops may enter homeostatic overload at this time (because the variability around this peak month median is greater, with higher extreme glucocorticoid values than the captive troop). The "park + lodge" troops, however, probably have a larger proportion of animals that enter homeostatic overload during their peak glucocorticoid response to food limitation and first estrus of the season. Although anthropogenic provisioning at tourist lodges may appear at first consideration to help mitigate dry season food limitation in these troops, we suspect that through its effect on concentrating mongoose movements and foraging around a highly concentrated food resource, it may actually present an ecological trap by inducing homeostatic overload. An ecological trap requires both habitat selection of poor quality habitat, and a demographic response to this habitat (Battin, 2004), and we need future research to determine whether mongoose troops select concentrated anthropogenic food resources at the second and third order (Johnson, 1980) and whether these troops have lowered fitness.

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Table 5.1. Model selection for mixed effects candidate models modeling fecal glucocorticoid metabolites (FGM), 11,17-dioxoandrostanes (11,17-DOA) (n = 1542 feces), in banded mongooses (*Mungos mungo*) in northeastern Botswana (2008 – 2011). We modeled troop identity (1|troop) and sampling event for each troop (1|event) as random effects in all models (random effects not shown in table). Fixed effects were the percentage organic matter of a bolus (org), the soil macrofauna density (macro), recent rainfall (rain), access to concentrated anthropogenic food sources (anth), breeding status (breed), troop size (size), and percent canopy cover (cc). We analyzed all subsets of the seven fixed effects but present only the best models ($\Delta\text{AIC}_c < 2$) here. We used all models for model averaging and parameter estimation.

	Model: *ln*(FGM, 11,17-DOA conc. ($\mu g\, g^{-1}$ org.)) $\sim$	log$L$	K	AIC$_c$	Δ	w_i
1	org + rain + anth	−2176.5	7	4367.1	0.0	0.06
2	org + rain	−2177.7	6	4367.4	0.4	0.05
3	org + rain + anth + breed	−2175.7	8	4367.6	0.5	0.04
4	org + rain + breed	−2176.8	7	4367.7	0.6	0.04
5	org + rain + anth + breed + macro	−2174.8	9	4367.8	0.7	0.04
6	org + rain + anth + macro	−2176.0	8	4368.1	1.0	0.03
7	org + rain + breed + macro	−2176.1	8	4368.3	1.2	0.03
8	org + rain + anth + size	−2176.2	8	4368.4	1.3	0.03
9	org + anth + macro	−2177.2	7	4368.5	1.4	0.03
10	org + rain + cc	−2177.2	7	4368.5	1.4	0.03
11	org + rain + anth + breed + size + macro	−2174.2	10	4368.5	1.4	0.03
12	org + rain + anth + breed + size	−2175.3	9	4368.7	1.6	0.02
13	org + rain + size	−2177.3	7	4368.8	1.7	0.02
14	org + rain + macro	−2177.4	7	4368.8	1.7	0.02
15	org + rain + breed + size	−2176.4	8	4368.8	1.7	0.02
16	org + rain + breed + cc	−2176.4	8	4368.9	1.8	0.02
17	org + rain + breed + size + macro	−2175.4	9	4369.0	1.9	0.02

Table 5.2. Model selection for a mixed effects candidate model and a random effects model, modeling fecal glucocorticoid metabolites (FGM), 11,17-dioxoandrostanes (11,17-DOA) (n = 584 feces), in a single free-ranging troop of banded mongooses (*Mungos mungo*) in northeastern Botswana (2008 – 2011). We modeled sampling event (1|event) as a random effect in both models. The only fixed effect was the percentage organic matter of a bolus (org).

	Model: ln(FGM, 11,17-DOA conc. ($\mu g\, g^{-1}$ org.)) $\sim$	log$L$	K	AIC$_c$	Δ	w_i
1	org + (1|event)	-783.8	4	1575.7	0.0	1.00
2	(1|event)	-797.5	3	1600.9	25.3	0.00

Table 5.3. Model selection for a mixed effects candidate model and a random intercept model, modeling fecal glucocorticoid metabolites (FGM), 11,17-dioxoandrostanes (11,17-DOA) (n = 86 feces), in a single captive troop of banded mongooses (*Mungos mungo*) in northeastern Botswana (2008 – 2011). We modeled sampling event (1|event) as a random effect in both models. The only fixed effect was the percentage organic matter of a bolus (org).

	Model: ln(FGM, 11,17-DOA conc. ($\mu g\, g^{-1}$ org.)) $\sim$	log$L$	K	AIC$_c$	Δ	w_i
1	org + (1|event)	-93.1	4	194.7	0.0	0.83
2	(1|event)	-95.8	3	198.0	3.2	0.17

Table 5.4. Fecal glucocorticoid metabolite ((FGM), 11,17-dioxoandrostanes (11,17-DOA)) concentrations during peak and nadir months in three groups of banded mongoose (*Mungos mungo*) troops in northeastern Botswana (2008 – 2011). Troop types differ by their level of anthropogenic disturbance. We report all concentrations as $\mu g\, g^{-1}$ org. content.

		Nadir month			Peak month		
	Troop type	n	Median ($\mu g\, g^{-1}$ org.)	IQR	n	Median ($\mu g\, g^{-1}$ org.)	IQR
1	Captive	8	0.14	0.12	34	5.49	5.23
2	Urban + lodge	15	0.41	1.29	19	5.28	8.93
3	Park + lodge	36	0.55	0.59	90	9.00	14.24

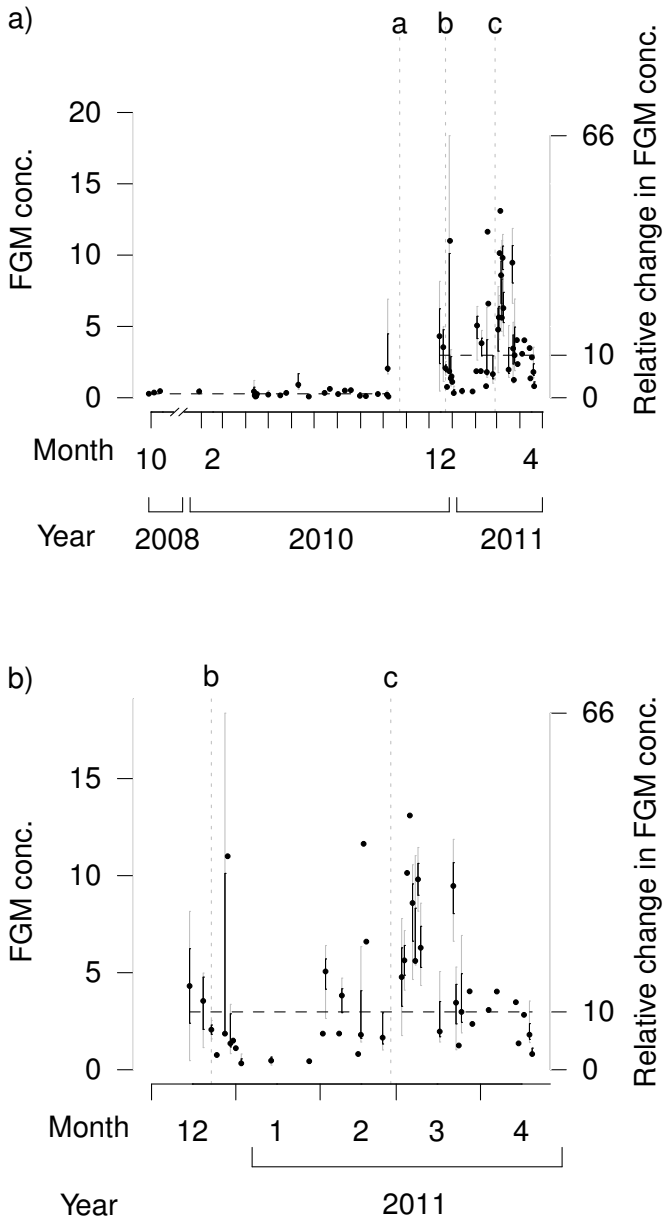


Figure 5.1. (a) Longitudinal profile of fecal glucocorticoid metabolites (FGM), 11,17-dioxoandrostanes (11,17-DOA), in a captive troop of four banded mongooses (*Mungos mungo*) (one female, three males) in northeastern Botswana (2008 – 2011). Prior to May 2010 we suppressed estrus in the female and we observed no reproductive behavior until the female conceived in October 2010 (at line a). (b) During their reproductive period, the female gave birth to two litters (at lines b and c) and the troop suffered from predation and troop invasion events. Dotted horizontal lines indicate median FGM concentrations for the non-reproductive (baseline) and reproductive periods. Dots indicate median FGM concentrations for each day of sampling. Tufte's quartile plots (Tufte, 2001) indicate variability for each sampling day with grey error bars representing values within 1.5*(interquartile range) of the first and third quartiles, and black lines representing the interquartile range. Secondary *y*-axis represents change relative to the non-reproductive baseline.

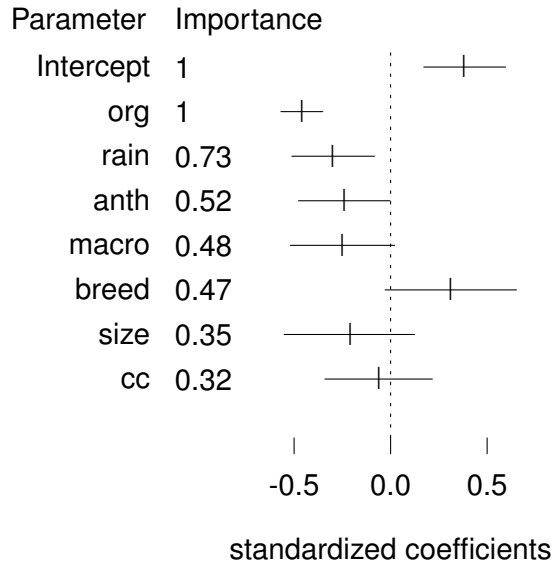


Figure 5.2. Intercept and effect sizes for standardized parameters (Gelman, 2008) with 85 % confidence intervals (Arnold, 2010), after model averaging of all candidate models. Parameters were the percentage organic matter of a bolus (org), the soil macrofauna density (macro), recent rainfall (rain), access to concentrated anthropogenic food sources (anth), breeding status (breed), troop size (size), and percent canopy cover (cc). After parameter labels, we report relative importance — sum of Akaike weights (Σw_i) over all models that include the parameter.

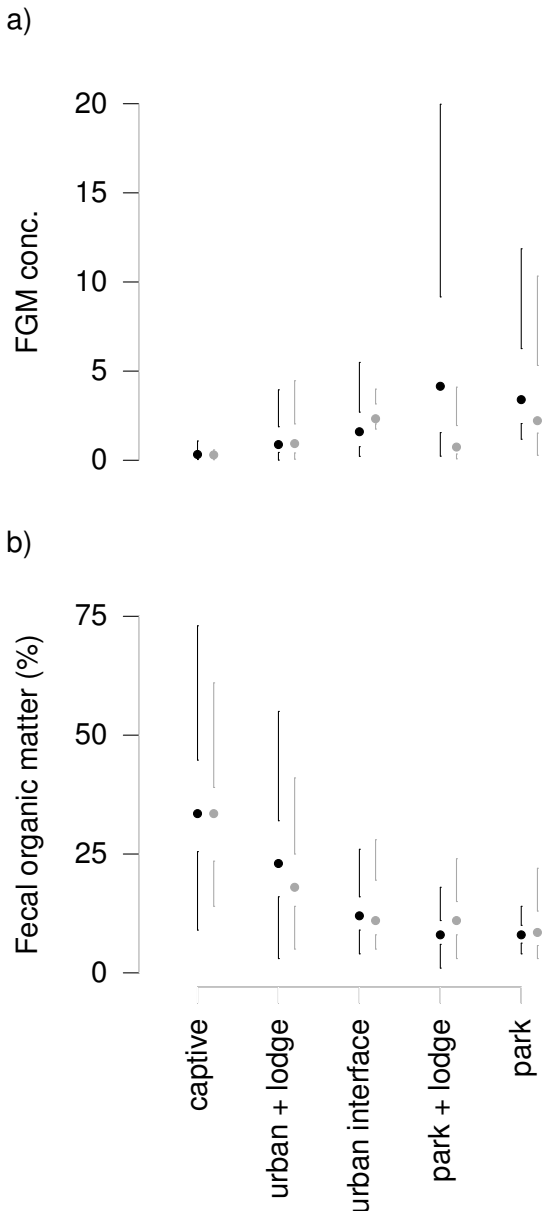


Figure 5.3. (a) Fecal glucocorticoid metabolite (FGM), 11,17-dioxoandrostane (11,17-DOA), concentration and (b) percentage fecal organic matter in banded mongoose (*Mungos mungo*) troops in northeastern Botswana (2008 – 2011). We grouped troops by level of exposure to anthropogenic disturbance (decreasing from left to right). Black quartile plots (Tufte, 2001) indicate dry season medians (dots) and variability (values within 1.5*(interquartile range) of the first and third quartiles). Grey quartile plots indicate wet season values. Comparing (a) to (b) illustrates the main effect of fecal organic matter. Comparing wet and dry season estimates within (a) broadly illustrates the effect of rainfall. Reading (a) and (b) from left to right illustrates the effect of access to anthropogenic resources.

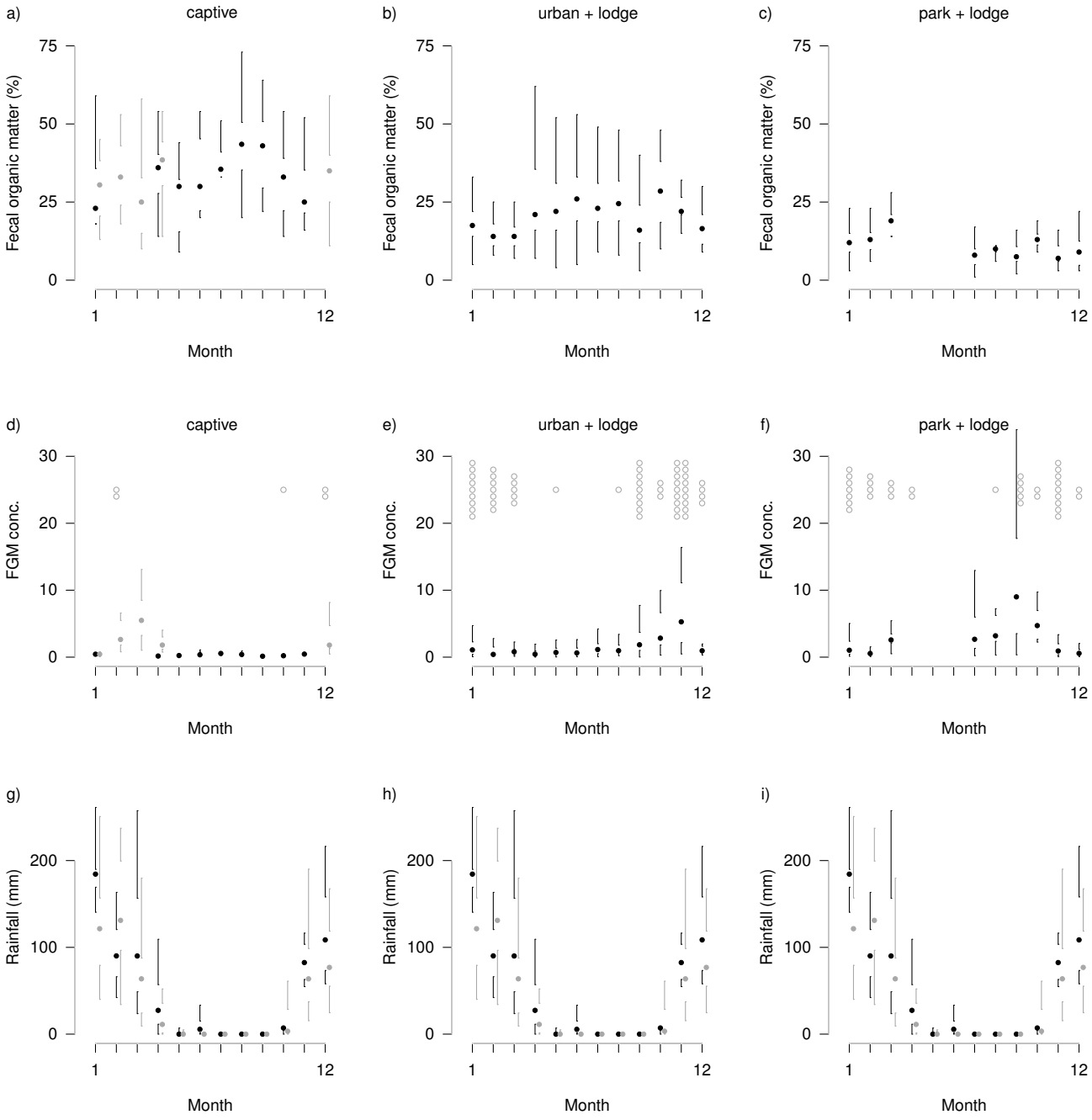
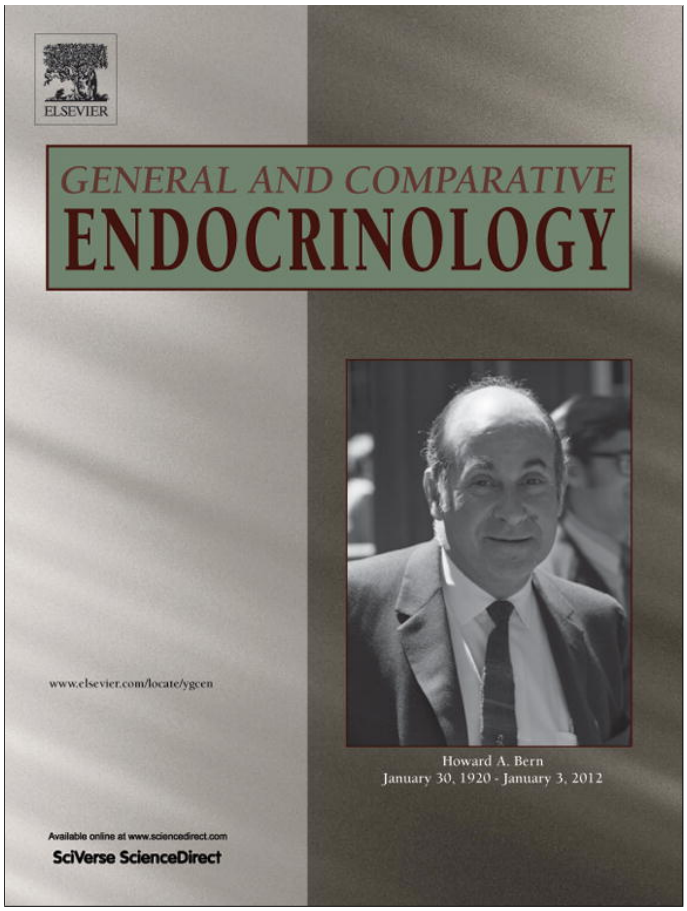


Figure 5.4. Quartile plots (Tufte, 2001) of percentage fecal organic matter (a – c), and fecal glucocorticoid metabolite (FGM), 11,17-dioxoandrostane (11,17-DOA) concentration (d – f), by month of the year for banded mongoose (*Mungos mungo*) troops in northeastern Botswana (2008 – 2011). We grouped troops by decreasing level of exposure to anthropogenic disturbance, from a captive troop (a and d) to urban troops with access to tourist lodges (b and e) to troops in the Chobe National Park with access to tourist lodges (c and f). For a single meteorological station in the study area, rainfall (g – i)) over our study period (black quartile plots) approximately matched long-term data (1994 – 2006, grey quartile plots). For a captive troop (a and d), we depict data for a non-reproductive period in black and for a reproductive period in grey. Open grey circles (d – f) depict reproductive events in a given month (each circle represents a single troop estrus or parturition on an arbitrary vertical axis).

Appendices

Appendix A

Peter N. Laver, André Ganswindt, Stefanie B. Ganswindt, Kathleen A. Alexander. 2012. Non-invasive monitoring of glucocorticoid metabolites in banded mongooses (*Mungos mungo*) in response to physiological and biological challenges. *General and Comparative Endocrinology* 179: 178 – 183.



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Non-invasive monitoring of glucocorticoid metabolites in banded mongooses (*Mungos mungo*) in response to physiological and biological challenges

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ABSTRACT

Free-ranging banded mongooses are infected by the novel pathogen, *Mycobacterium mungi* in northern Botswana. A reliable method for determining stress-related physiological responses in banded mongooses will increase our understanding of the stress response in *M. mungi* infection. Therefore, our aim was to examine the suitability of four enzyme immunoassays (EIAs) for monitoring adrenocortical endocrine function in captive and free-ranging banded mongooses based on fecal glucocorticoid metabolite (FGM) analysis. A conducted adrenocorticotropic hormone challenge revealed suitability of a valid measurement of FGM levels in banded mongoose feces for all four tested EIAs, with an 11-oxoetiocholanolone assay detecting 11,17-dioxoandrostanes (11,17-DOA) performing best. Subsequent analyses using only this EIA showed the expected decrease in FGM concentrations 48 h after administering dexamethasone sodium phosphate. Furthermore, captive mongooses showed higher FGM concentrations during reproductive activity, agonistic encounters and depredation events. Finally, a late-stage, tuberculosis-infected moribund mongoose in a free-ranging troop had a 54-fold elevation in FGM levels relative to the rest of the troop. Measurements of gastrointestinal transit times and FGM metabolism post-defecation indicate that the time delay of FGM excretion approximately corresponded with food transit time and that FGM metabolism is minimal up to 8 h post-defecation. The ability to reliably assess adrenocortical endocrine function in banded mongoose now provides a solid basis for advancing our understanding of infectious disease and endocrinology in this species.

1. Introduction

Probably still the most widely used approach to monitoring endocrine function is the measurement of circulating hormones, but obtaining blood samples from wild, free-ranging animals is often difficult or impossible and may introduce confounding effects of capture- or handling-induced stress [46]. Non-invasive hormone monitoring, especially through excretions such as feces, urine and saliva, is already a well-established approach [14,46,45,28,34,24,51,32]. It is not, however, without problems [51] and validation of non-invasive assays is vital [47]. This is especially true when applied to a species for the first time, since the particular circulating glucocorticoids (e.g. cortisol versus corticosterone), and their metabolism and routes of excretion are species- [37] and sex-specific [48,37].

Banded mongooses (*Mungos mungo*) are small, diurnal, cooperatively breeding mammals in the order Carnivora and family Herpestidae, distributed widely in sub-Saharan Africa. Free-ranging banded mongooses in our study population in northern Botswana are infected with the novel *Mycobacterium mungi* which has caused seven outbreaks in the population since 2000 [3]. A viable non-invasive approach to monitor glucocorticoid output in banded mongooses would facilitate research on the possible links between the stress response, allostatic overload and *M. mungi* infection dynamics. Unfortunately, no test system to monitor adrenocortical endocrine function based on fecal glucocorticoid metabolite (FGM) analysis has been validated to date for banded mongooses.

Therefore, our objectives were to (a) validate an enzyme immunoassay for measuring FGMs in banded mongooses using physiological/pharmacological challenges and (b) biological challenges associated with the reproductive cycle and chronic disease. Furthermore, we (c) determined the gastrointestinal transit time and the rate of metabolism of FGMs post-defecation to further evaluate the approach of non-invasive hormone measurements as a tool to provide information on the level of stress experienced in this species.

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2. Materials and methods

2.1. Study area and animals

We conducted this study on 13 troops (troop size range 5–64) of free-ranging banded mongooses and four (one female and three males) captive animals for almost 4 years from October 2007 to June 2011. We monitored free-ranging mongoose troops over an area of approximately 120 km^2 in the towns of Kasane and Kazungula (approx. 17 km^2 of developed land) and surrounding areas of the Chobe National Park (approx. 30 km^2) and Kasane Forest Reserve (approx. 73 km^2) in northeastern Botswana, with our study site centered at approximately 25.163°E and 17.828°S.

This area has seasonal rainfall, with a wet season from November to March and annual mean (SE) rainfall in Kasane (1975–2005) of 574 (30) mm [5]. Soil groups in the study included gleysols, fluvisols, luvisols and arenosols [1] and the altitude was between 927 and 1012 m above mean sea level.

The captive troop was housed together in an outdoor enclosure (approx. 95 m^2) at the Center for African Resources: Animals, Communities and Land use (CARACAL) research facility in Kasane. The four animals were raised in captivity from approximately 2 weeks of age and were adults of approximately 3 years of age at the time of the pharmacological challenges.

We conducted this study under approval and in accordance with the guidelines of the Virginia Tech Institutional Animal Care and Use Committee (07-146-FIW).

2.2. Observations and sample collection

For free-ranging troops we made approximately 3600 troop observations between October 2007 and June 2011, recording spatially-referenced weather, habitat, behavioral, clinical and other relevant ecological data. For troops marked with radio collars, we found their evening dens in the morning before sunrise and returned to collect fresh feces later in the morning. We collected feces from unmarked troops opportunistically during observations, or after following troops to their dens the evening prior to fecal collection. To minimize disturbance [51] we waited until after any over-marking had been completed [33,21–23] and the mongooses had left the den site on foraging bouts before collecting feces. We avoided additional diurnal variation by collecting feces only in the morning [51] or by using time of day as a factor in analyses. We collected samples using transparent plastic bags and estimated by touch, the temperature, consistency and relative water content of the collected material to estimate the freshness of the sample. Fresh feces were warm (within a few minutes of defecation), wet and pliable. We excluded old feces that were dry and friable. We placed collected material on ice for a maximum of 4 h prior to storage at −20 °C. We collected 25 samples from a free-ranging troop at two sampling events in conjunction with observed putative stressful events which we used for the biological validation of our enzyme immunoassay.

We collected a total of 304 fecal samples from the four captive mongooses over 97 sampling events. Collection took place daily at 8 AM between April 2010 and May 2011. We collected fecal samples following the protocol for sample collection for free-ranging troops (above) but froze feces within approximately 1 h of defection at −20 °C until further processing.

2.3. Determination of gastrointestinal transit (GIT) time

To estimate GIT time for banded mongooses, we conducted an experiment using food-grade dyes and uncooked rice, adding these ingredients to the regular food of the four captive mongooses. We fed them daily at 8 AM as per their normal regimen and measured the time lag between consumption of the marked meal and the excretion of marked feces.

2.4. Post-defecation metabolism

Especially for the free-ranging troops, fecal sample collection was often impossible directly after defecation, therefore we assessed the metabolism rate of FGMs post defecation to ensure reliable data interpretation. We performed a post-defecation metabolism experiment according to the procedure described by [31]. Therefore, we collected fecal samples from four captive mongooses and divided each sample into five subsamples which we placed under environmental conditions (full sunlight) for 0, 2, 4, 6, or 8 h prior to storage at −20 °C. Maximum (27–33 °C) ambient temperatures varied slightly during the experiment and we recorded no rainfall.

2.5. Steroid extraction and analysis

We lyophilized, pulverized and sifted fecal samples using a mesh strainer to remove fibrous material as described by [17]. We then extracted approximately 0.05 g of the fecal powder with 80% ethanol in water (1.5 mL) according to the procedure described by [17] and additionally determined the organic content of each sample according to the procedure described by [16]. All steroid concentrations are expressed henceforth per mass of dry organic fecal matter. We measured the resulting extracts for immunoreactive FGMs using cortisol, corticosterone and two different 11-oxoetiocholanolone EIAs. Details of the EIAs including cross-reactivities of the antibody are described by [36,30]. The 11-oxoetiocholanolone EIA detecting 11,17-DOA that we used for the majority of our analyses, had an intra-assay CV of 2.8–4.0% and an inter-assay CV of 12.1–16.8%. The sensitivity of the assay at 90% binding was 3 pg/well. We performed assays on microtiter plates according to the procedure described by [15].

2.6. ACTH challenge test

We intramuscularly injected long-acting ACTH preparation (Tetracosactide 1 mg/mL: Synacthen Depot; Novartis, Basel, Switzerland, Lot S0892) into three captive banded mongooses using doses of 200 μg/kg for one adult female and one adult male and 133 μg/kg for a second adult male. We additionally injected (i.m.) an equivalent volume (0.3 mL) of sterile isotonic saline solution as a control into an additional adult male. We collected samples on the day of and for 3 days after the ACTH injection, checking for feces in the enclosure hourly during daylight hours when mongooses are typically active.

2.7. Dexamethasone suppression test

Using the same experimental setup as for the ACTH challenge test (see Section 2.6), we injected (i.m.) 105.6 μg/kg dexamethasone sodium phosphate (DEX; 2.64 mg/mL: Dexa 0,2 Phenix; Virbac RSA, Centurion, South Africa, Lot BJ77) into the three animals of the experimental group 21 days after the ACTH challenge test. We administered an equivalent volume (0.06 mL) of saline into our control male.

2.8. Suppression of reproductive activity

To suppress estrous in the captive female mongoose, we administered an orally-delivered progestin contraceptive, megestrol acetate (Ovarid; Schering-Plough Corporation, Kenilworth, USA) between September 2008 and May 2010. During this time, neither

the males nor the female in the captive group engaged in any reproductive behavior and the animals were not visited or invaded by any free-ranging mongooses. After May 2010, we observed estrous behavior and parturition as well as the troop invasions and depredation events that coincided with these reproductive events.

2.9. Data analysis

We standardized FGM concentrations relative to a predefined baseline to give relative differentials. Baseline FGM concentrations were determined as the median of the four captive animals (a) on the day of ACTH injection (day 0 ACTH), (b) on the day of DEX injection (day 0 DEX) and (c) during their pre-reproductive period from 29 January 2010 to 8 October 2010. Furthermore, we used as baselines the starting FGM concentration for each captive mongoose (hour 0) in the post-defecation metabolism study and the median FGM concentration for the clinically healthy troop members of the sick animal in the disease comparison. For the ACTH and DEX analyses, we used the median FGM concentration for each animal on each day (when a mongoose defecated multiple times on a given morning). We then report medians with interquartile range for the relative differentials for the experimental mongooses. The sample sizes for the post-defecation metabolism study were small (four mongooses with each feces sampled at five sampling events) and hence we could not reliably assess normality for the data. Thus, we tested for differences in the distributions of FGM concentrations among sampling events using Friedman's rank sum test using R [39].

3. Results

3.1. Gastrointestinal transit (GIT) time

Gastrointestinal transit time for the four captive mongooses based on the use of food-grade dyes and uncooked rice was a minimum of 24 h (first clearance of marked food items), although residual marking of the feces continued for up to 72 h (final clearance of marked food items).

3.2. ACTH challenge test

Using the 11-oxoetiocholanolone EIA detecting 11,17-DOA, median FGM concentrations increased more than 2-fold above pre-injection levels 24 h post ACTH administration (i.e. 300% of the starting values). The median baseline, peak and nadir values were 0.81 μg/g org content, 2.89 μg/g org content and 0.63 μg/g org content, respectively. In comparison, the remaining three assays tested revealed a 1-fold elevation (i.e. 200% of starting values) in median FGM concentrations 24 h post ACTH injection (Fig. 1). Their median baseline, peak and nadir values were 1.5 μg/g org content, 2.73 μg/g org content, 0.77 μg/g org content (11-oxoetiocholanolone EIA detecting 5β-3α-ol-11-one), 58.4 ng/g org content, 104.2 ng/g org content, 62.3 ng/g org content (cortisol) and 1.03 μg/g org content, 2.12 μg/g org content, 0.67 μg/g org content (corticosterone). In all cases, median FGM levels returned to pre-injection baseline levels at 48 h (Fig. 1). Therefore, only the 11-oxoetiocholanolone EIA measuring 11,17-DOA was used for any further analysis.

Interestingly, the saline-injected control animal showed a similar response to that of the experimental animals (Fig. 1), indicating that handling was presumably the prominent stressor in this experiment.

3.3. Dexamethasone suppression test

The administration of dexamethasone revealed an almost 1-fold decrease (15% of starting values) in median FGM levels in the experimental group 48 h post-injection, before FGM concentrations returned approximately to their pre-injection baseline at 72 h (Fig. 2). In contrast to the conducted ACTH challenge, no handling-related increase in FGM concentrations could be detected in the control animal 24 h post-injection. FGM levels of that mongoose, however, increased substantially 72 h after handling (Fig. 2).

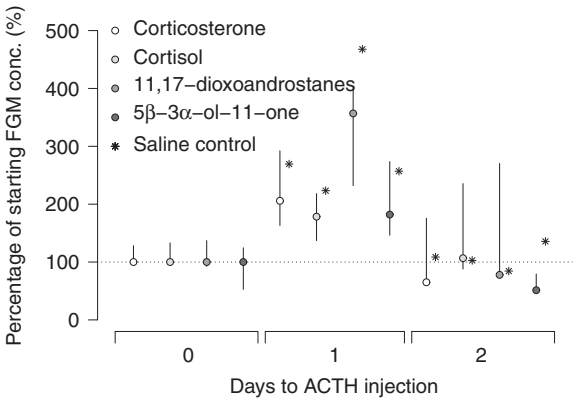

Fig. 1. FGM levels (median and interquartile range) for three captive banded mongooses (one female, two males) in response to ACTH administration. Levels are expressed relative to the pre-ACTH levels (day of injection, 100%). The saline control male's FGM response to the handling and saline injection is indicated by the asterisks.

3.4. Biological validation

3.4.1. Suppression of reproductive activity

We observed several, partly reproduction-related, potentially stressful events in the captive mongooses after administration of contraceptives was stopped. These events were associated with increases in FGM levels relative to the baseline FGM concentration while the progestin contraceptive was administered (2010/01/29–2010/10/08). We observed the following range of FGM concentrations for the three different observation periods with the captive troop: contraceptive period, $n = 98$, 0.03–6.91 μg/g org content; reproductive period, $n = 104$, 0.08–18.37 μg/g org content; phar-

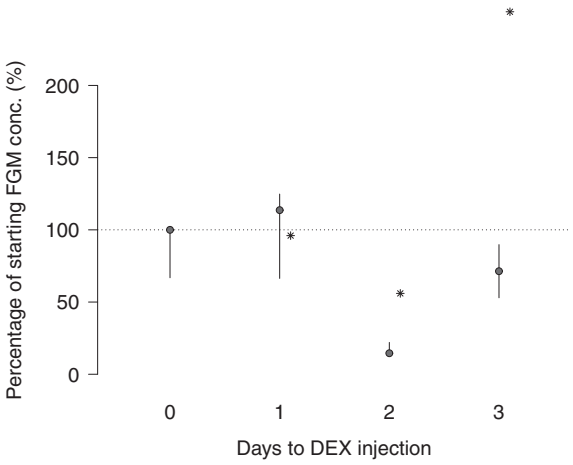

Fig. 2. FGM (11,17-DOA) levels (median and interquartile range) determined with an 11-oxoetiocholanolone EIA for three captive banded mongooses (one female, two males) in response to a dexamethasone (DEX) suppression test. Levels are expressed relative to the pre-DEX levels (day of injection, 100%). The saline control male's FGM response to the handling and saline injection is indicated by the asterisks.

macological validation period (ACTH and DEX), $n = 99$, 0.11–43.01 μg/g org content.

Case A. After contraception was stopped, troop median FGM concentration was 2.98 μg/g org content (2010/12/15–2011/04/20, $n = 104$). This was 10-fold higher compared to the median FGM level (0.28 μg/g org content) in 2010 while contraception was administered (i.e. 1100% of the baseline). Troop FGM concentrations increased leading up to and after the birth of the first litter. Three days prior to parturition, the median male FGM concentration increased to 3.55 μg/g org content (a 12-fold increase relative to the pre-reproductive period, or 1300% of baseline). One week prior to parturition and on the day of parturition, the female's median FGM concentrations were 4.32 μg/g org content and 2.07 μg/g org content, respectively, which reflect 15- and 7-fold increases relative to the pre-reproductive period (i.e. 1600% and 800% of baseline). For the month shortly after parturition and the loss of the first litter, troop median FGM concentration was 0.45 μg/g org content (2011/01/03–2011/01/28, $n = 14$, range: 0.08–1.11 μg/g org content) before FGM concentrations again increased towards the second parturition. Of these 14 samples, 12 had FGM concentrations below the upper 95% confidence limit of the pre-reproductive baseline and four samples were below the baseline median of 0.28 μg/g org content.

Case B. On 24 December 2010 an African rock python (*Python sebae natalensis*) entered the enclosure and predated at least two pups before it was discovered and removed a day later. On the 26th of December, three free-ranging male mongooses entered the enclosure, at which time no more pups were observed. All males, including the resident ones, fought over access to the female, before the invading males were trapped and removed 1 day later. We observed guarding and mating behavior, as well as putative estrus of the female, which ended on the 30th of December. During this time span, the female's FGM concentrations were elevated up to 18.37 μg/g org content (28th of December) which was a 66-fold increase relative to the 2010 troop pre-reproductive baseline levels (6700% of baseline) and an 8-fold increase relative to the female's FGM concentrations detected on the day of putative parturition (median FGM 2.07 μg/g org content). By the 31st of December her FGM concentrations had decreased to the level seen at parturition (1.38–1.62 μg/g org content) which were still 4- to 5-fold higher than the troop pre-reproductive baseline FGM concentrations (i.e. 500–600% of baseline).

Case C. In the 10 days after the second parturition, the female's median FGM concentration was 7.98 μg/g org content, which was a 28-fold increase relative to pre-reproductive baseline (i.e. 2900% of baseline). During this time, three of the four pups died. For the month after this period, her median FGM concentration dropped to 3.56 μg/g org content, a 12-fold increase relative to the pre-reproductive baseline (i.e. 1300% of baseline) and a 0.6-fold decrease relative to the post-parturition period (i.e. 44% of the post-parturition period).

3.4.2. FGM levels in relation to disease

The FGM concentration of a free-ranging mongoose diagnosed (after necropsy) with a late stage *M. mungi* infection was 188.16 μg/g org content. This was a 54-fold increase (i.e. 5500% of baseline) relative to the median FGM concentration for the remaining 24 putatively healthy troop members of the infected animal (3.4 μg/g org content, range: 0.5–15.5).

3.5. Post-defecation metabolism

FGM concentrations in four feces decreased 0.38-fold over the first 2 h after defecation (i.e. to 62% of the starting FGM concentration), but remained at approximately the same level up to 6 h thereafter (Fig. 3). Using the Friedman rank sum test, we failed to reject the null hypothesis that the FGM distributions were the same across the repeated measures (sampling times), $\chi^2 = 3, df = 4, p = 0.5$.

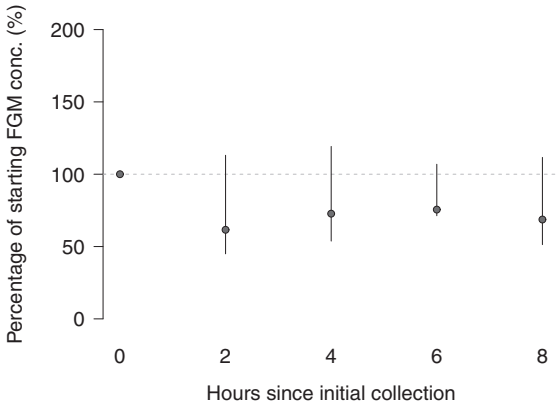

Fig. 3. Median and interquartile range of FGM (11,17-DOA) levels determined with an 11-oxoetiocholanolone EIA in four banded mongoose fecal samples from one female, three males over time since defecation. Levels are expressed relative to the starting concentration (100% at $t = 0$ h). Using the Friedman rank sum test, we failed to reject the null hypothesis that the FGM distributions were the same across the repeated measures (sampling times), $\chi^2 = 3, df = 4, p = 0.5$.

4. Discussion

We have shown that an 11-oxoetiocholanolone EIA can be reliably used to measure biologically relevant changes in glucocorticoid metabolite concentrations in banded mongoose feces. We validated this assay by conducting an ACTH challenge and dexamethasone suppression test as well as by monitoring putative stressful events in captive and free-ranging animals as a form of biological validation.

Although our chosen assay detects the expected stimulation and suppression of the HPA axis by ACTH and dexamethasone (respectively) in terms of relative changes in fecal glucocorticoid metabolite output, the revealed signal might be underestimated due to the suboptimal sampling regimen in our experiments, which was unavoidable due to the logistical setup at the research facility in Kasane and might have resulted in missing the peak samples during the partly one-off collection per day. Also the use of suboptimal doses for the pharmacological challenges is conceivable, especially if individual susceptibility has to be taken into account. Differing exercise regimens [11] and differing exposure to chronic stress [42] can lead to individual differences in adrenal sensitivity to exogenous ACTH. Further, there are numerous pathways in addition to ACTH that could play a role in regulating glucocorticoids [7]. Individual variation could occur in any of these, leading to individual variation in glucocorticoid production in response to exogenous ACTH.

During the ACTH challenge, the control male showed a similar response to that of the experimental animals suggesting that the signal was induced through handling – not through a pharmacological response to the ACTH. Similarly, in African buffalo (*Syncerus caffer*), anesthesia alone induced a glucocorticoid response equal to that of ACTH [10]. This response was not induced 21 days later, however, when we performed the DEX suppression test although we used the same handling and injection protocols. It is possible that in our study stress responses may be socially transmitted (i.e. a social contagion), perhaps via a chemosignal from the experimental mongooses in response to ACTH and DEX which in turn induces similar behavioral and glucocorticoid responses in the control male. This was first demonstrated in Sprague–Dawley rats

exposed to a stressor in which a chemosignal induced a behavioral response in control subjects [2]. This chemosignal was not produced by hypophysectomized rats but was produced by sham-operated rats and by hypophysectomized rats treated with ACTH, suggesting that the pituitary and ACTH may play a key role in such chemosignals [2]. In addition, chemosignals from stressed rats have been shown to increase glucocorticoids in conspecifics housed in the same facility but not subjected to the same stressor [13].

Reproductive behavior in banded mongooses appears to be associated with increased production of glucocorticoids. Our captive troop was fed a constant diet throughout the study and always had access to drinking water and dens. Further, there was no clinical indication of disease in the troop. Aside from seasonal temperature changes, these mongooses were not exposed to any seasonal dietary, denning or reproductive constraints while the adult female's estrus was suppressed. As a result, their 2010 baseline FGM concentrations were low, with low variability. Once the female came into estrus, after ceasing the birth control treatment, FGM concentrations in the entire captive troop increased dramatically. These increases were possibly related to several correlated factors, including (1) increases in the female's glucocorticoid production associated with estrus, pro-estrus, parturition and lactation, (2) increases in male glucocorticoid production associated with guarding and mating the female, (3) increases in the troop's glucocorticoid production in response to other stressors brought about by the reproductive cycle: (a) attraction of predators to and depredation of juveniles, (b) post parturition attraction of invading male mongooses to the estrous female and (c) non-depredation-related juvenile mortality. The pattern shown here by banded mongooses was approximately similar to that shown in meerkats (*Suricata suricatta*) where female FGMs were low at conception and increased to parturition in females that did not have postpartum conception [4]. Female meerkats with postpartum conception, however, had lowered FGMs in the last 2 weeks of gestation [4]. In contrast, the captive female in our study did conceive after her first litter but her FGMs appeared to remain high until parturition.

It is interesting to note that the female only became estrous in late October, nearly 5 months after stopping birth control treatment and had only 2 l, with the second one in late February. Free-ranging females in our study area generally display estrus in the late dry season each year (approximately mid-September). Pregnancy can be identified within about 30 days of conception from swelling of the female's abdomen and nipples (approximately mid-October) with the first parturition of the season roughly coinciding with the first significant rainfall event and associated termite eruption (approximately mid-November). Parturition of the final litter of the season is early to mid-March. The captive mongooses were fed a consistent diet (volume and nutrient content) throughout this time period. We suggest that cues other than seasonal dietary changes are responsible for triggering estrus and reproductive activity in banded mongooses in this population.

In addition to physiological and biological challenges monitored in the captive troop, we were also able to detect biologically relevant differences in FGM concentrations in a monitored free-ranging troop through the assessment of a late stage TB case – confirmed on clinical signs, gross pathology and histopathology [3]. An animal exposed to a severe and chronic biological challenge such as a chronic disease is expected to have elevated FGM concentrations. The adrenal response to infection by ectoparasites and nematodes has been equivocal in wildlife species with decreased glucocorticoids in response to parasite reduction in female blue tits (*Cyanistes caeruleus*) [26], *Peromyscus* spp. [38] and cliff swallows (*Petrochelidon pyrrhonota*) [40], but no effect on glucocorticoids after parasite reduction in raccoons (*Procyon lotor*) [29] and Rocky Mountain big horn sheep (*Ovis canadensis canadensis*) [18]. Chronic infection by *Mycobacterium tuberculosis* in humans, however, leads to increased glucocorticoids [43], possibly mediated by cytokine activation of the HPA axis during the immune response to infection [8,9,41]. Further, increases in FGMs have been detected in mice innoculated with mouse scrapie as the disease approaches late stage [49]. Thus, although glucocorticoids are often implicated in immuno-suppression, they may also be altered in response to parasitism [25] and this positive feedback may result in "vicious circles" of susceptibility, infection and transmission within an individual and within a social group or population [6].

FGM excretion time approximately matched the GIT time in banded mongooses, a finding similarly shown in other species [20,27,48,12,44,50,19,35].

We found FGM levels to be stable over time since defecation, indicating that samples collected within 8 h post-defecation should give a reliable reflection of FGM concentrations in the collected feces.

5. Conclusions

Banded mongoose stress response can be reliably assessed non-invasively using an 11-oxoetiocholanolone enzyme immunoassay which specifically detects 11,17-dioxoandrostanes. Gastrointestinal transit time in captive banded mongooses is at least 24 h. FGM concentrations in banded mongoose remain stable up to 8 h after defecation. In spite of having a consistent diet, captive banded mongooses appear to become reproductively active at the same time of the year as free-ranging banded mongooses. FGMs appear to increase in both male and female captive banded mongooses as they approach parturition. Parturition may be associated with other putatively stressful events such as predator attraction, predation and agonistic troop encounters. Late stage tuberculosis-infected banded mongooses may show an over 50-fold elevation in FGM levels relative to putatively healthy troop members.

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