DEMOGRAPHIC, BEHAVIORAL AND PHYSIOLOGICAL RESPONSES OF WILDLIFE TO REINTRODUCTION

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The undersigned, appointed by the dean of the Graduate School, have examined the dissertation entitled

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DEMOGRAPHIC, BEHAVIORAL AND PHYSIOLOGICAL RESPONSES OF WILDLIFE TO REINTRODUCTION

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ABSTRACT

The growing field of reintroduction biology focuses on the process of restoring extirpated species to previously occupied habitat. Beyond academic interest, these fields have facilitated the reintroduction of charismatic and ecologically important mammals, which have been inspiring to wildlife conservation and critical to restoration of ecosystem functions. However, fewer than 11% of all reintroduction attempts have successfully reestablishing extirpated wildlife populations. To improve the outlook for conserving species and to strengthen natural resource management, I evaluated factors influencing reintroduction success.

Success can be defined in several ways. Often, it is defined demographically, wherein reintroduced populations need to reach a critical size before they are considered successfully recovered. The black-footed ferret, one of the rarest mammals in North America, is a leading example of a species having difficulty in recovering demographically after reintroduction. Following extirpation in the wild, over 3,000 ferrets have been released at 19 reintroduction sites in the western United States as well as in Canada to Mexico. However, ferrets at only four of these sites have succeeded in maintaining self-sustaining populations. I compiled and analyzed data collected over the 21-year history of the ferret reintroduction program to test hypotheses about

environmental and management factors that might influence demographic success. I found that ferrets are likely to establish self-sustaining populations only at reintroduction sites that contain large populations of their primary prey. Successful sites were only located on prairie dog complexes >4300 ha in size regardless of prairie dog density. This finding is important, given that prairie dog populations are declining across the western United States, and that all reintroduction efforts over the past 10 years have been at sites <4300 ha in size. Thus, management efforts should focus on preservation of the declining prey base prior to future reintroductions of this critically endangered carnivore.

For large and potentially dangerous species like African elephants, the success of wildlife reintroduction can be defined in terms of how well the animals adjust behaviorally to their translocation. Once nearly extirpated from South Africa, reintroduction of African elephants has become increasingly common, yet behavioral problems have occurred post-release that have been linked to physiological condition and elevated stress hormone concentrations. However, there is uncertainty about the generality of a stress response in reintroduced elephant populations and the amount of time needed to physiologically adjust (i.e. decline of stress hormones from elevated to basal levels). I assessed stress hormone concentrations in elephants that were reintroduced into five reserves in South Africa over a six year period to evaluate the relationship of temporal, climatic, and social factors to elephant physiological state. I found that variation in stress hormones across the five reserves was best explained by the number of years that elapsed since initial release. Fecal glucocorticoid stress hormones were 10% lower 10 yrs after release, and 40% lower 24 yrs after release in comparison to 1 yr after release. Thus, regardless of reintroduction site conditions, elephants will require an extended period of time to physiologically adjust to their new surroundings and managers should prepare for prolonged pathological consequences of chronic stress responses.

Chronic stress in elephants following reintroduction can have multiple behavioral consequences. I compared space use patterns of reintroduced elephant populations in differing physiological states and found that elephants in a chronic physiological state exhibited refuge behavior. Refuge behavior was associated with elephants exhibiting smaller home ranges than expected and only utilizing a restricted portion of the reserve away from human disturbance. Therefore, the provision of refugia away from human disturbance following release is likely critical to limiting dangerous human-elephant interactions.

At a finer scale, I found that elevated stress hormone concentrations influence elephant movement behavior. Understanding elephant movement is critical to reserve design, as well as predicting elephant responses to management conditions. I found that the physiological state of elephants can affect fine-scale movement based on memory and environmental conditions. Elephants in iSimangaliso Wetland Park in elevated physiological states were less likely to utilize areas away from refugia and cover provided by commercial forest plantations compared to when they were in basal physiological states. These findings reveal connections between typically hidden physiological states and observed elephant behaviors, and provide useful information to managers on how to predict and potentially mitigate future human-elephant conflicts.

Collectively, results from my dissertation advance our understanding of reintroduction biology by illuminating factors that influence reintroduction success demographically, while also advancing our understanding of behavioral and physiological responses of wildlife to reintroduction. An understanding of these factors is needed if current and future reintroduction attempts are to succeed in restoring extirpated species and their ecosystems.

CHAPTER 1

INTRODUCTION

The eminent ecologist E.O. Wilson predicts that after centuries of human persecution and extinction, the current century will viewed as the “era of restoration” (Wilson 1992). This unique and exciting time in the evolution of wildlife conservation and management is highlighted by the relatively recent birth of the fields of wildlife restoration and reintroduction biology, which focus on investigating factors that contribute to the establishment and persistence of reintroduced populations (Armstrong and Seddon 2008).

Beyond academic interest, these fields have facilitated the reintroduction of charismatic and ecologically important mammals, which have been inspiring to wildlife conservation and critical to restoration of ecosystem functions. This area of research and management contains many of the greatest conservation success stories, such as the recovery of Arabian oryx (*Oryx leucoryx*) in Saudi Arabia (Ostrowski et al. 1998) and gray wolves (*Canis lupus*) in the Yellowstone ecosystem in North America (Bangs and Fritts 1996). However, fewer than 11% of reintroduction attempts have been successful at reestablishing extirpated populations (Beck et al. 1994). Of those that succeed demographically, management concerns can arise due to impacts reintroduced species have on the ecosystems to which they are reintroduced (Ripple and Beschta 2003, Kerley et al. 2008).

For species with a low probability of achieving self-sustaining populations after reintroduction, there is an urgent need to evaluate what conditions are required to increase survival and post-release reproductive success. Captive breeding and translocation programs can be extremely costly, and the continued augmentation of a species in a single area requires a large investment in ex-situ conservation resources of zoos or captive breeding centers, and it restricts the ability of managers to allocate individuals to other suitable sites. The black-footed ferret (*Mustela nigripes*) is a classic example of a costly and labor-intensive reintroduction effort that has resulted in limited demographic reintroduction success (Lockhart et al. 2006, Jachowski and Lockhart 2009). Despite over 18 years of reintroduction attempts and the release of over 3,000 individual captive-reared ferrets, only 4 of 18 reintroduction sites have been successful in establishing self-sustaining populations (Jachowski and Lockhart 2009). In Chapter two, I report the first quantitative analysis of reintroduction attempts for the critically endangered black-footed ferret by evaluating how habitat quality, disease and reintroduction strategies are related to reintroduction success.

Beyond monitoring population-level demographic responses to reintroduction, there is a need to better understand how wildlife species respond behaviorally and physiologically to reintroduction. Research into behavioral responses of species to reintroduction can provide insights into factors that might limit reintroduction success (Kleiman 1989), such as dispersal or disease susceptibility (Dickens et al. 2010). An understanding of behavioral responses also is critical for assessing how reintroduced species might influence trophic interactions (Ripple and Beschta 2003, Fortin et al. 2005), habitat conditions (Kerley et al. 2008), and species interactions (Slotow et al.

2001). Space use and movement metrics are among the most commonly used techniques for quantitatively evaluating the behavioral response of wildlife (Nathan et al. 2008). Another technique used to evaluate how individuals respond to perturbations (such as reintroduction) is the monitoring of internal or physiological responses (Romero and Butler 2007, Busch and Hayward 2009).

The emerging field of conservation physiology shows great promise for evaluating the influence of management strategies and environmental conditions on wildlife populations (Wikelski and Cooke 2006). Physiological tools, such as the measure of stress hormone levels, are particularly valuable for assessing the response of animals to reintroduction (Teixeira et al. 2007). The process of translocating and releasing animals typically induces a chronic or elevated stress response, yet the intensity of response and amount of time it takes to recover from chronic stress vary by species (Dickens et al. 2010). While rarely the direct cause of translocation or reintroduction failures, chronic stress usually leads to other problems and might make individuals more prone to disease, reproductive failure, predation, starvation or dispersal away from the release site (Dickens et al. 2010). In Chapter three, I compare the physiological responses of five populations of African elephants (*Loxodonta africana*) that differ in their reintroduction conditions to identify factors correlated with heightened stress hormone levels post-reintroduction.

Space use and movement patterns also can provide valuable insight into the responses of wildlife to reintroduction. After release, reintroduced species tend to select for distinct resources (Rodriguez et al. 1995, Biggins et al. 2006a). Over time post-reintroduction, certain species can develop recurrent movement patterns based on memory (i.e. cognitive maps) (Fryxell et al. 2008). Elephants reintroduced into fenced reserves likely exhibit specific, predictable movement patterns similar to wild elephants (Douglas-Hamilton et al. 2005, Loarie et al. 2009). However, we do not yet know if reintroduced elephants establish repeated movement patterns. Further, most studies linking movements to perception of the environment by a species attempt to do so at the population scale and can only infer causative relationships between perception of the surroundings and movements of a population, without addressing the question of why individuals move (Nathan et al. 2008). The physiological state or memory of previous states could influence space use, where animals likely respond via movement and avoidance or escape behaviors in an attempt to limit chronic exposure to physiological stressors (Wingfield and Ramenofsky 1997, Ricklefs and Wikelski 2002). In Chapter four, I evaluate if an elephant population in a chronic physiological state is more likely to exhibit these types of refuge behaviors in comparison to reintroduced elephant populations in basal physiological states.

At a finer spatial scale and over shorter periods of time, movement paths of an animal might be influenced by its physiological state. Technological and analytical advances in movement ecology have resulted in a variety of movement models inferring behavioral responses to internal state based on the location of successive data points (Morales et al. 2004, Schick et al. 2008). However, such inferential models are indirect, and the navigational capacity and internal states that drive animal movement remain relatively unknown in movement ecology (Getz and Saltz 2008, Holyoak et al. 2008, Patterson et al. 2008). Existing efforts to link stress hormone production with animal movement have focused on laboratory settings (Wingfield and Romenofsky 1997, Breuner et al. 1998) or large-scale patterns in animal space use (Breuner and Hahn 2003, Addis et al. 2011). To date, there have been no examinations of the effects of stress hormones on the fine-scale movement behavior of wild animals. In Chapter five, I assess how the physiological state of elephants relates to fine-scale movements by comparing the movements of animals in two physiological states (elevated and basal) in response to environmental conditions, memory of prior habitat use, and the social effect of belonging to a family group.

Collectively, the findings of this dissertation are expected to improve our understanding of factors that limit the success of wildlife reintroduction. The two species studied here have widely different natural histories and obstacles to achieving reintroduction success. Despite these differences, both species share a common link of being difficult to successfully reintroduce. Further, they both are excellent examples of the complex demographic, physiological and behavioral responses of wildlife to reintroduction. It is my hope that managers can use the findings I report here to guide future management plans for the restoration of their particular species of concern, whether it is one that is difficult to recover demographically (such as the black-footed ferret) or that presents behavioral problems following release (such as the African elephant).

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CHAPTER 2

FACTORS THAT INFLUENCE THE ESTABLISHMENT OF SELF-SUSTAINING BLACK-FOOTED FERRET POPULATIONS

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ABSTRACT

Objective evaluations of wildlife reintroductions are vital for increasing the success of future efforts to re-establish endangered species. Attempts to reintroduce one of the most endangered mammals in North America, the black-footed ferret (*Mustela nigripes*), have been ongoing for 18 years with no quantitative assessment of factors related to reintroduction success. We examined relationships between ferret reintroduction success and factors associated with disease outbreaks, release strategies, and the distribution and abundance of their primary prey, prairie dogs (*Cynomys* sp.), at 11 reintroduction sites.

The most important factor related to ferret reintroduction success was a cumulative metric incorporating both size of the area occupied by prairie dogs and density of prairie dog burrows within that area. Each of the 4 successful sites had prairie dog populations that occupied an area of at least 4,300 ha. No sites with < 4,300 ha of prairie dogs were successful in maintaining ≥30 adult individual ferrets over multiple years without augmentation even if they had a high prairie dog burrow density. The overarching importance of the availability of high-quality habitat suggests managers should prioritize actions that maintain and enhance the availability of large areas with high prairie dog burrow density, which are becoming increasingly rare due to anthropogenic impacts and disease outbreaks.

INTRODUCTION

Successful reintroductions are essential for the conservation and recovery of many endangered species (Griffith et al. 1989), but reintroduction attempts often fail (Beck et al. 1994, Armstrong and Seddon 2008). Despite this lack of success, few attempts have been made to evaluate factors that differentiate successful and unsuccessful reintroductions (Griffith et al. 1989, Wolf et al. 1996, 1998, Fischer and Lindenmayer 2000). Further examinations of these factors are essential because objective evaluations of reintroduction attempts strengthen our understanding of reintroduction biology and provide important information for decision-makers (Sarrazin and Barbault 1996, IUCN 1998). Such examinations can help conservation biologists prioritize future reintroduction efforts so that available funding is focused on recovery sites and strategies that have a high likelihood of success (Gusset et al. 2008a).

Evaluations of carnivore reintroductions are particularly important given the political and biological challenges involved with these species. For example, many carnivore reintroductions face public opposition due to economic cost and perceived threats to human activities or interests (Reading and Clark 1996, Breitenmoser et al. 2001). Availability of suitable sites and feasibility of re-establishing populations are limited because carnivore species often are characterized by low population densities (Carbone and Gittleman 2002), large home ranges (Woodroffe and Ginsberg 1998), sensitivity to habitat fragmentation (Schadt et al. 2002), complex social structures (Gusset et al. 2008a, Somers and Gusset 2009), and dependence on specific prey species (Steury and Murray 2004). These complexities underscore the importance of evaluating factors that might influence success of carnivore reintroduction attempts. However, data often are insufficient for thorough evaluation of individual reintroduction attempts, and factors affecting reintroduction success throughout the range of a species might not be evident in results from a single site. Consequently, comparative approaches can be insightful when reintroductions have been attempted at multiple sites (Gusset et al. 2008a).

The black-footed ferret (*Mustela nigripes*, hereafter referred to as the ferret), among the most endangered mammals in North America (Clark 1987), has been the subject of one of the highest profile reintroduction programs (Lockhart et al. 2006). Following the capture of the last 18 wild ferrets and subsequent captive breeding in 1987, reintroduction attempts were initiated (Thorne and Williams 1988, Seal et al. 1989, Clark 1997) and implemented through a cooperative effort with the Black-footed Ferret Recovery Implementation Team (BFFRIT), a group composed of federal, state, tribal, and private stakeholders. There have been 18 attempts to reintroduce ferrets during the last 18 years (Jachowski and Lockhart 2009). Although >2,900 ferrets have been released, results across release sites have been mixed. Most reintroduced populations have not grown consistently, but a few attempts have established relatively large populations of ferrets (Livieri 2006, Grenier et al. 2007). Factors differentiating successful and unsuccessful reintroductions have not been evaluated.

We developed hypotheses about biological conditions and release strategies that might contribute to successful reintroduction, and used results of ferret reintroduction attempts to test our hypotheses. Ferrets are extremely specialized carnivores, occurring only on prairie dog (*Cynomys sp.*) colonies (Henderson et al. 1969, Biggins et al. 2006a), and depending on prairie dogs for >90% of their diet (Sheets et al. 1972, Campbell et al. 1987). Therefore, we hypothesized that success of reintroduction attempts would be greater at sites where abundance of prairie dogs was high, as measured by the total area occupied by prairie dogs, prairie dog density, or a combination of both metrics. Ferrets are extremely vulnerable to an exotic disease, sylvatic plague (hereafter referred to as plague), which acts as a catastrophic stochastic factor within the ecosystem (Williams et al. 1994, Rocke et al. 2008). In addition to the direct effect of plague on ferrets, plague epizootics result in 85-100% declines in prairie dog populations (Rayor 1985, Ubico et al. 1988, Cully and Williams 2001, Pauli et al. 2006). Once a plague epizootic has occurred in an area, outbreaks are likely to reoccur at 5-10 year intervals (Barnes 1982). Therefore, we hypothesized that successful sites have not had an epizootic occur or have had fewer incidences of epizootic plague.

Reintroduction efforts must assess how many animals need to be released to establish a population (Armstrong and Seddon 2008) and when to halt releases (Schaub et al. 2009). Sometimes, sustained release of individuals is unlikely to contribute to population persistence and reintroduction success (Griffith et al. 1989, Steury and Murray 2004). However, the release of too few individuals might result in populations that do not persist due to demographic stochasticity or Allee effects (Armstrong and Seddon 2008, Somers et al. 2008). The continued release of animals over multiple years might augment populations and overcome these effects (Smith and Clark 1994, Gusset et al. 2009). Therefore, we hypothesized that the number of animals released and the number of years reintroductions took place at a site are positively correlated with reintroduction success.

STUDY AREAS

From 1991 to 2008, approximately 2,964 captive-born ferrets were released and 157 wild ferrets were translocated to initiate or supplement populations of ferrets at 18 sites in the United States and one site in Mexico (Jachowski and Lockhart 2009), all within the historical range of the species. Reintroduction sites were identified by the BFFRIT based on habitat conditions, a local commitment to monitoring and management of ferrets, and socio-political conditions favorable to their conservation. Sites typically were geographically isolated from each other such that movement of ferrets between sites was rare or non-existent. We limited our analyses to those reintroduction sites where > 5 years had elapsed from the initial release of ferrets until December 2008 (*n*=11) (Fig. 1). This five-year threshold is consistent with general recommendations for evaluating carnivore reintroductions (Breitenmoser et al. 2001) and is appropriate for ferrets given their short generation time and potential for rapid population growth (Grenier et al. 2007), making it biologically plausible for a ferret population to grow fast enough to meet the threshold for being considered "recovered" (see Methods section below) after 5 yrs.

METHODS

Field monitoring

To evaluate reintroduction attempts, we used data provided by BFFRIT members responsible for overseeing the post-release monitoring of ferrets at each reintroduction site. The BFFRIT expected site managers to conduct annual standardized spotlight surveys to assess the status of ferret populations (Biggins et al. 2006b). During spotlight surveys, ferrets were located, captured in traps, anesthetized and uniquely marked with passive-integrated-transponder microchips (Fagerstone and Johns 1987, Grenier et al., 2009). Survey results were used to calculate an index of ferret abundance, expressed as the minimum number known alive (Krebs 1966). Although this index does not account for incomplete and variable detectability, the field protocol generally is believed to detect >82% of the adult ferrets present, making this index adequate for our examination (Biggins et al. 2006b).

Habitat quantity and quality at reintroduction sites were assessed using standardized techniques to measure the maximum extent of prairie dog colonies (aggregations of prairie dog family groups defined by the maximum extent of burrow systems) (Hoogland 1995) and the mean burrow density of prairie dogs through density transects (Biggins et al. 1993). The standardized monitoring of prairie dog populations also provided an indirect index of epizootic outbreaks of plague, which were apparent due to rapid, large-scale mortality of prairie dog populations (Collinge et al. 2005, Wagner et al. 2006). We used this information to determine if a plague epizootic had occurred following a reintroduction attempt and tallied the number of years epizootic outbreaks were observed since reintroduction.

Data analyses

We established biological criteria for defining reintroduction success based on the concept that the principal aim of a species reintroduction project is to establish a self-sustaining population that requires minimal long-term intervention (IUCN 1998). The goal of the current ferret recovery plan for down-listing the species from Endangered to Threatened status is the establishment of 1,500 free-ranging, breeding adult ferrets distributed in >10 populations over the historical range of the species, with ≥30 breeding adults in each population (U. S. Fish and Wildlife Service 1988). Therefore, we categorized a reintroduction site as successful if it directly contributed to down-listing by maintaining a population of ≥30 adult ferrets. We further refined this definition by requiring sites to have documented the ability to maintain a population of ≥30 adult ferrets for >2 years without augmentation of captive-bred or translocated animals prior to December 2008.

We examined how 11 variables differed between successful and unsuccessful reintroduction sites (Table 1). We focused on variables in 3 categories: (1) variables related to prairie dogs (area occupied by prairie dogs, colony number, colony size, prairie dog burrow density, a composite index based on both area occupied by prairie dogs and burrow density, and species); (2) disease or plague-related variables (epizootic plague occurrence and the number of epizootics); and (3) ferret reintroduction variables (number of years released, number of years since initial reintroduction, and cumulative total number released).

In this analysis, our composite index of prairie dog biomass was calculated by multiplying the total area occupied by prairie dogs in each site by the average density of prairie dog burrows per ha, a metric we termed the prairie dog index. Prior to conducting analyses, we hypothesized that this index would be a more accurate measure of the total prey base for ferrets than either area occupied by prairie dogs or density alone. For example, black-tailed prairie dogs (*Cynomys ludovicianus*) typically occur at higher densities than white-tailed (*C. leucurus*) or Gunnison's prairie dogs (*C. gunnisoni*) (Cully and Williams 2001), while the areas occupied were much larger, on average, at reintroduction sites occupied by one of the latter 2 species. The prairie dog index standardized total prey biomass across reintroduction sites regardless of which one of these species was present. We calculated a prairie dog index for each year at sites where both the area occupied by prairie dogs and burrow density data were available. Because data were not available for each metric every year for some sites, we averaged all recorded data across years to generate a single mean value of each metric for each site. We used site averages versus a summary parameter that down-weighted extreme values (e.g., median) because a year of especially high or low prairie dog abundance could have a disproportionately strong effect in determining whether the population achieved a sufficient minimum population size to escape limiting effects of demographic stochasticity and other extinction factors.

We focused on univariate comparisons of each variable between successful and unsuccessful reintroduction sites due to the low number of reintroduction sites available (*n*=11). For numeric variables we used the Wilcoxon Rank-Sum test to examine whether the distributions of each variable differed between successful and unsuccessful sites, and we computed 90% non-parametric confidence intervals for the difference in the location parameter for the 2 distributions (R Core Development Team 2008). For non-numeric variables, we used univariate logistic regression to examine whether there was evidence that the proportion of successful sites differed among categories of each variable. We used a likelihood ratio test to determine if there was a statistically significant effect of each categorical variable (i.e., by comparing the univariate model to an intercept-only null model). Our sample size was constrained by the number of existing ferret reintroduction sites, but such low sample sizes characterize almost all reintroduction efforts (Seddon et al. 2007). To appropriately balance our ability to detect relationships of conservation significance *vs*. our need to minimize Type I error, we set $\alpha = 0.10$ for evaluating statistical significance and we focused on 90% confidence intervals for evaluating biological significance.

RESULTS

Four of the 11 reintroduction sites we examined met our criteria of success as of December 2008. Successful sites were located in Arizona, South Dakota and Wyoming, on privately and publicly-owned lands (Fig. 1). Of the 7 sites that did not meet our criteria for success, 2 no longer contained ferrets by December 2008 (40-Complex and Ft. Belknap Indian Reservation), while the remaining 5 sites contained small populations or were periodically augmented with captive-reared or translocated individuals (i.e., UL Bend, Coyote Basin, Wolf Creek, El Cuervo, and Badlands).

The average total area occupied by prairie dogs and the prairie dog index value were higher on successful than unsuccessful sites (Table 1). Four of the 5 sites with a prairie dog index >150,000 were successful (Table 1, Fig. 2). None of the reintroductions on sites where prairie dog populations occupied <4,300 ha were successful even if the site had a high density of prairie dogs (Fig. 2). Average colony size, prairie dog burrow density, and species of prairie dog did not differ between successful and unsuccessful sites (Tables 1 and 2). Successful reintroductions included sites occupied by each of the 3 prairie dog species.

The documented occurrence of plague epizootics and the number of epizootics did not differentiate successful and unsuccessful sites (Table 2). Two of the 4 successful reintroduction sites had at least 1 plague epizootic, but their ferret populations persisted or recovered sufficiently to meet our criteria of success. Successful and unsuccessful sites were not differentiated by the number of ferrets released, the number of years in which releases occurred, or the number of years since first reintroduction. Both successful and unsuccessful sites on average received over 200 captive-reared ferrets over approximately 10 years (Table 1).

DISCUSSION

We evaluated how successful and unsuccessful ferret reintroduction sites differed in spatial extent and burrow density of prairie dogs, occurrence of plague epizootics, and the intensity of ferret release efforts. Successful reintroductions occurred on sites where the total area occupied by prairie dogs was large and the total relative biomass of prairie dogs was high. These metrics directly measure habitat availability and quality for ferrets because ferrets require prairie dogs as prey and use their burrows for security and denning (Forrest et al. 1988, Jachowski et al. 2010). Therefore, our results show that habitat characteristics were the most important factor affecting success of reintroduction attempts for ferrets.

Our findings support a common paradigm in reintroduction biology that emphasizes habitat quantity and quality as critical factors determining whether self-sustaining populations can be re-established and avoid extinction (Griffith et al. 1989, Kleiman 1989, Wolf et al. 1996). The availability of high-quality habitat is essential for conserving endangered carnivores (Wikramanayake et al. 1998, Dinerstein et al. 2007) and predicting extinction risk (Woodroffe and Ginsberg 1998). Our work suggests that such metrics similarly are important for reintroduction success. For ferrets, the best measure of habitat quality integrates the spatial extent of suitable habitat and the density of the primary prey. Prairie dog density alone, as indexed by burrow density, did not differentiate successful and unsuccessful sites. Rather than focusing solely on area of suitable habitat (Woodroffe and Ginsberg 1998) or density of prey within a defined area (Steury and Murray 2004, Hetherington and Gorman 2007), managers should consider both the size and quality of the habitat when potential ferret reintroduction sites are being evaluated.

In contrast to the general assumption that increasing the number of animals released improves the likelihood of carnivore reintroduction success (Breitenmoser et al. 2001), the number of ferrets and duration of release attempts did not differ between successful and unsuccessful sites. Most endangered large carnivores are characterized by high longevity, long generation time, and small litter sizes (Purvis et al. 2000). In contrast, ferrets have exhibited a high potential rate of increase that has resulted in rapid population growth from relatively small (5-25 individual) founder populations when high-quality habitat is available (Grenier et al. 2007). Ferrets and possibly other small carnivores with high potential intrinsic rates of increase may increase in abundance rapidly if habitat quality and quantity is sufficient. The rapid population growth possible when habitat is sufficient can allow a reintroduced population to increase rapidly to a level at which demographic and genetic stochastic processes and factors such as Allee effects pose little threat to the population's persistence. Further, our findings suggest that the number of individuals released at most ferret reintroduction sites might have been high enough such that habitat availability was the primary factor determining likelihood of success. This would indicate that there are diminishing returns with continued releases.

Emerging infectious diseases pose a severe threat to reintroduced wildlife populations (Daszak et al. 2000), and carnivore reintroductions in particular (Scheepers and Venzke 1995, Schmidt-Posthaus et al. 2002, Wild et al. 2006). Epizootic outbreaks of sylvatic plague were not related to ferret reintroduction success during our period of study, but future management of reintroduced ferret populations requires a better understanding of the long-term impacts of this emerging infectious disease. In particular, results to date suggest that subsequent examinations of ferret reintroduction attempts should examine the hypothesis that there is an interaction between habitat availability and the threat posed by plague. Of the 3 ferret reintroduction sites with a longer history of exposure to sylvatic plague, 2 small reintroduction sites (Ft. Belknap and 40-Complex) suffered massive prairie dog die-offs and the extirpation of ferret populations (Antolin et al. 2002, Jachowski and Lockhart 2009). In contrast, a single large site (Shirley Basin) persisted through a plague epizootic without management intervention and subsequently supported a large ferret population (Grenier et al. 2007). Persistence of ferrets at the Shirley Basin site suggests that the effects of plague epizootics can be overcome without intervention when sufficient habitat is available. However, there are additional complications in assessing past effects and the relative threat posed by plague and its interaction with habitat. For example, new research has suggested that plague persists between epizootic outbreaks at an enzootic level which has a limiting effect on prairie dog (Biggins et al. 2010) and ferret (Matchett et al. 2010) populations. Therefore, further work is needed to assess the need for and effectiveness of plague intervention in large prairie dog populations, the sustainability of such intervention, and the long-term effects of this disease as it spreads to new areas and re-occurs in epizootic and inter-epizootic (i.e. enzootic) forms in areas where it already is present. We are not saying plague is unimportant; rather our results indicate that habitat availability is of overriding importance when compared with epizootic outbreaks, given information to date.

Efforts to increase success of carnivore reintroductions typically focus on improving release strategies (Rodriguez et al. 1995) and managing small populations of re-introduced individuals to address competition, conflict with humans, and disease (Vucetich and Creel 1999, Gusset et al. 2008a). In the 18 years since the ferret reintroduction program began, a great deal of attention and funding for enhancing ferret reintroduction success has focused on captive breeding and preconditioning of ferrets prior to release (Biggins et al. 1999), potential consequences of low genetic diversity (Wisely et al. 2008), and development of vaccines for ferrets (Williams et al. 1996, Rocke et al. 2008). However, if insufficient habitat is present, the probability of success is low regardless of the availability of strategies for intensive management of ferret populations. When determining whether to attempt reintroductions at sites with a low probability of success, program managers need to consider whether the benefits in terms of public relations, partnerships or conservation (e.g., research or the establishment of populations that could be used to augment other populations decimated by a catastrophe) make the effort a worthwhile use of resources.

Given the continued decline and fragmentation of prairie dog populations in most areas throughout North America (Miller and Cully 2001), managers concerned with recovering black-footed ferret populations should focus on increasing the size and density of prairie dog populations. Prairie dogs historically were among the most abundant mammals in North America (Forrest 2005), but numbers have declined to the point that the black-tailed prairie dog, Gunnison's prairie dog and white-tailed prairie dog have been petitioned repeatedly for listing under the U.S. Endangered Species Act. While successful ferret reintroductions occurred on all 3 prairie dog species, no successful reintroduction sites contained prairie dog populations occupying areas that were less than 4,300 ha in size. Beyond this threshold, habitat quality appeared to be determined by the interaction of relative prairie dog burrow density and the size of the area occupied by prairie dogs. Successful sites included those where prairie dogs occupied large areas at low relative density, and moderately large areas where prairie dogs occurred at fairly high relative densities. However, there were no successful sites where prairie dogs occupied small areas at high densities. Therefore, management for higher local prairie dog densities is of low utility except, perhaps, where the area occupied by prairie dogs is > 4,000 ha in size. Areas occupied by black-tailed prairie dogs, which typically contain moderate densities of prairie dogs burrows (30-80 per ha), likely need to be large in spatial extent (>4,000 ha) to meet minimum biological requirements for successful ferret populations. Areas occupied by Gunnison's prairie dogs and white-tailed prairie dogs, which typically contain lower densities of prairie dog burrows (10-30 per ha), likely need to be even larger. However, prairie dog populations that occupy an area >4,000 ha are exceedingly rare (Proctor et al. 2006), with only 2 such sites identified by the BFFRIT that have not already hosted ferret reintroduction attempts. Therefore, in order to reach the recovery goal of establishing 10 populations of >30 adult ferrets, managers need to increase the number of large prairie dog populations beyond what is currently available.

Although increasing the size and density of prairie dog populations might seem like a straightforward objective, it would be highly controversial and difficult to implement. Most evaluations of social-political aspects of carnivore reintroduction focus on negative local attitudes toward the carnivores themselves (Wilson 2004, Lindsey et al. 2005, Gusset et al. 2008b). However, ferret reintroductions face the unique problem that the prey on which they depend, rather than the ferrets themselves, are widely regarded as pests (Reading and Kellert 1993, Miller et al. 2007). Therefore, improving the suitability of reintroduction sites for ferrets, and ultimately recovering the species in the wild, only can be achieved by changing public attitudes and management policies related to their declining prey. Government-sponsored subsidies recently have been made available to local landowners to protect prairie dogs on public and private land (Miller and Reading 2006). However, such approaches are slow to succeed at a large scale when faced with widespread and deeply ingrained prejudice against prairie dogs by many private landowners (Miller et al. 2007). Although the ecological role of prairie dogs in grassland systems is well-documented (Koford 1958, Sharps and Uresk 1990, Miller et al. 1994, Hoogland 1995, Ceballos et al. 1999), the ethical, sociological, and economic arguments that have been developed and used to help justify protection for charismatic carnivores (e.g., such as increasing tourism; Hayward et al. 2007), also need to be applied to prairie dogs. When the prey on which an endangered carnivore depends is itself at risk, conservation of that prey is essential to the long-term success of recovery efforts for the carnivore.

Ferret recovery efforts, whether biological or socio-political in nature, depend on a foundation of scientific information. This study faced low sample sizes that typify most reintroduction situations. Yet, managers need to learn from experience despite these statistical limitations. Our analyses of broad patterns across all reintroduction sites complements the results of more detailed analyses at the few individual recovery sites where more detailed information permits finer-scale assessments (e.g. Holmes 2008, Grenier et al. 2007). However, our ability to examine relationships in greater detail was hindered by the lack of data based on more rigorous and structured monitoring. For improving our conservation effectiveness, conservation activities need to be continually re-assessed in an adaptive management approach that involves testing a priori hypotheses in an iterative fashion (Armstrong et al. 2007, Gusset et al. 2009). Our results should lead to refined hypotheses about factors affecting recovery success - and help managers identify specific monitoring goals to evaluate such refined hypotheses and advance ferret recovery.

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Table 1. Summary (mean value or percentage of sites and standard deviation) and parameter estimate contrasting successful and unsuccessful reintroduction sites (with 90% confidence intervals and significance) of variables hypothesized to influence reintroduction success of black-footed ferrets (*Mustela nigripes*) at 11 sites in North America.

				90% CI		
Parameter	Successful sites[a]	Unsuccessful sites	Estimate [b]	Lower Bound	Upper Bound	P
Habitat						
Average size of area occupied by prairie dogs(ha)	9,406.6 (4,681.0)	3,879.6 (3,427.1)	5,530	161	11,079	0.07
Average number of colonies	54.9 (75.9)	23.3 (21.6)	3.9	-11.4	144.0	0.92
Average colony size (ha)	619.4 (680.9)	1,431.1 (3,435.4)	220	-279	1336	0.40

Average prairie dog density (burrows/ha)	47.2 (29.8)	50.9 (33.8)	-1.6	-46.6	40.0	0.92
Average total prairie dog index	346,127.1 (176652.8)	97,551.1 (55,926.2)	207,563	90,158	486,044	0.02
Prairie dog species (number of sites)	*Cynomys ludovicianus* (2) *C. leucurus* (1) *C. gunnisoni* (1)	*C. ludovicianus* (5) *C. leucurus* (2)	0.4 [c]	0.05	3.42	0.48
Disease						
Number of sylvatic plague epizootics	1.8 (2.9)	0.9 (1.5)	0.0	-1.0	5.0	0.75
Occurrence of sylvatic plague epizootic	50% had plague epizootic	40% had plague epizootic	1.3	0.2	10.6	0.82

Ferret releases						
Total years released	7.3 (2.5)	5.1 (2.2)	2.2	0.0	6.0	0.20
Years since 1st reintroduction	12.3 (3.7)	9.7 (3.1)	3.0	-2.0	7.0	0.29
Total ferrets released	294.0 (161.6)	216.3 (64.1)	60.9	-72.0	274.0	0.57

[a] Success was defined by documented ability of a site to maintain a population of ≥30 adults more than 2 years after halt of augmentation. Successful reintroduction sites included Aubrey Valley (Arizona), Shirley Basin (Wyoming), Cheyenne River (South Dakota), and Conata Basin (South Dakota).

[b] For Prairie dog species and Plague epizootic, results are based on univariate logistic regression models for the probability that a site is successful, including the estimated odds ratio (odds of being a successful site for the first category of each variable / odds for the second category), confidence interval for this odds ratio, and significance of a likelihood ratio test comparing a logistic regression model with the variable included to an intercept-only model. For other variables, results are the difference (successful – unsuccessful

sites), confidence interval for this difference, and significance of the difference based on the Wilcoxon Rank Sum non-parametric analog of the 2-sample t-test.

[c] Comparing black-tailed prairie dogs (*Cynomys ludovicianus*) to both white-tailed (*C. leucurus*) and Gunnison's prairie dogs (*C. gunnisoni*).

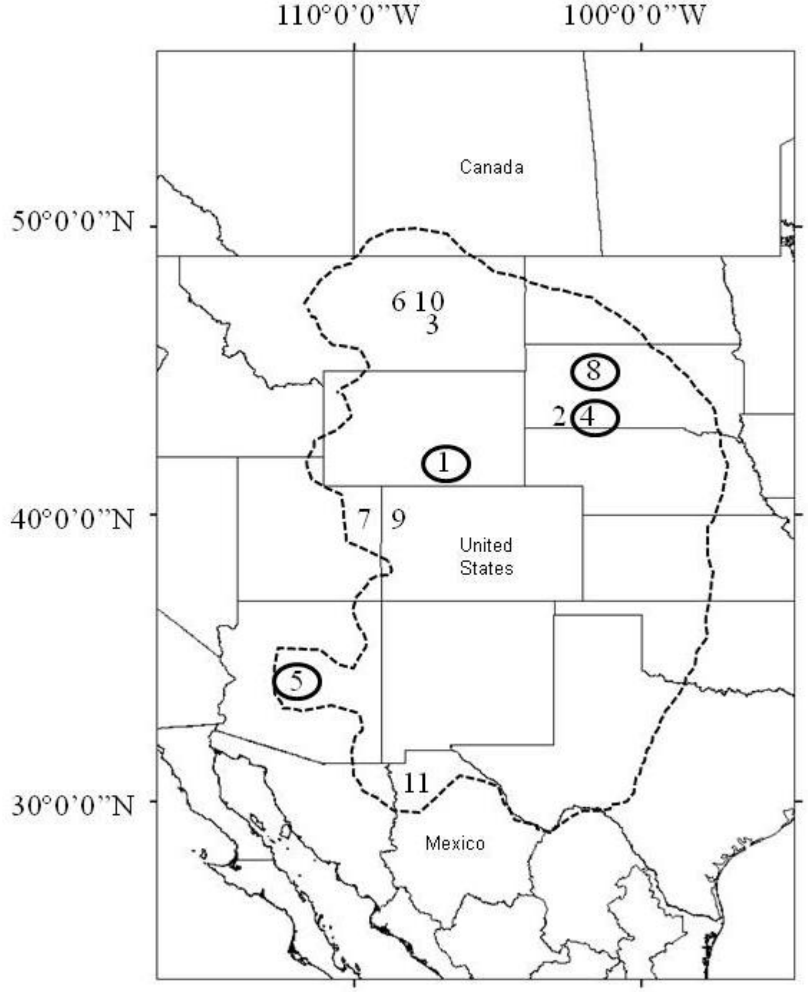

Figure 1. Great Plains of North America, with state and international boundary lines, showing the historical range of the black-footed ferret (*Mustela nigripes*) (dashed line). The 11 ferret reintroduction sites that we used in our analysis (i.e. all reintroduction sites where ≥5 years had elapsed from the initial release of ferrets until December 2008) are numbered in sequential order based on year of first release: (1) Shirley Basin, (2) Badlands, (3) UL Bend, (4) Conata Basin, (5) Aubrey Valley, (6) Ft. Belknap, (7) Coyote Basin, (8) Cheyenne River, (9) Wolf Creek, (10) 40-Complex, and (11) El Cuervo. Sites where reintroduction met our criteria for success are circled.

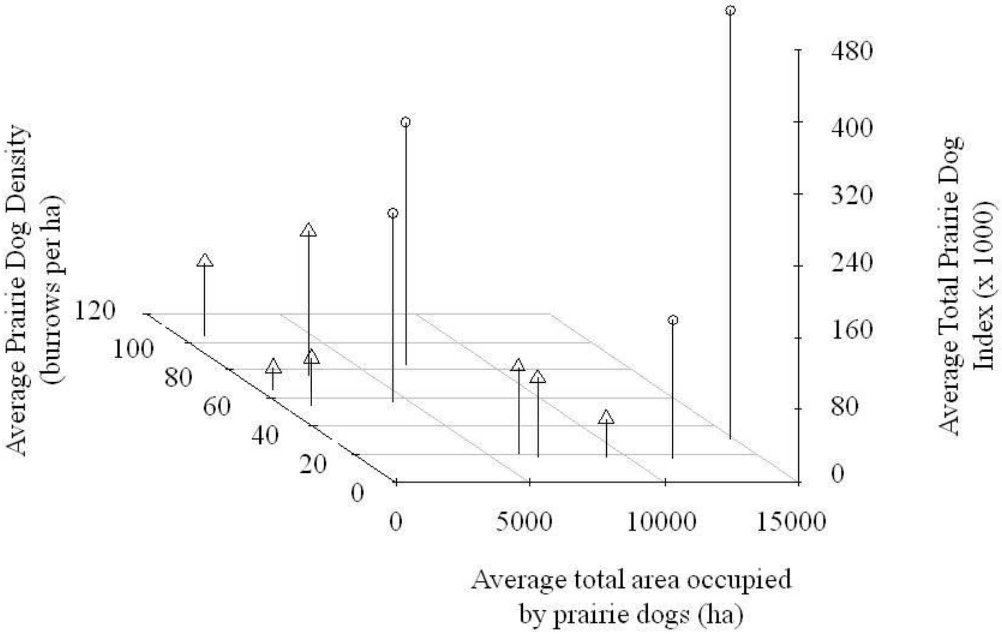

Figure 2. The average total prairie dog (*Cynomys sp.*) index value (calculated as the area that the prairie dog population covers multiplied by burrow density) shown in relation to the average total area (ha) occupied by prairie dogs and the average density of prairie dog burrows per ha for 11 black-footed ferret (*Mustela nigripes*) reintroduction sites in North America. Unsuccessful sites are denoted by triangles. Successful sites, denoted by circles, contained prairie dog index values greater than 150,000 and were greater than 4,000 ha in size.

CHAPTER 3

PHYSIOLOGICAL RESPONSES OF AFRICAN ELEPHANTS TO REINTRODUCTION

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ABSTRACT

Capturing, translocating and releasing animals for reintroduction typically causes an immediate stress response that has been linked to behavioral problems and pathological conditions that limit the success of wildlife reintroductions. Reintroduction of African elephants (*Loxodonta africana*) has become increasingly common, yet behavioral problems have been encountered post-release that have been linked to physiological condition and elevated stress hormone concentrations. However, there is uncertainty about the generality of a stress response in reintroduced elephant populations and the amount of time needed to physiologically adjust (i.e. decline of stress hormones from elevated to basal levels). The objective of this study was to evaluate (1) the relationship of temporal, climatic, and social factors to stress hormone concentrations in African elephant populations following reintroduction; and (2) variation in stress hormone concentrations among elephant family groups within reintroduced populations. We determined fecal glucocorticoid metabolite concentrations (FGMs) in 1,567 fecal samples collected from elephants reintroduced to 5 fenced reserves with differing reintroduction histories in South Africa during 2000-2006. Variation in FGMs across the 5 reserves was best explained by the number of years that elapsed since initial release. Compared with FGMs 1 year after release, FGMs were 10% lower 10 years after release, and 40% lower 24 years after release. Across all reserves, FGMs were consistently highest in the dry season, although daily and monthly temperature and rainfall were not as important as other factors. FGMs did not vary solely in relationship to reserve size or elephant density, and FGMs were similar among family groups within reserves. Collectively, our results suggest that regardless of reintroduction site conditions, elephants will require an extended period of time to physiologically adjust to their new surroundings. Managers should prepare for prolonged behavioral and pathological consequences of long-term elevated stress responses following reintroduction, such as restricted space use and aggressive behavior.

INTRODUCTION

Despite the increasing use of reintroduction as a method of restoring wildlife species to their former range, success is typically low (Griffith et al. 1989). The failure of populations to become established post-release has, in part, been linked to a physiological stress response that typically follows the process of capturing, translocating and releasing animals for reintroduction (Armstrong and Seddon 2008, Dickens et al. 2009, Dickens et al. 2010). Chronic stress responses have been hypothesized to be a major cause of reintroduction failure by increasing susceptibility to disease, reproductive failure, predation, starvation, or dispersal away from the release site (Teixeira et al. 2007, Dickens et al. 2010). Animals with elevated stress hormone concentrations also can be more prone to aggression (Muller and Wrangham 2004) or avoidance behaviors (Koolhaas et al. 2009). Therefore, a greater understanding of reintroduction practices or environmental factors that influence the physiological stress response could enhance reintroduction success, provide guidelines to mitigate exposure to potential stressors, and help managers better prepare to manage stress response induced behaviors.

The reintroduction of African elephants (*Loxodonta africana*) has become an increasingly common and controversial tool to reduce the size of large elephant source populations and to augment or restore small or extirpated populations (Grobler et al. 2008). Whereas reintroductions have been highly successful at restoring elephant populations into portions of their historic range (Garaï et al. 2004), behavioral issues have arisen following release that have been linked to elephant physiology. For example, following early attempts to reintroduce elephants in South Africa, aberrant and destructive behaviors were observed, such as the goring of >100 white rhinoceroses (*Ceratotherium simum*) and several critically endangered black rhinoceroses (*Diceros bicornis*) by young adult male elephants (Slotow et al. 2001). These attacks, linked with abnormally elevated testosterone levels and prolonged periods of musth in young bulls, were remedied by the introduction of large adult bulls that suppressed the musth patterns in younger bulls (Slotow et al. 2000). Problems are not limited to young bulls, because in at least 4 reintroduced populations normally non-aggressive female elephants have killed people (Slotow et al. 2008). These patterns of female aggression, combined with reclusive behavior, have been linked to elevated glucocorticoid stress hormone concentrations (Jachowski et al. 2012). To mitigate the occurrence of such physiological and behavioral problems in the future, there is increasing interest in building a more complete understanding of the physiological responses of elephants following reintroduction.

Past studies of African elephants suggest variations in the duration and intensity of physiological responses to translocation. Pinter-Wollman et al. (2009) observed no difference in stress hormone concentrations in wild elephants that had been translocated to a new area in Kenya (where elephants were already present) in comparison to the resident donor elephant population. In South Africa, in the case of at least 2 attempts to move elephants to new environments, stress hormone levels increased dramatically during translocation and subsequently declined to baseline conditions within 30 days post-translocation (Millspaugh et al. 2007, Viljoen et al. 2008). However, these studies were limited to populations of captive working elephants (Millspaugh et al. 2007) or wild elephants allowed to navigate back to their original territory (Viljoen et al. 2008). In the case of at least 1 translocated wild elephant population that was a true reintroduction (i.e. animals released into an area where a population was previously extirpated) into a fenced environment, stress hormone values remained elevated for up to 6 years following reintroduction (Jachowski et al. 2012). Thus, there is still uncertainty surrounding the generality of an elevated stress response in translocated elephant populations, and the amount of time needed for reintroduced elephant populations to physiologically adapt (i.e. decline from elevated stress hormone levels).

A number of local stressors might affect stress hormone responses, including climate and social factors. Seasonal climatic conditions frequently have been found to be overriding features influencing the physiological status of large herbivores (Chinnadurai et al. 2009). For large herbivores in temperate regions, seasonal differences in stress hormone levels vary, being consistently elevated during the winter (Dalmau et al. 2007, Huber et al. 2003) or summer (Millspaugh et al. 2001) depending on the predominant stressor in the environment. For large herbivores in tropical southern Africa, stress hormone values sometimes vary in response to seasonal rainfall patterns and the resulting availability of forage (Chinnadurai et al. 2009). In addition, for elephant populations where access to drinking water is limited during periods of low rainfall (i.e. dry season), seasonal limitations in water availability (Foley et al. 2001, Burke 2005, Woolley et al. 2009), and rainfall (Gobush et al. 2008), are likely to increase stress hormone concentrations. At an even finer time scale, high daily maximum temperature has been linked to decreases in stress hormone levels in elephant family groups (Pretorius 2004, Burke 2005), although the mechanism for such a response remains unclear.

Social factors are known to influence physiological states of vertebrates (McEwen and Wingfield 2003), particularly in mammalian species that live in groups (Creel 2001, Creel 2005). Given the importance of social structure in elephant populations, animals typically are captured and translocated as family groups (Grobler et al. 2008). Following reintroduction, elephants face a greater likelihood of encountering unrelated family groups or individuals, a social factor that has been linked to increased stress hormone concentrations (Munshi-South et al. 2008). The potential for social stressors is likely greatest in small fenced reserves, where elephant density and the likelihood of interaction is highest post-release. Stress hormone concentrations also vary among family groups in relation to social structure (Gobush et al. 2008) and group size (Foley 2002). Therefore, variation in stress hormone levels among populations following reintroduction could potentially be related to differences better explained at the level of family group, rather than population.

The objective of this study was to evaluate if temporal, climatic, and social factors were related to stress hormone concentrations in African elephants following reintroduction. We evaluated support for competing hypotheses associated with time since release, season, temperature, rainfall, reserve size and elephant density in 5 reintroduced elephant populations in South Africa. Within reserves, we evaluated whether elephant family groups differed in their stress hormone concentrations. While the use of stress hormone measures are an increasingly common tool to evaluate animal responses to reintroduction (Teixeira et al. 2007), no previous attempts have been made to compare physiological responses across multiple reintroduced populations of the same species. Further, to our knowledge, there have been no previous examinations of stress hormone responses of wildlife to reintroduction for an extended period of time (> 1-3 years) after release. This study was designed to shed light on the long-term physiological responses of a long-lived social species that could provide key insights into the process of physiological adaptation to reintroduction.

STUDY AREAS

We studied elephants reintroduced to 5 fenced reserves in South Africa: Pilanesberg National Park, Phinda Private Game Reserve, iSimangaliso Wetland Park, Mabula Game Reserve, and Hluhluwe-Umfolozi Game Reserve (Figure 1). Most elephants within our 5 study sites were animals translocated from Kruger National Park (Slotow et al., 2005). Entire family groups typically were captured and translocated. Exceptions included 10 elephants at Phinda Private Game Reserve brought in 1993 from Gonarhezou in Zimbabwe (Grobler et al. 2008) and 6 individuals (2 from US captive populations, two from Namibia, and 2 from Mabula Game Reserve) that were released in Pilanesberg National Park (Burke, 2005). Reserves differed in climate, size, elephant density and when elephants were reintroduced, where the earliest elephant population was initiated in Pilanesberg in 1981 and the most recent was in iSimangaliso in 2000.

Pilanesberg National Park, located in North West Province, is 560 km^2 in size and is composed of hilly terrain containing a mix of open grasslands and closed *Acacia* and broad-leaf bushveld (Burke et al. 2008). In addition to occasional hunting (Burke et al. 2008), human disturbance in Pilanesberg was primarily limited to game drive tourist activity and routine management. Tourist activity was primarily focused on roads in the central basin and southern half of Pilanesberg that were open to the public, and was relatively high (> 21 vehicles/hr) compared to the other reserves we studied due to proximity of the park to the large cities of Johannesburg and Pretoria (Burke 2005). One third of the park is closed to the public and designated as a wilderness area. Fifty-eight male and 37 female elephants were reintroduced from 1981 and 1998, primarily from Kruger National Park (Slotow et al. 2000). Currently there are at least 16 family groups (Shannon et al. 2008) and approximately 180 individual elephants in Pilanesberg, a density of 0.32 elephants per km^2.

Phinda Private Game Reserve, located in KwaZulu-Natal Province, is 180 km^2 in size and contains a range of habitats that include sweet lowveld bushveld, Natal low bushveld, and coastal bushveld (Low and Rebelo 1996). In Phinda, human disturbance primarily was limited to tourist activity. Extensive roadways through the reserve enabled access to nearly every portion of the reserve, but were limited to management and 18 guided game viewing vehicles belonging to the 2 lodges within the reserve. Lodges also offered walking safaris, which were taken by approximately 700 people annually. Managers released 54 orphan and 4 adult elephants in 1992-1994, and 3 mature adult bulls in 2003 (Druce et al. 2008). In 2007 there were at least 5 family groups and the current total population of elephants is estimated to be 98 individuals (Druce et al. 2008, Lagendijk et al. 2011), a density of 0.54 elephants per km^2.

iSimangaliso Wetland Park, part of the iSimangaliso World Heritage Site, is located on the eastern coast in KwaZulu-Natal Province. Mkhuze Game Reserve is technically managed as part of iSimangaliso Wetland Park, but contains a separate population of fenced elephants to the north of our study area, and was not included in this study. Our study focused on the southern portion of iSimangaliso Wetland Park, hereafter referred to as iSimangaliso, that was 602 km^2 in size and is composed of the Eastern Shores section (273 km^2) bordered by fencing to the North and South, and by the Indian Ocean to the east and St. Lucia Estuary to the west; and the Western Shores section (329 km^2) bordered by the St. Lucia Estuary to the east and electrified fence along its other boundaries. Human disturbance in iSimangaliso differed between the Eastern and Western Shores. The Eastern Shores was open to the public and received high amounts of tourist activity on an established road system. There was considerably less tourism activity on the Western Shores, but that area received frequent human activity in commercial forest plantations that compose ~50% of the land area (Jachowski et al. 2012). The reintroduction of elephants to iSimangaliso was initiated in 2000 with the translocation of a single group of elephants from Hluhluwe-Umfolozi Park (originally from Kruger National Park) and in subsequent years with 2 additional family groups directly from Kruger National Park. During this study approximately 45 elephants were present in iSimangaliso (van Aarde et al. 2008), a density of 0.07 elephants per km^2. Female elephants primarily formed into 4 family groups, with occasional temporary aggregated groupings.

Mabula Game Reserve, located in Limpopo Province, is 85 km^2 in size and is defined geographically by mountains in the north and plains with rocky outcroppings in the south (Bredenkamp and van Rooyen 1990). Vegetation is characterized as sour and mixed brushveld which varies from short bushveld to open savanna (Low and Rebelo 1998). Mabula had an average of 800 visitors per day and extensive road systems, with no area > 2 km^2 from the nearest road (Pretorius 2004). Six juvenile elephants (2 bulls and 4 cows) were translocated to Mabula from Kruger National Park in 1992. In 2002, 2 of the 4 cows were put on contraception. During this study approximately 10 elephants were present in Mabula (Pretorius 2004), a density of 0.12 elephants per km^2.

Hluhluwe-Umfolozi Game Reserve, located in KwaZulu-Natal Province, is 960 km^2 in size and is composed of the Hluhluwe section in the north (300 km^2) and Umfolozi section in the south (660 km^2). It is characterized by hilly topography covered by Zululand thornveld tropical forest and tropical bush savanna (Acocks 1988). Human disturbance in Hluhluwe-Umfolozi is primarily limited to tourist activity, where the reserve maintains a road system that is open to the public for vehicle-restricted game drives. The Umfolozi portion of the reserve contains a large wilderness area closed to the public. The first release of 8 elephants occurred in 1981. Since then a total of 184 elephants have been released in the reserve (Dominy et al. 1998). As of 2009, the elephant population was estimated to be around 450 individuals (D. Druce, Hluhluwe-Umfolozi Game Reserve, personal communication), a density of 0.47 elephants per km^2.

METHODS

Stress hormone analyses

From 2000 to 2006 in each of the 5 reserves, elephant fecal samples were collected and used to assay fecal glucocorticoid metabolite concentrations (FGMs). Trained employees of the reserves and students/staff from the University of KwaZulu-Natal collected fecal samples in the field during elephant monitoring surveys. They attempted to collect samples from all family groups in each reserve. In Pilanesberg, they opportunistically collected fecal samples at daily, and in some cases weekly, intervals between August 2000 and February 2005, on average collecting 0.43 samples per day (SE=0.03, *n*=706). In Hluhluwe-Umfolozi, they surveyed and opportunistically collected fecal samples at daily or weekly intervals between March and August 2002, on average collecting 0.37 samples per day (SE=0.06, *n*=67). In Mabula, they surveyed for elephants at daily intervals and opportunistically collected fecal samples between March and September 2002, on average collecting 0.92 samples per day (SE= 0.14, *n*=194). In iSimangaliso and Phinda, they tracked family groupings and collected fecal samples linked specifically to them. In iSimangaliso, they tracked each of the 4 elephant family groups present and opportunistically collected fecal samples at daily intervals between August 2001 and August 2002, and then again at weekly or monthly intervals between September 2005 and November 2006, on average collecting 0.52 samples per day (SE=0.05, *n*=405). In Phinda, they tracked each of the 5 elephant family groups at daily intervals and collected fecal samples in March and April 2003, and then again between September 2003 and June 2005, on average collecting 0.27 samples per day (SE=0.03, *n*=195). To avoid pseudoreplication, they did not collect multiple samples from the same individual elephant on the same day. When they were not able to confirm the identity of individual elephants producing the samples, we determined if samples belonged to unique individuals within the larger group or reserve on a given day based on comparing bolus size, a method commonly used to differentiate sex and age classes in elephants (Morrison et al. 2005, Burke et al. 2008, Woolley et al. 2008).

For each fecal sample, time of collection, approximate age of the sample and location of collection, and whenever possible identified the individual or family group that deposited the sample was recorded. Samples were only collected if $<$ 72 hrs had passed since deposition. Samples for laboratory analysis were collected by opening the bolus and taking a portion from its center (Burke 2005). After collection, samples were treated with a 2% acetic acid solution to kill potential pathogens and prevent disease transmission (Millspaugh et al. 2003), and then frozen for shipment. In the laboratory, samples were freeze-dried, ground, and sifted through a stainless steel mesh. FGMs were extracted from the feces using corticosterone I^{125} radioimmunoassay kits (MP Biomedicals, Solon, OH) following established protocols that have been validated for elephants (see Wasser et al. 2000, Millspaugh et al. 2007). Assay accuracy and precision was confirmed by conducting a standard assay validation, including assessment of parallelism, recovery of exogenous analyte, intra- and inter-assay precision, and assay sensitivity (Jeffcoate 1981, O'Fegan 2000, Millspaugh et al. 2007). Inter-assay variation for 21 assays was 8.1% and average intra-assay variation was 4.4%.

Prior to data analysis, for each fecal sample, we estimated the time period when FGM concentrations represented exposure to potential stressors. Based on inspection of fecal samples in the field, we used the estimated age of each fecal sample to estimate the deposition time (Burke et al. 2008). Because there is a 36 hr delay following exposure to an acute stressor before elevated FGMs are expressed in fecal samples (Wasser et al. 2000, Ganswindt et al. 2003), we assumed that FGMs reflected the physiological state of the elephant 36 hrs prior to deposition, and used this calculation to define the time of inference for each fecal sample (Burke et al. 2008).

Statistical analyses

We used a repeated measures analysis of variance (ANOVA) to test whether FGM concentrations differed by reserve and season. Given that multiple samples were collected from populations on individual days, we treated day as the repeated effect. We classified seasons based on temperature and rainfall, where the wet season occurred from November to April, and the dry season occurred from May to October (Burke 2005, Shannon et al. 2006).

To evaluate support for the hypothesized influence of time since release, temperature, rainfall, reserve size and elephant density on FGMs, we used linear mixed models (SAS PROC MIXED (Littell et al. 2006)) and an information theoretical framework (Burnham and Anderson 2002). We calculated time since release as the amount of time that had elapsed between the first release of elephants into the reserve and the time of inference for the FGM sample. We estimated the maximum daily temperature and daily rainfall at the reserve level based on data provided by the nearest South African Weather Service remote weather station (http://www.weathersa.co.za/) within or adjacent to each reserve. We also calculated average maximum temperature and rainfall at monthly intervals. We included the following 2 interactions. First, because the effects of limited rainfall (and lower water availability) are likely to be exacerbated by increases in temperature, we included an interaction between daily temperature and rainfall as well as monthly temperature and rainfall. Second, because smaller reserves could result in elephants maintaining increased FGMs due to the greater probability of coming into close proximity to stressors such as roads or human disturbance (Burke 2005) or unrelated family groups or individuals (Munshi-South et al. 2008), we included a fixed effect of reserve size and calculated the density of elephants within each reserve during our study. Prior to model fitting, we standardized our continuous covariates and tested our response variable (FGM) for normality.

Within our mixed models, the day of sample collection was the repeated effect, the reserve sampled was a random effect, and all other independent variables hypothesized to influence FGMs were fixed effects. We fit models that individually evaluated the effect of each hypothesized factor, as well as models that contained combinations of the two types of factors hypothesized to influence FGMs; reserve-specific conditions (i.e. reserve size, elephant density, and time since release) and climatic factors (i.e. daily and monthly rainfall and temperature) (Table 1). We used restricted maximum-likelihood (REML) to select the most appropriate covariance structure to the data based on the lowest AIC_c scores (Littell et al. 2006), which we identified to be compound symmetry. Therefore, we fit all subsequent models with a compound symmetry structure. Because REML AIC_c values are not comparable across models with different fixed effects, we used a maximum-likelihood approach to rank models using ΔAIC_c (Diggle et al. 1994).

We compared model performance within both stages of analysis by calculating the percent of variation explained. To calculate the percent of variation explained, we used maximum likelihood covariance parameter estimates for each model in each stage by using the formula:

$$\%\text{ variation explained} = \left| \left(\frac{\sigma^2_{\text{process}} - \sigma^2_{\text{residual}}}{\sigma^2_{\text{process}}} \right) x\ 100 \right|$$

where $\sigma^2\ process$ = variance component estimate for the intercept-only model, and the $\sigma^2\ residual$ = variance component estimate for the model in question (Doherty et al. 2010).

Given that we were only able to link fecal samples with elephant family groups in Phinda and iSimangaliso, we evaluated if differences existed in FGMs among family groups within populations only in those 2 reserves. We used a repeated measures ANOVA, where day was the repeated effect, to evaluate if differences existed in FGM concentrations among family groups in each of the 2 reserves. We also evaluated the amount of variability within family groups by calculating the seasonal (i.e., wet and dry season) coefficient of variation in

FGMs within family groups during our study, as well as the coefficient of variation within family groups within a given day (limited to days when samples were simultaneously collected from multiple members of a family group). This analysis allowed us to determine if family groups differed in FGMs within reserves, and if this variation potentially explained some of variation in population-level differences we observed among reserves.

RESULTS

We collected and assayed 1,567 samples from the 5 reserves between 2000 and 2006. FGMs were 23% higher during the dry season than the wet season ($F_{1,1560}$=30.55, P<0.0001). Elephant FGMs differed among reserves ($F_{4,93}$=41.66, P<0.0001) (Figure 2). Samples originating from iSimangaliso (during both seasons) and Mabula (during the dry season) were typically > 40 ng/g, indicative of an elevated physiological state (Wasser et al. 2000, Jachowski et al. 2012). By contrast, samples from Phinda and Pilanesberg tended to have FGMs 16-45% lower, and on average below 40 ng/g, indicative of elephant populations in basal physiological condition (Wasser et al. 2000, Jachowski et al. 2012).

Across-park variation

Variation in FGM concentrations across the 5 reserves was best explained by the number of years that elapsed since initial release and the interaction of monthly average maximum temperature and average monthly rainfall (Table 1). All models containing time since release explained > 85% of the variation (Table 1), where FGMs were predicted to decrease by 1.18% each year following release

(Figure 3). FGMs for elephants in iSimangaliso 1 year after release ($\bar{x}$ = 48.47, sd = 26.32, range = 9.87 - 123.70) were 10% greater than values for elephants in Phinda and Hluhluwe-Umfolozi reserves 10 years after release ($\bar{x}$ = 43.41, sd = 14.17, range = 19.28 - 91.35), and were 40% greater than values in Pilanesberg 24 years following release ($\bar{x}$ = 27.00, sd = 6.65, range = 17.13 - 42.06) (Figure 4). In addition, the variability of FGMs decreased over time, where the coefficient of variation in FGMs in elephants in iSimangaliso 1 year following reintroduction (0.5429) was nearly twice as high as elephants in Pilanesburg 24 years following reintroduction (0.2462) (Figure 4).

Despite observing an overall effect of season on FGMs across all reserves, when the effect of time since release was removed, daily and monthly rainfall and temperature patterns did not explain a large amount of observed variation in FGMs (Table 1). The interaction of monthly average maximum temperature and total rainfall, while a component of the most supported model, individually explained only 29% and 15% of the variation in FGMs respectively (Table 1). Based on our top-ranked model, we predicted a 3.49% decrease in FGMs for every 20 mm increase in monthly rainfall, and a 0.03% decrease in FGMs for every 1 degree increase in average maximum monthly temperature (Figure 5). Maximum daily temperature and rainfall were even poorer predictors of FGMs, explaining 0-16% of the variation and were not retained in our top ranked model.

We failed to find support for a direct effect of reserve size and elephant density on FGMs (Table 1). In contrast to our hypothesized negative relationship between reserve size and elephant FGMs, reserve size alone explained 0.36% of the observed variation. One of the largest reintroduction sites (iSimangaliso) was also the most recently initiated, and its elephants consistently had the highest FGMs (Figure 2). With the exception of Mabula, elephant density was inversely correlated to time since release for the reintroduced populations we studied. Therefore, despite remaining lower than the density of elephants at the donor site (Kruger National Park, 0.63 elephants/km^2; van Aarde et al. 2008) and receiving a low amount of model support (Table 1), elephant density explained 45% of the observed variation in FGM concentrations.

Elephant family group variation

We assayed 406 fecal samples assigned to specific elephant family groups within Phinda (n=114, 5 family groupings) and iSimangaliso (n=292, 5 family groupings) between 2002 and 2006. All family groups exhibited similar FGM concentrations within each of these reserves ($F_{4,10}$=0.65, P=0.64; $F_{4,2}$=7.41, P=0.12) (Figure 6). However, there was a difference in the variability of FGMs among family groups when comparing between, rather than within reserves. Similar to reserve-level average FGMs (Figure 2), variation in FGM concentrations within family groups was highest in elephants of the most recently reintroduced population (iSimangaliso), where we found that seasonal coefficient of variation values for FGM concentrations within family groups were on average 32% higher in iSimangaliso ($\bar{x}$=0.417, SE=0.043, range=0.303-0.682) than Phinda ($\bar{x}$=0.317, SE=0.042, range=0.190-0.675). In addition, even within a given day, coefficient of variation values for FGM concentrations within family groups were on average 42% higher at iSimangaliso ($\bar{x}$=0.251, SE=0.016, range=0.047-0.682) compared to Phinda ($\bar{x}$ =0.177, SE=0.032, range=0.007-0.627).

DISCUSSION

Our study shows that physiological adaptation can require an extended period of time for wild elephants following reintroduction into fenced reserves. Elevated stress responses are not uncommon following wildlife reintroduction (Teixeira et al. 2007), but previously little was known about how long stress hormones remain elevated post-release (Dickens et al. 2010). Generally, the duration of elevated stress response following release has been linked to the sensitivity of a species, the intensity and duration of stressors, and the number of stressors encountered (Dickens et al. 2010). Within the reserves we studied, elephant FGMs have been shown to vary in relationship to fluctuations in the availability of key nutrients in their forage (Woolley et al. 2009), human disturbance both in the form of tourism (Pretorius 2004, Burke 2005) and hunting (Burke et al. 2008), as well as stochastic events such as catastrophic fires (Woolley et al. 2008). However, regardless of the presence of acute, reserve-specific stressors, our data suggest that a relatively long-term (>10 years), population-level, elevated stress response is likely to occur to following reintroduction.

Elevated stress hormones can have multiple pathological and behavioral consequences that should be of concern to managers (Romero 2004). While pathological implications of elevated stress hormone concentrations in elephants are not well understood, elephants in an elevated physiological state can exhibit refuge behavior (Woolley et al. 2008, Jachowski et al. 2012) that in turn could limit tourist viewing opportunities (one of the primary reasons for reintroducing elephants), lead to aggressive elephant behavior when encountering humans (Jachowski et al. 2012), and potentially cause extensive habitat modification (Lagendijk et al. 2011, Skarpe et al. 2004). In contrast to environmental and seasonal stressors that can be difficult to manage, human disturbance is known to elicit a physiological stress response (Pretorius 2004, Burke 2005) and can be more easily managed. Therefore, to mitigate human-elephant conflict, we suggest that managers ensure that reintroduced elephants have access to refugia away from human disturbance, and limit human access to refugia to avoid potential aggressive encounters (Jachowski et al. 2012).

During physiological adaptation, seasonal patterns in the availability of water and forage likely influence elephant FGMs similar to other large vertebrate species. Within a given reserve, short term elevations in stress hormones have been observed in response to acute environmental stressors (Burke et al. 2008, Woolley et al. 2008), including daily maximum temperature and rainfall (Burke 2005). Such reserve-specific environmental conditions are likely important in evaluating FGMs at the individual or population level over short periods of time, but our study shows that variation in FGMs across multiple populations over an extended period of time is better explained by translocation history. Nevertheless, during physiological adaptation, FGMs varied in response to longer-term monthly patterns of rainfall and maximum temperature that were representative of wet and dry season conditions. Seasonal differences in stress hormone concentrations are commonly observed in herbivores (Millspaugh et al. 2001, Chinnadurai et al. 2009). Although the mechanism underlying such seasonal variations in tropical systems remains unclear, the positive influence of dry season on FGMs in large herbivores in South Africa is likely due to variations in water availability and forage quality (Chinnadurai et al. 2009). In contrast to previous findings that suggest elephant FGMs are elevated seasonally in response to decreases in water availability (Foley et al. 2001), elephants reintroduced to fenced reserves typically have year-round access to natural or man-made water sources. Therefore, it is likely that the seasonal variations in FGM concentrations we observed were due primarily to nutritional stress associated with lower forage quality during the dry season (Woolley et al. 2009).

Our findings suggest that at both the individual family group and population scale, any fine-scale differences in FGMs related to social stressors were likely of less importance than the overriding factor of adjustment time post-release. Persistent social stressors that are likely to be represented at the population level in fenced reserves, such as overcrowding and more frequent interactions with unrelated family groups (Munshi-South et al. 2008), were not likely to have been major factors during our study (where <25 years had elapsed since release) due a combination of lag time in elephant population growth and proactive population control (Pretorius 2004, Druce et al. 2011). Further, the consistent practice of translocating entire family groups (a practice initiated across South Africa in 1993) likely helped maintain the social structure and group size needed to ameliorate group-specific social FGM responses as seen in disturbed wild populations (Gobush et al. 2008).

The trend of decreasing FGMs within elephant populations and family groups over time suggests that elephants were psychologically adapting to their new surroundings. Translocated elephants must physiologically and psychologically adjust to dealing with novel environments and isolation from their original families and matriarchs (Bradshaw et al. 2005). Given that the sensitivity of elephants to threats is related to the age and experience of its matriarch (McComb et al. 2011), it is likely that an extended period of time is required for elephants to build site-specific knowledge and psychologically adapt to their new surroundings. Our finding that time since translocation better explains physiological state compared to environmental conditions, and that physiological state of elephants declined over time post-release, support this hypothesis and suggests that psychological adjustment is slowly occurring. Further, declining FGM variability within populations and family groups over time also suggests that elephants generally psychologically adapted to their new surroundings regardless of reserve-specific stressors. Thus, with a sufficient amount of time, elephants can likely physiologically and psychologically adapt to stressors specific to each reserve.

Failure to observe long-term elevated physiological stress responses in previous studies of wild elephants subject to translocation (e.g. Viljoen et al. 2008, Pinter-Wollman et al. 2009) was likely primarily due to both the lack of long-term monitoring, as well as to differences in the practice of reintroduction from other types of translocation. Previous studies of elephant physiological responses to translocation that found only short-term (0-30 day) elevations in FGMs were limited to translocations where individuals were allowed to return to their home range (e.g., Viljoen et al. 2008) or where resident populations were already present at the release site (e.g., Pinter-Wollman et al. 2009). By contrast, elephants reintroduced into fenced reserves during this study were restricted in their ability to navigate back to their original territory and there was no resident population with which to interact following release. Therefore, the discrepancy we observed from previous translocation studies is potentially attributed to a key difference between the practice of reintroduction from other types of translocation; where in true reintroductions, individuals are moved to a portion of their historic range where conspecifics are no longer present (Armstrong and Seddon 2008). This suggests that while previous assessments have summarized physiological responses of wildlife to translocation and reintroduction collectively given exposure to similar stressors (e.g. capture, handling, and release into new environment) (Teixeira et al. 2007, Dickens et al. 2010), those considering reintroductions likely need to be particularly concerned about the potential for long-term elevated physiological stress responses following release.

For elephants and other species subject to reintroduction, because the establishment phase following release is critical to overall success or failure (Armstrong and Seddon 2008), it is important to identify the amount of time needed for animals to adjust following release. In addition to monitoring stress hormone concentrations for extended periods of time post-release, there is a need to better understand behavioral and pathological consequences associated with sustained elevated physiological states (Dickens et al. 2010). In this case, and likely other vertebrate reintroduction programs, such information can be used to guide decisions that mitigate human-wildlife conflict and facilitate long-term reintroduction success.

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Table 1. Support for models based on time since release, social and environmental reserve-specific attributes explaining observed FGM concentrations of African elephants in 5 reserves in South Africa between 2000 and 2006.

Model	log (l)	K	ΔAIC_c	AIC_c weight	σ^2_{model} [a]	Absolute variation explained
Time since release + Average monthly rainfall * Average monthly temperature	1524	8	0	0.8134	0.0005	97%
Average monthly rainfall * Average monthly temperature	1529	7	2.948	0.1863	0.0118	29%
Average monthly temperature	1546	5	16.032	0.0003	0.0126	25%
Time since release + Maximum daily temperature	1557	6	28.798	0.0000	0.0016	90%
Time since release +	1556	8	32.318	0.0000	0.0016	90%

Maximum daily temperature * Total daily rainfall						
Maximum daily temperature * Total daily rainfall	1564	7	38.115	0.0000	0.0140	16%
Average monthly rainfall	1574	5	44.186	0.0000	0.0192	15%
Time since release	1586	5	56.011	0.0000	0.0025	85%
Time since release + Size of reserve	1585	6	56.963	0.0000	0.0017	90%
Time since release + Total daily rainfall	1586	6	57.775	0.0000	0.0025	85%
Density of elephants in reserve	1593	5	62.681	0.0000	0.0091	45%
Intercept only model	1595	4	63.584	0.0000	0.0167	-
Total daily rainfall	1595	5	65.357	0.0000	0.0167	0%
Size of reserve	1595	5	65.564	0.0000	0.0166	0%
Maximum daily temperature	1731	5	201.302	0.0000	0.0228	37%

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[a] σ^2_{model} = covariance parameter estimate

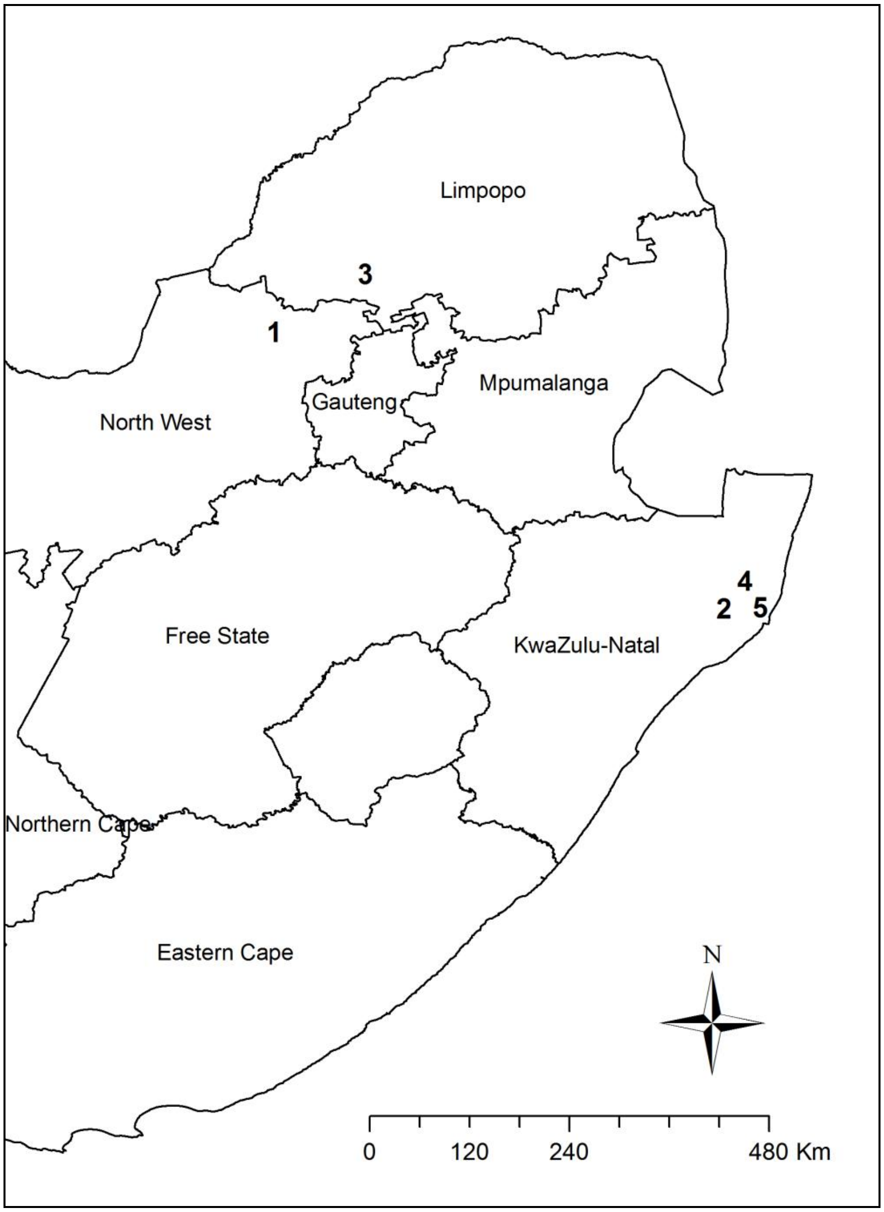

Figure 1. Location of the 5 reserves (1, Pilanesberg National Park; 2, Hluhluwe-Umfolozi Game Reserve; 3, Mabula Game Reserve; 4, Phinda Private Game Reserve, 5, iSimangaliso Wetland Park) monitored during this study within provinces of eastern South Africa.

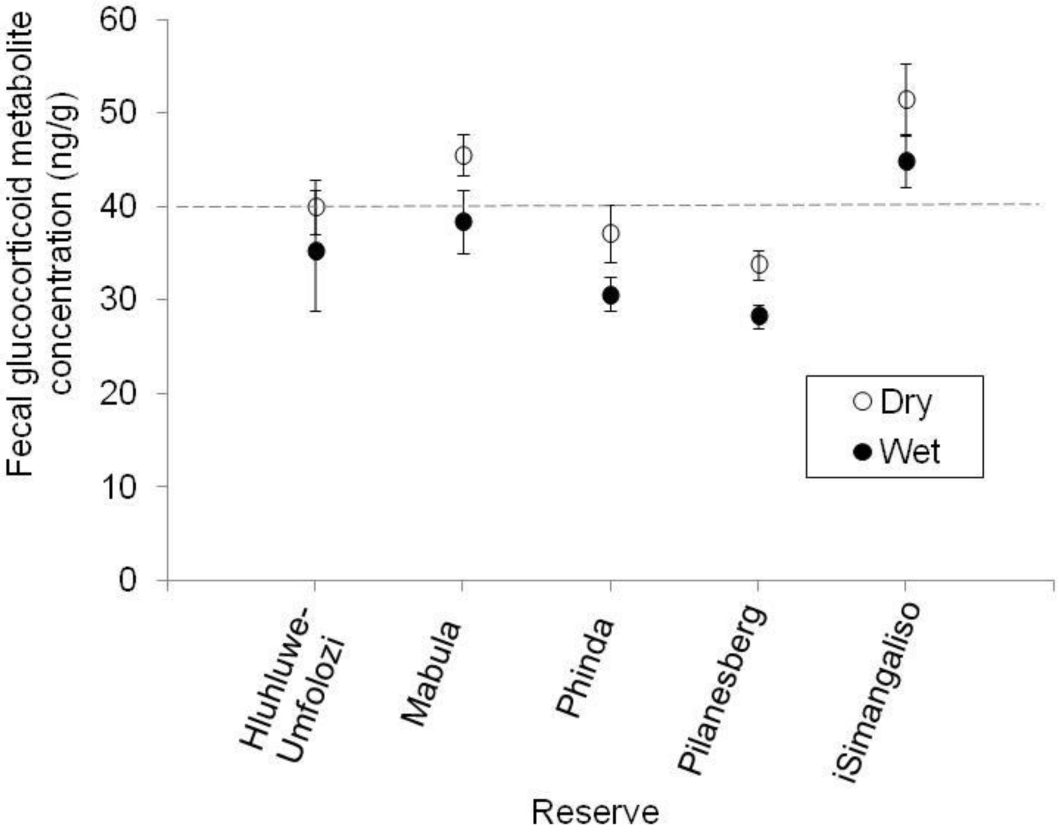

Figure 2. Mean (with 95% confidence intervals) seasonal fecal glucocorticoid metabolite (FGM) concentrations of elephants by reserve. FGM values >40 ng/g (dashed line) are typical of elephants in an elevated physiological state (Wasser et al. 2000, Jachowski et al. 2012).

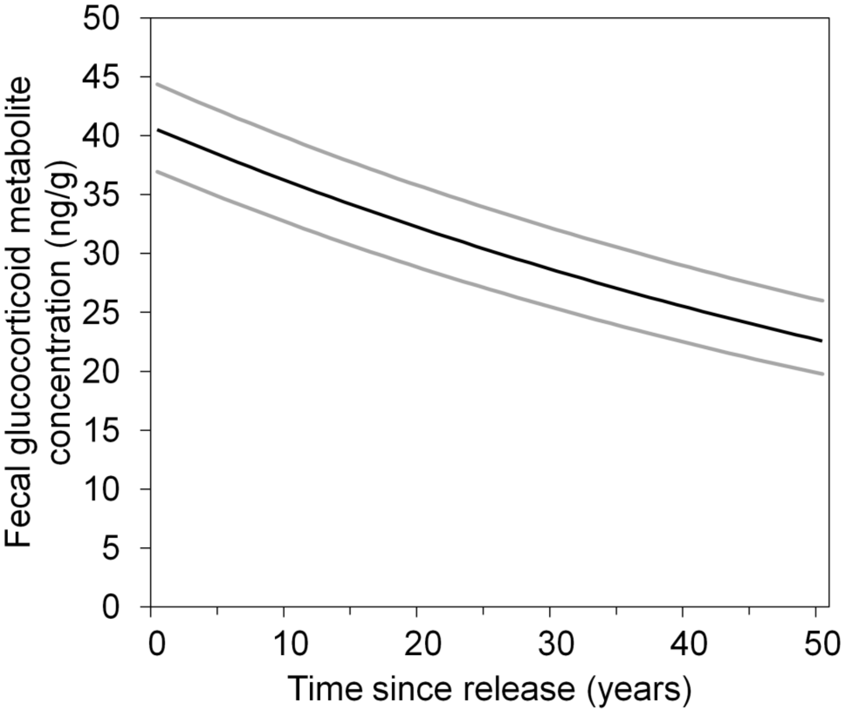

Figure 3. Predicted response of fecal glucocorticoid metabolite concentrations in elephants as a function of time elapsed since initial release based on the top ranked across-population level model. Grey lines indicated 95% confidence intervals.

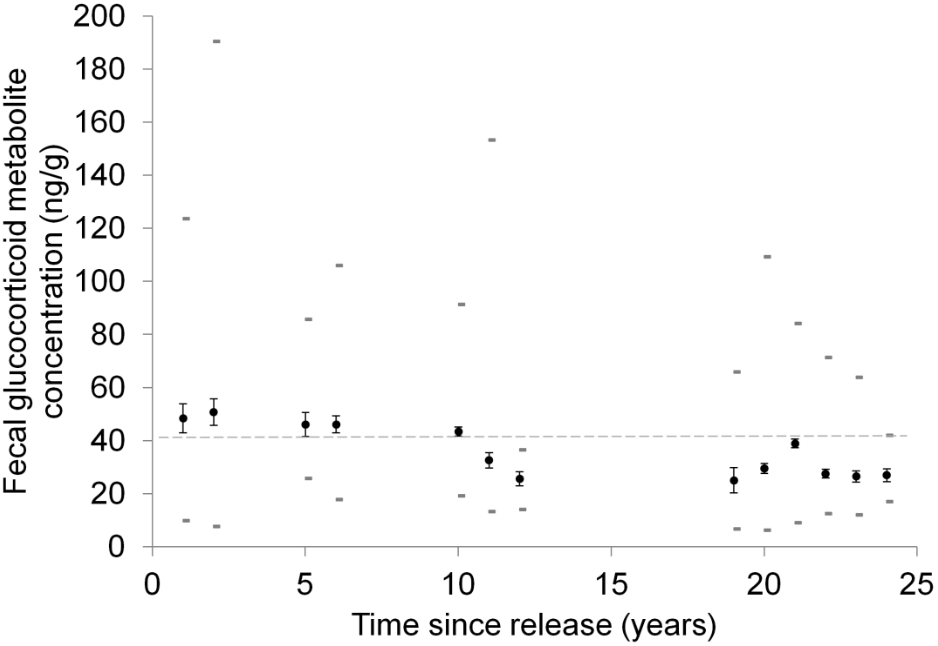

Figure 4. Mean (hollow circles) and 95% confidence intervals, as well as maximum and minimum (dashes) values of fecal glucocorticoid metabolite concentrations across 5 elephant populations over time post-release. FGM values >40 ng/g (dashed line) are typical of elephants in an elevated physiological state (Wasser et al. 2000, Jachowski et al., 2012).

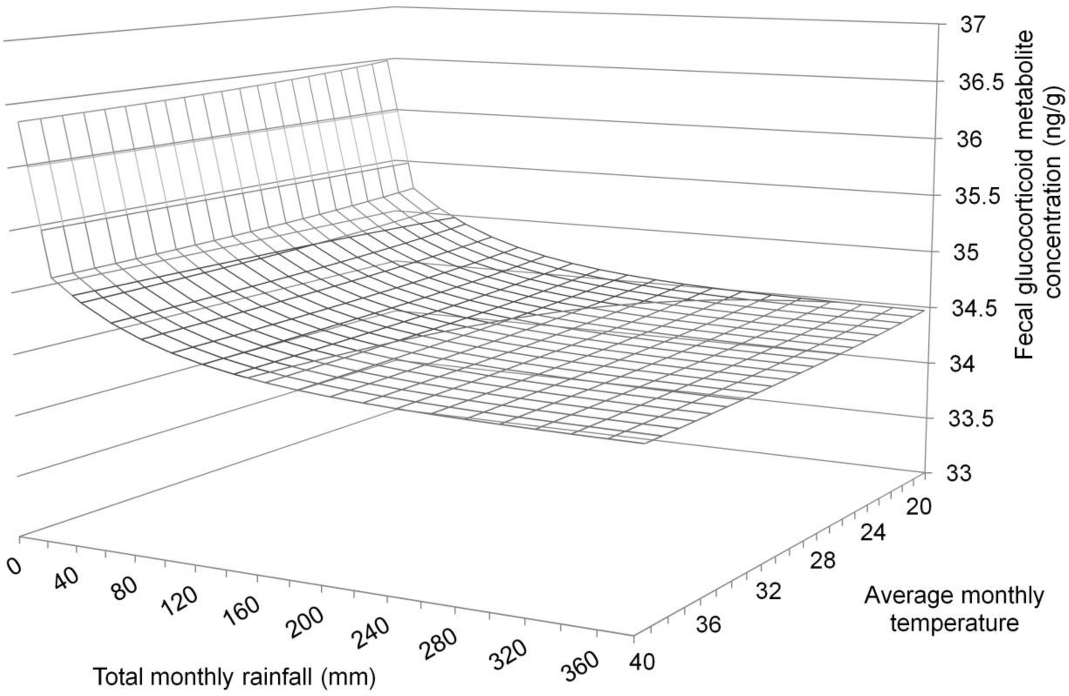

Figure 5. Predicted response of fecal glucocorticoid metabolite concentrations in elephants as a function of the interaction of average maximum monthly temperature and total monthly rainfall based on top ranked population level model.

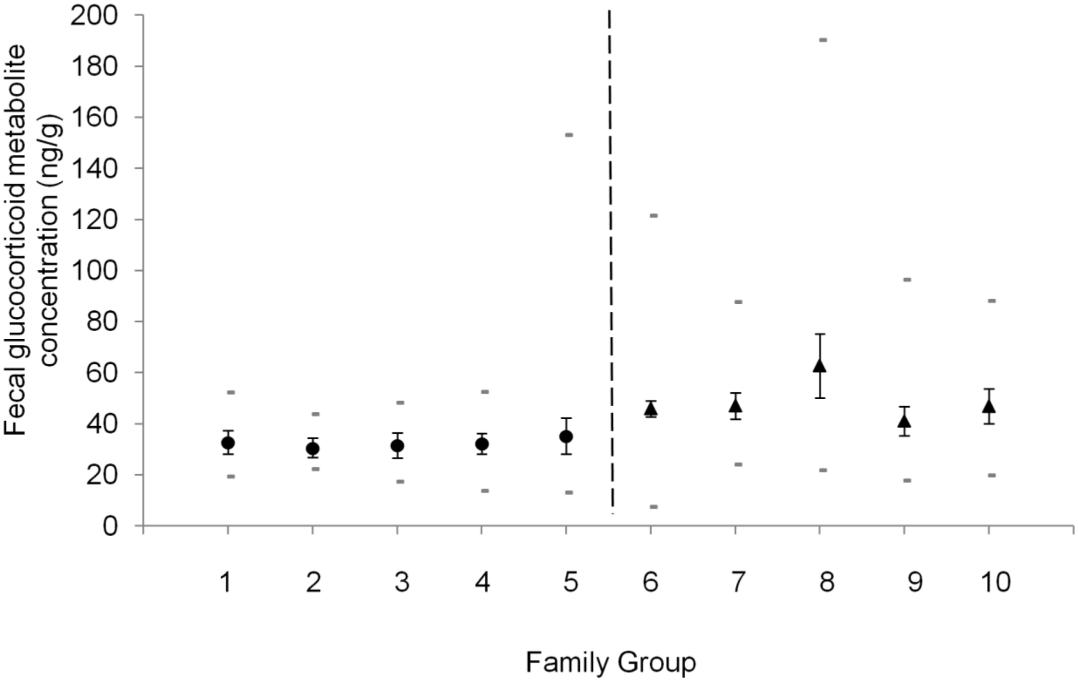

Figure 6. Mean (circle, with 95% confidence intervals), as well as maximum and minimum (dashes) fecal glucocorticoid stress hormone values for elephant family groups present in Phinda Private Game Reserve (1-5) and iSimangaliso Wetland Park (6-10).

CHAPTER 4

ADAPTATION OF SPACE USE PATTERNS BY REINTRODUCED AFRICAN ELEPHANTS

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ABSTRACT

Physiological stress responses allow individuals to adapt to changes in their status or surroundings, but chronic exposure to stressors could have detrimental effects. Increased stress hormone secretion leads to short-term escape behavior; however, no studies have assessed the potential of longer-term escape behavior, when individuals are in a chronic physiological state. Such refuge behavior is likely to take two forms, where an individual or population restricts its space use patterns spatially (spatial refuge hypothesis), or alters its use of space temporally (temporal refuge hypothesis). We tested the spatial and temporal refuge hypotheses by comparing space use patterns among three African elephant populations maintaining different fecal glucocorticoid metabolite (FGM) concentrations. In support of the spatial refuge hypothesis, the elephant population that maintained elevated FGM concentrations (iSimangaliso) used 20% less of its reserve than did an elephant population with lower FGM concentrations (Pilanesberg) in a reserve of similar size, and 43% less than elephants in the smaller Phinda reserve. We found mixed support for the temporal refuge hypothesis; home range sizes in the iSimangaliso population did not differ by day compared to nighttime, but elephants used areas within their home ranges differently between day and night. Elephants in all three reserves generally selected forest and woodland habitats over grasslands, but elephants in iSimangaliso selected exotic forest plantations over native habitat types. Our findings suggest that chronic stress is associated with restricted space use and altered habitat preferences that resemble a facultative refuge behavioral response. Elephants can maintain elevated FGM levels for ≥ 6 years following translocation, during which they exhibit refuge behavior that is likely a result of human disturbance and habitat conditions. Wildlife managers planning to translocate animals, or to initiate other management activities that could result in chronic stress responses, should consider the potential for, and consequences of, refuge behavior.

INTRODUCTION

In responding to real or perceived threats, vertebrates initiate a physiological stress response that has broad implications if stress levels are maintained at a high level (i.e., chronic) (Romero 2004). The production of stress hormones is a key physiological step in balancing the expenditure of energy, and facilitates the ability of an individual to survive exposure to a stressor (McEwen and Wingfield 2003, Romero and Butler 2007). While this response is effective in the presence of short-term stressors, chronic levels of stress can result in various pathological dysfunctions, including an increase in blood glucose, or the inhibition of reproduction, immune function, or growth (Romero 2004, Dickens et al. 2010). Therefore, while short-term releases of stress hormones help a vertebrate adapt to its surroundings, over extended periods of time, chronic release of hormones should be minimized to reduce deleterious effects (McEwen and Wingfield 2003).

Vertebrates limit chronic exposure to stressors through three kinds of facultative behavioral responses (Wingfield and Romenofsky 1997): (1) the individual exhibits escape behavior away from the perturbation; (2) the individual remains in the area, but identifies and uses a refuge to avoid the perturbation; and (3) the individual identifies and uses a refuge, but will move outside the refuge during periods of non-disturbance. Many studies have focused on short-term escape behavior away from disturbances (Wingfield and Romenofsky 1997, Breuner et al. 1998). The latter two kinds of responses have received considerably less attention. Previous studies have suggested that use of refugia typically is temporary, and that normal space use continues once the disturbance passes (Astheimer et al. 1992, Wingfield and Romenofsky 1997). However, to our knowledge, there has been no research to evaluate if long-term use of refugia is likely to occur if the animal does not adjust to the source of perturbation, and maintains a chronic physiological state.

Descriptions of wildlife use of “refuges” or “refugia” are increasingly widespread in ecology and conservation biology. In the ecological literature, refugia frequently are defined by fine-scale spatial responses of animals to perturbations (Elliot 2000, Riegl and Piller 2003, Ultsch 2006). While particular behaviors and space use patterns have been reported as refuge behavior, little is known about the facultative process behind those observations. Initiation of refuge behavior is an active process involving an external cue (i.e. the stressor), internal physiological response, and active movement and selection of refugia (Breuner and Hahn 2003). The extent to which physiological state influences the timing and duration of refuge behavior is poorly understood, despite its potential importance in predicting when, where, to what extent, and for how long refuge behavior will occur.

The refuge behavior of African elephants (*Loxodonta africana*) is relatively well documented through long-term behavioral studies. Elephants are long-lived with high cognitive ability for spatial memory (Hart et al. 2008) that allows them to adapt space use patterns based on the location of resources (Loarie et al. 2009), boundaries (Vanak et al. 2010), or past experiences (Druce et al. 2008). Behavioral observations suggest that elephants exhibit at least two facultative behavioral responses indicative of spatial and temporal refuge behavior. Firstly, humans have restricted elephant movements, and fragmented habitat, through the creation of real (e.g., electric fences) or perceived (e.g., human land use and disturbance) boundaries (Hoare and Du Toit 1999). In response, elephants have restricted space use patterns and have identified, used, and rarely occurred outside of protected areas or refugia (Douglas-Hamilton et al. 2005). Secondly, in addition to restricting movements spatially, space use can be modified temporally to avoid areas during periods of disturbance (Theuerkauf et al. 2003, Hebblewhite and Merrill 2008). This pattern of spatio-temporal refuge behavior allows elephants to reoccupy habitats when humans are absent (Graham et al. 2009, Wedge et al. 2010).

In South Africa, where elephants are being reintroduced to relatively small fenced reserves, there is a particular need to consider the potential for refuge behavior. Elephants have been translocated for reintroduction into over 58 reserves in South Africa (Garaï et al. 2004). The process of translocation is well established and designed to be as unobtrusive to the animals as possible (Grobler et al. 2008), but still results in an elevated physiological stress response for up to 10 years post-release (Chapter 3). However, little is known about the potential for longer-term stress responses in elephants following translocation (Poole and Granli 2011), despite the need to understand how they habituate to their new surroundings, and if they exhibit aberrant behavior that poses a risk to elephants, other animals and people (Grobler et al. 2008). To facilitate acclimatization, it has been suggested that managers provide “refuge areas” to allow translocated elephants freedom from harassment (Pretorius 2004). Thus, there is interest in identifying when and where refuge behavior occurs, to mitigate potential human-elephant conflict.

In this study, we evaluated spatial and temporal hypotheses of refuge behavior in elephants by comparing space use patterns among three restored elephant populations. These populations maintained different levels of physiological stress, including one with chronic levels. Under the spatial refuge hypothesis, where individuals restrict space use when stress hormone levels are elevated, we expected elephant populations that were chronically stressed to avoid disturbance by exhibiting restricted space use patterns. Therefore, we examined two metrics: home range size, and the proportion of the area used by elephant family groups in each reserve. Under the temporal refuge hypothesis, where individuals temporally alter their use of space when stress hormone levels are elevated, we expected elephant family groups in a state of chronic stress to restrict their use of space during the day, when human disturbance existed, and increase their use of space at night, when disturbance ceased. We tested support for the temporal refuge hypothesis by evaluating whether elephant family group home range sizes were smaller during the day than at night, whether family groups used the same areas during the day and night, and whether seasonal resource selection differed between night and day. By comparing these metrics across elephant populations in different physiological states, we were able to link stress with refuge behavior.

STUDY AREAS

We selected three reintroduced elephant populations in South Africa, which were each contained by electrified boundary fences: Pilanesberg Game Reserve (25°8'-25°22'S, 26°57'-27°13'E), iSimangaliso Wetland Park (28°49'-27°55'S, 32°68'-32°22'E), and Phinda Private Game Reserve (27°92'-27°68'S, 32°44'-32°20'E). Most individual elephants within our three study sites were translocated from Kruger National Park, or were the offspring of such animals (Slotow et al. 2005). Exceptions were 10 individuals at Phinda Private Game Reserve brought in 1993 from Gonarhezou in Zimbabwe (Druce et al. 2008), and six individuals (two from US captive populations, two from Namibia, and two from Mabula Game Reserve) that were released in Pilanesberg National Park (Burke 2005).

Pilanesberg National Park (hereafter referred to as Pilanesberg), located in the North West Province, is 560 km^2 in size and is composed of hilly terrain containing a mix of open grasslands and closed *Acacia* and broad-leaf bushveld (Burke 2005). We classified habitats based on seven major vegetation types in the park (Vanak et al. 2010, Brockett 1993): (1) *Acacia caffra* woodland, (2) *A. karoo* woodland, (3) *A. mellifera* woodland, (4) *Combretum* woodland, (5) *Faurea* woodland, (6) mixed *Acacia* woodland, and (7) grassland. Fifty-eight male and 37 female elephants were reintroduced from 1981 to 1998, primarily from Kruger National Park (Slotow et al. 2000). In 2004 there were at least 16 family groups (Shannon et al. 2008), and by 2009 there were approximately 180 individual elephants in the park (S. Dell, Pilanesberg National Park, personal communication).

Phinda Private Game Reserve (hereafter referred to as Phinda), located in the KwaZulu-Natal Province, is 180 km^2 in size and contains a range of habitats that include sweet lowveld bushveld, Natal low bushveld, and coastal bushveld (Low and Robelo 1996). We used existing land use and vegetation maps created by Noel van Rooyen and Simon Morgan for reserve management to classify habitats into eight categories: (1) *Acacia* woodland, (2) human habitation, (3) open grassland, (4) closed woodland, (5) riverine and wetland, (6) sand forest, (7) Lebombo thicket, and (8) palmveld. Managers released 54 orphan elephants in 1992-1994 and 3 mature adult bulls in 2003 (Druce et al. 2008). In 2009, there are at least five family groups, and the total population in 2010 was estimated to be 93 individuals (T. Burke, Phinda Private Game Reserve, personal communication).

The iSimangaliso Wetland Park is located on the eastern coast in KwaZulu-Natal Province. It is 602 km^2 in size and is composed of the Eastern Shores section (273 km^2) bordered by fencing to the north and south, by the Indian Ocean to the east and the estuary of Lake St. Lucia to the west; and the Western Shores section (329 km^2) bordered by Lake St. Lucia to the east and electrified fence along its other boundaries. We used existing vegetation and land use maps created by Noel van Rooyen for park management to classify iSimangaliso into eight major habitat types: (1) tree plantations (composed of either *Eucalyptus globulus* or *Casuarina equisetifolia*), (2) dry forest, (3) lowland forest, (4) marsh and swamp, (5) freshwater lake, (6) grassland, (7) human settlement, and (8) open beach. We did not consider the estuarine Lake St. Lucia as available habitat in our analysis. The reintroduction of elephants to iSimangaliso was initiated in 2001 with the translocation of a 24 elephants (15 females and 9 males) from Hluhluwe-iMfolozi Park (originally from Kruger National Park), and in 2002 and 2003 with two additional family groups directly from Kruger National Park.

METHODS

Stress hormone data

From 2000 to 2006, we sampled FGM concentrations of elephants in each of the three reserves. In the field, fecal samples were collected opportunistically by trained employees of the reserves or by the University of KwaZulu-Natal. On average, samples from Phinda, Pilanesberg, and iSimangaliso were collected within 30 min, 10 hrs, and 20 hrs respectively. Across all reserves, time between deposition and collection for all samples used was < 72 hrs, and similar to other FGM-based studies on elephant (Millspaugh et al. 2007, Burke et al. 2008, Ahlering et al. 2010). We recorded the approximate age of the sample as well as the location of collection, but were unable to consistently identify which individual or family group deposited the sample. Samples for laboratory analysis were collected by opening, and taking a portion from the center of the bolus (Millspaugh et al. 2007, Burke et al. 2008). After collection, samples were immediately treated with a 2% acetic acid solution and frozen for shipment (Millspaugh et al. 2003). In the laboratory, samples were stored at -80° C, freeze-dried, ground, and sifted through a stainless steel mesh. We extracted FGM from the feces using corticosterone I^{125} radioimmunoassay kits (MP Biomedicals, Costa Mesa, CA) following validated and established protocols (Wasser et al. 2000). Inter-assay variation for 11 assays was 7.3% and average intra-assay variation was 3.9%.

We conducted a nested analysis of variance (ANOVA) to determine if significant differences occurred in mean FGM concentrations of elephants among the three reserves, and if differences within reserves occurred between years. In addition, we evaluated if FGM concentrations followed a pattern of variation between seasons (wet and dry) similar to that seen in previous studies (Foley et al. 2001, Woolley et al. 2009). We partitioned data into annual wet and dry seasons based on rainfall patterns for our study areas, where the wet season occurred from November to April, and the dry season occurred from May to October (Burke 2005, Shannon et al. 2006).

Location Data

From 2004 to 2007, GPS collars were placed on a single adult female individual in each of 14 family groups (iSimangaliso n = 3, Phinda n = 5, Pilanesberg n = 6). Because adult female elephants live in cohesive family units, we assumed that GPS collars deployed on adult female elephants capture the movements of an entire family group (Douglas-Hamilton et al. 2005). All collars were programmed to record elephant locations at predetermined intervals (ranging from 30 min to 12 hrs depending on the individual elephant) and to transmit coordinates by Global System for Mobile Communications (GSM) cell phone signal or satellites to a ground station where they were stored on a master computer (Druce et al. 2008). We omitted locations in Pilanesberg from September 2005 to September 2006 due to a catastrophic fire that altered elephant space use patterns (Woolley et al. 2008). We also omitted locations in Phinda prior to September 2005 due to removal of a section of fence at that time that allowed for expansion of the reserve (Druce et al. 2008). While locational data were not validated, location error was relatively low (< 50 m) based on evaluations of similar GPS collars on elephants (Loarie et al. 2009, Vanak et al. 2010).

Analysis of Space Use Patterns

In analyzing elephant space use, we first wanted to identify distinct, biologically meaningful time intervals among which we could compare space use patterns by family groups over time. Elephant space use patterns consistently vary between two annual seasons based on rainfall (i.e. wet and dry seasons) (Loarie et al. 2009, Ngene et al. 2010). Given that elephants at the donor site (Kruger National Park) also exhibit distinctive wet and dry season movement patterns (DeKnegt et al. 2010), we predicted that translocated elephants at our study sites would similarly exhibit seasonal movement patterns (Shannon et al. 2006, Shannon et al. 2010).

We developed seasonal utilization distributions (UDs) (van Winkle 1975) to estimate space use for each season during which an elephant continuously wore a GPS collar. Between 2004 and 2007, within each season we captured $\geq$300 locations ($\bar{x}$ =303.51, SE =8.33, range = 90 - 370) of elephants separated by 12$\pm$2 hours in each of our three study sites. We represented space use by each elephant family group during each season by creating 95% fixed kernel UDs using the plug-in method of bandwidth selection (Gitzen et al. 2004). Because elephant space use is limited by hard boundaries (i.e. electric fences) at each reserve, we trimmed each UD by the reserve boundary and standardized the remaining UD value so that cell values in each UD summed to 1.0.

To evaluate the spatial refuge hypothesis, that elephant family groups with high FGM levels exhibit restricted space use, we compared the proportion of a reserve utilized by elephants among reserves. Because each reserve was completely fenced around the entire perimeter (except for portions of iSimangaliso bordered by lake or ocean) and fences created an edge effect influencing elephant movement (Vanak et al. 2010), home range size was likely influenced by reserve size (Shannon et al. 2006). Therefore, because reserves were different sizes (180 km^2 to 602 km^2), we evaluated elephant space use based on the percent of the reserve occupied by the UD contour in addition to home range size estimates. We evaluated the home range size and proportion of the reserve utilized by each family group during each season for normality and compared among reserves, family groups, years, and seasons using a nested factorial ANOVA. In the ANOVA, reserve, year and season were fixed effects, elephant family group was nested within reserve, and home range size or proportion of the reserve utilized was the dependent variable.

To evaluate the temporal refuge hypothesis, that elephants with elevated FGM levels exhibit different behavioral patterns in day vs. night, we compared day home range size to night home range size within each reserve. Given that tourist game drive traffic and, in the case of iSimangaliso, forestry operations, primarily occur during daylight, we hypothesized that there might be differences in day and night space use by elephants. We categorized locations into day or night separately for each season and computed UDs for each family group using procedures described above. We defined day as between 0800 and 1900, and night as between 2100 and 0600. We omitted locations between 1900 and 2100 and 0600 and 0800 due to seasonal variations in the time of sunrise and sunset, and because some guided tourism viewing occurs during those periods. We computed home range sizes for both day and night within each season for each elephant, and evaluated if there were significant differences in home range size between day and night within each reserve individually using a factorial nested ANOVA. In the ANOVA, elephant family group was a fixed effect, season was nested within elephant family group, time (in terms of UDs based on daytime or nighttime locations) was nested within season and elephant family group, and home range size was the dependent variable.

To determine the extent to which elephants used the same area by day as by night, we evaluated space use overlap by individual family groups between day and night within each season using a volume of intersection (VI) analysis (Seidel 1992, Millspaugh et al. 2004). The VI index measures overlap in space use between two UDs (as distinct from polygon overlap). Volume of intersection scores range from 0 – 1, where a VI score of 1 indicates perfect overlap of the UDs. Therefore, we interpreted higher VI scores as evidence of the repeated use of space between day and night. To account for potential day-night variation in highly utilized areas, we computed VI scores for both the home range scale of 95% fixed-kernel UDs, and core area scale of 50% fixed-kernel UDs (Barg et al. 2005). We log-transformed VI scores and used a nested ANOVA to test the null hypothesis that no difference occurred in VI scores among reserves (Millspaugh et al. 2000). In the ANOVA, reserve, year and season were fixed effects, elephant family group was nested within reserve, and the VI score for comparing day vs. night space use was the sampling unit.

Analysis of resource selection

We assessed resource selection by elephants in each reserve using a weighted compositional analysis (Millspaugh et al. 2006). We utilized the 95% fixed-kernel UD for each day and night period and summed UD values for each habitat type. We divided the summed UD values for each habitat type by total UD score to get weighted proportional use of each habitat type by an elephant. We substituted 0.5% for 0 for all non-used habitats (Bingham and Brennan 2004) and subtracted log-transformed use data from log-transformed availability data (at the reserve level) for each elephant at each sampling interval to calculate the difference in log-ratios (Millspaugh et al. 2006, Aebischer et al. 1993). We evaluated if overall selection occurred using Wilk's lambda statistic to test if the mean vector of log-ratio differences differed from a vector of zeros, and when selection occurred, we ranked habitats based on their relative utilization (Aebischer et al. 1993). We tested for effects of season (wet vs. dry) and time of day (day vs. night) on log-ratio difference values for each habitat type in each reserve using a nested multivariate analysis of variance (MANOVA) (Smith et al. 2004). In the MANOVA, elephant family group was the fixed effect, season was nested within elephant family group, time of day was nested within season and elephant family group, and the log-ratio differences were the sampling unit.

RESULTS

From 2000 to 2006, we collected and assayed 709 fecal samples from elephant populations in the three reserves included in this study (Phinda Private Game Reserve n = 195; iSimangaliso Wetland Park n = 366; Pilanesberg National Park n = 148). Fecal glucocorticoid metabolite concentrations were significantly higher for elephants in iSimangaliso than for elephants in the other two reserves ($F_{2, 708} = 80.17$, $P < 0.0001$) (Figure 1). Elephants in iSimangaliso consistently had FGM concentrations around 50 ng/g, indicative of a chronic stress response (Millspaugh et al. 2007, Wasser et al. 2000). In comparison, elephants in Phinda and Pilanesberg had relatively moderate FGM concentrations (25-35 ng/g), typical of baseline levels in elephants (Millspaugh et al. 2007, Wasser et al. 2000) (Figure 1). Across all reserves, FGM values were 20% higher in the dry season than in the wet season ($F_{1, 705} = 23.20$, $P < 0.0001$). We observed differences in FGM levels among years ($F_{5, 700} = 2.79$, $P = 0.0167$). However, annual differences primarily occurred in FGM concentrations of elephants in Phinda; FGM levels of elephants in iSimangaliso were consistently elevated across all years (Figure 1).

In support of the spatial refuge hypothesis, from 2004 to 2007 elephants in iSimangaliso maintained smaller home ranges and used a smaller proportion of the reserve compared to elephants in the other two populations. Despite iSimangaliso being slightly larger (602 km^2) than Pilanesberg (560 km^2) (Figure 2), elephant home range size was twice as large in Pilanesberg than in iSimangaliso ($F_{2, 52} = 48.45$, $P < 0.0001$). Within all reserves, home range size was consistent across years ($F_{4, 52} = 1.66$, $P = 0.1744$), but on average 65 km^2

larger during the wet as opposed to the dry season ($F_{1, 52} = 18.47$, $P < 0.0001$). When scaled in proportion to the total area available within the reserve, elephant home ranges in iSimangaliso occupied 20% less of the available area ($\bar{x} = 0.35$, SE = 0.04, range = 0.13-0.56), than in the similarly-sized Pilanesberg ($\bar{x} = 0.55$, SE = 0.03, range = 0.17-0.74), and 43% less than in the smaller Phinda (180 km^2) ($\bar{x} = 0.78$, SE = 0.02, range = 0.63-0.98) (Figure 2). Elephants utilized more of the available area during each season in the relatively small Phinda reserve than in the other two reserves ($F_{2, 52} = 49.29$, $P < 0.0001$) (Figure 3). Similar to home range size, scaled home ranges were consistent across years ($F_{4, 52} = 2.02$, $P = 0.1059$), but on average elephants utilized 9% more of the reserve during the wet as opposed to the dry season ($F_{1, 52} = 21.14$, $P < 0.0001$).

We found mixed support for our temporal refuge hypothesis. We found no difference between day and night home range sizes of elephants within any reserve (iSimangaliso, $F_{6, 16} = 0.20$, $P = 0.9706$; Pilanesberg, $F_{12, 48} = 0.27$, $P = 0.9921$; Phinda, $F_{10, 18} = 0.39$, $P = 0.9324$) (Figure 2). However, across reserves, we observed significantly less day vs. night space use overlap in iSimangaliso compared to Pilanesberg and Phinda (Figures 3 and 4), at both the home range ($F_{2, 47} = 7.52$, $P = 0.0015$) and core area ($F_{2, 46} = 8.26$, $P = 0.0009$) scales. In iSimangaliso, we observed 66.6% overlap in daytime and nighttime space use at the home range scale and 55% space use overlap at the core area scale (Figure 4). By contrast, we observed 7 – 10 % more overlap in daytime and nighttime space use in Pilanesberg and Phinda at the home range scale, and 8 – 10% more at the core area scale. The amount of day-night space use overlap did not differ by season (home range, $F_{1,47} = 0.42$, $P = 0.5225$; core area, $F_{1,46} = 0.04$, $P = 0.8346$) or year of investigation (home range, $F_{1,47} = 1.74$, $P = 0.1573$; core area, $F_{1,46} = 1.32$, $P = 0.2751$).

In terms of resource selection patterns, in iSimangaliso, with the exception of dry forest, elephants selected forest plantation over all other habitat types (Table 1). This pattern was consistent across seasons (Pillai's Trace = 1.3258, $F_{21,30} = 1.13$, $P = 0.3713$) and time of day (Pillai's Trace = 1.4295, $F_{42,78} = 0.58$, $P = 0.9718$), suggesting that elephants generally tended to select forest plantation in favor of most native habitat types regardless of time of day or their relative availability (Figure 5). In contrast, in Phinda and Pilanesberg where tree plantations were not present, elephants exhibited differing seasonal resource selection patterns that favored native forest habitats (Table S1). In Phinda, we observed seasonal differences in resource selection (Pillai's Trace = 2.0965, $F_{35,80} = 1.65$, $P = 0.0338$), where elephants selected sand forest and closed woodland over all other habitat types during in the dry season, and selected *Acacia* woodland in the wet season (Table 1). Similar to iSimangaliso, we did not observe differences in resource selection between day and night (Pillai's Trace=2.0032, $F_{70,126} = 0.72$, $P = 0.9328$), and resource use did not consistently correspond with the relative availability of habitats within the reserve (Figure 5). In Pilanesberg, resource selection differed between seasons (Pillai's Trace = 1.0712, $F_{36,276} = 1.67$, $P = 0.0128$), but was consistent between day and night (Pillai's Trace = 1.0270, $F_{72,276} = 0.79$, $P = 0.8812$), similar to iSimangaliso and Phinda. Elephants in Pilanesberg tended to select *Combretum*, *Faurea*, and

Acacia caffra woodland over other habitat types during both the wet and dry seasons, but varied in their selection of grassland and mixed *Acacia* woodland among seasons (Table 1). Furthermore, in contrast to iSimangaliso and Phinda, resource selection more closely followed the relative availability of habitats (Figure 5). Overall, despite the failure to observe temporal day vs. night differences in resource selection in iSimangaliso that would provide support for our temporal refuge hypothesis, the differences in resource selection patterns we observed among reserves provides further support for our spatial refuge hypothesis. In particular, selection of forest plantation and dry forest in favor of available habitat in iSimangaliso, regardless of season, suggests that restricted space use patterns are related to the avoidance of a particular area rather than to the availability of suitable habitat.

DISCUSSION

Our study suggests that chronic stress hormone concentrations are associated with restricted space use and altered habitat preferences that resemble a facultative refuge behavioral response. The elephant population in iSimangaliso displayed FGMs indicative of chronic stress and used a smaller portion of this reserve throughout the year. These results contrast with findings for other translocated populations with lower FGM concentrations, and other wild elephant populations (Shannon et al. 2006, Loarie et al. 2009, Ngene et al. 2010, Shannon et al. 2010). Restricted space use patterns indicative of refuge behavior have been documented for a variety of species, but few previous studies have linked the internal physiological status and selection of refugia (Elliot 2000, Kauffman et al.

2007). Our results suggest that if stressors are persistent and result in a chronic physiological state, populations will restrict space use and occupy refugia for an extended period of time.

Chronic stress response by elephants in iSimangaliso following translocation could be a consequence of delayed acclimatization. Previous studies on elephants have documented short-term elevations in FGM concentrations associated with poaching (Gobush et al. 2008), hunting (Burke et al. 2008), fire (Woolley et al. 2008), tourism (Pretorius 2004), and translocation (Millspaugh et al. 2007, Viljoen et al. 2008). Elephants selected for translocation to iSimangaliso exhibited baseline FGM concentrations prior to capture; however, FGM values did not return to baseline conditions within 30 days after the translocation event as found in previous studies of FGM responses to translocation of working elephants (Millspaugh et al. 2007) and elephants allowed to navigate to their original home range (Viljoen et al. 2008). One potential explanation is that it simply takes an extended period of time for wild elephants to acclimatize. For example, those elephant populations in our study with lower FGM concentrations were in reserves where initial translocations occurred 10 - 20 years prior to the initiation of the study, so it is possible that > 6 years is required for physiological acclimatization following translocation to a new environment.

The spatial refuge behavioral response of elephants that we observed in iSimangaliso is potentially a consequence of avoiding the area associated with translocation and release. Because previous behavioral research has shown that there are sometimes long-term sociological and behavioral effects of traumatic events on elephants (Bradshaw et al. 2005, Bradshaw and Shore 2007), a persistent stress response could be attributed to the experience of a population or family group with the process of capture and translocation. Elevated stress responses to translocation have been reported with subsequent dispersal away from the release site for multiple species (Dickens et al. 2010), including elephants (Viljoen et al. 2008). Similarly, upon translocation to iSimangaliso, elephants were released in the Eastern Shores section, but quickly dispersed to the Western Shores section. All three separately introduced elephant family groups have subsequently resided in the latter section for 6 years post-release. This suggests that, given elephants were released on the Eastern Shores section, they could be avoiding the location associated with a translocation, a highly stressful event (Dickens et al. 2010). This avoidance following dispersal might be compounded by the presence of Lake St. Lucia, which could act as a barrier to movement between the two sections. However, during our study elephant family groups easily traversed the lake, crossing it 20 times to visit the Eastern Shores section for short periods (typically 24 - 48 hours) before returning to the Western Shores section.

The timing and frequency of human disturbances within iSimangaliso also could influence the refuge behavior pattern we observed. Wild elephant populations, similar to most wildlife, avoid areas associated with persistent interactions with humans (Douglas-Hamilton et al. 2005). The Eastern Shores section of iSimangaliso is open to the public and receives a consistently high level of tourism visitation, a factor known to elicit a physiological stress response in elephants (Pretorius 2004). By contrast, the Western Shores section is closed to the public, yet contains forest plantations that occasionally have a high amount of human disturbance by plantation workers, but which is localized to a particular stand. It is likely that elephants make trade-offs between relative risks associated with human disturbances within their environment. That is, elephants in iSimangaliso might utilize forest plantations, which are only intermittently visited by humans, and which occur in a matrix of native forest habitat that provides opportunities for the animals to escape disturbance, in favor of the Eastern Shores section, which more consistently receives human disturbance.

In addition to human disturbance, restricted foraging by elephants in iSimangaliso in dry forests and tree plantations could have influenced chronic FGM levels. Elevated elephant FGM concentrations might be related to nutritional stress and overall diet quality, where FGM concentrations are inversely related to the amount of nitrogen present in their diet (Woolley et al. 2009). The restricted space use patterns we observed in iSimangaliso, where elephants selected for and likely primarily consumed browse in dry forest and non-native tree plantations, likely further limited access to high quality forage regardless of season, and might have contributed to elevated FGM levels in that reserve. Therefore, in addition to potential human disturbance stressors, the impact of nutritional stress on chronic FGM concentrations is an area in need of research in translocated elephant populations.

The differences we observed in elephant space use patterns among populations did not correspond with our current understanding of how reserve shape, competition, and resource selection could restrict elephant space use. Elephants within fenced reserves have been shown to avoid areas in proximity to boundary fences (Vanak et al. 2010), thus the shape of the reserves could influence elephant movement and space use. However, elephants in the most elongated and narrowest reserve (Phinda) utilized the highest portion (78%) of their reserve. By comparison, elephants in the round reserve (Pilanesberg), where we would expect less of an edge effect, utilized 55% of their reserve. African elephant family groups establish age- or size-related dominance hierarchies (Archie et al. 2006), which could result in competition between elephants that restricts space use patterns when populations are highly abundant and/or resources are limited (Wittemyer et al. 2007). While individual family groups could have exhibited greater competition and avoidance in iSimangaliso than the other two reserves, in general we would expect greater competition in reserves with higher elephant density (Wittemyer et al. 2007). In contrast, the reserve that exhibited the most restricted space use patterns contained the lowest elephant density (iSimangaliso, 0.04 elephants per km^2) compared to the other two reserves (Phinda, 0.51 elephants per km^2; Pilanesberg, 0.32 elephants per km^2). The distribution of dry native forest and tree plantations within iSimangaliso, which were primarily limited to the Western Shores where we observed restricted spaces use patterns, suggests that these habitats could be limiting and selected over grasslands habitats that dominated the Eastern Shores. However, elephants generally are considered habitat generalists (Laws 1970, Owen-Smith 1988). Further, given that grasslands likely contained higher quality forage most similar to the donor site (Kruger National Park; Smit et al. 2007), particularly during the wet season (Codron et al. 2006), we feel selection of tree plantations and dry forests of the Western Shores is more likely due to elephants avoiding open areas (i.e. grasslands) and sources of human disturbance than nutritional attributes that typically drive habitat-related patterns in elephant movement (Codron et al. 2006).

The differences we observed in space use overlap between day and night in iSimangaliso suggest that elephants might slowly be adapting temporal refuge behavior in response to human disturbance. Despite restricting their use of space to the Western Shores, elephants in iSimangaliso continue to exhibit a state of chronic physiological stress. If utilizing forest plantation is a persistent stressor to elephants in iSimangaliso, under the concept of allostasis an individual or population should continue to adapt and change its behavior to minimize the likelihood of encountering stressors (McEwen and Wingfield 2003). However, elephants tend to be slow in developing novel movement patterns in response to changes in their environment (Druce et al. 2008). Therefore, given variation in the timing and location of disturbances in forest plantations, developing reliable movement patterns is likely difficult and elephants might only slowly be adapting to stressors in the Western Shores. It also is possible that the elephants have finer-scale refuge behavior that we were unable to detect at the scale of our analysis, such as avoidance of roads during periods of peak use by plantation workers. Future research is needed to evaluate if elephants in iSimangaliso continue to maintain an elevated physiological state, and if they modify their fine-scale spatial and temporal behavior over time.

Chronic stress and elephant refuge behavior could have a number of potential short and long-term consequences to elephant health, human safety, tourism, ecosystem processes, and biodiversity. Two months following the initial release of elephants in iSimangaliso, mortality of an 8 month-old male calf occurred, likely as a result of stressors associated with translocation and long, continuous movement of the family group post-release. This incident suggests that chronic stress is likely to be a problem for young animals, and that providing refugia to limit continuous movements could reduce the risk of future mortalities. The history of human deaths caused by elephants in the reserves included in this study, while anecdotal, suggests that chronic stress and refuge behavior might be linked to incidences of elephant aggression toward humans. In iSimangaliso, despite closure of the Western Shores to the public, elephants in a single family group have killed two reserve workers. Also, in Phinda, within 3-5 years after introduction, a female elephant killed a human. In Pilanesberg, by contrast, although there have been a number of elephant attacks on humans and one person has been killed, all attacks were by male elephants of which most if not all were in musth. Given that it is comparatively less common for female elephants to be aggressive (Twine and Magome 2008), and stress associated with socially disruptive events like translocation have previously been associated with incidences of elephant aggression (Bradshaw and Shore 2007, Twine and Magome 2008), our findings collectively suggest that chronic stress and refuge behavior following translocation are at least loosely linked to elephant aggression toward humans. Refuge behavior by elephants also limits their tourism value.

Elephants frequently are seen by tourists in Pilanesberg and Phinda, but rarely are seen in iSimangaliso, where the opportunity for viewing elephants was one of the primary reasons for their reintroduction. Finally, the repeated use of refugia by elephants over an extended period of time could lead to extensive habitat modification (Skarpe et al. 2004) and potentially to loss of biodiversity (Kerley et al. 2008). In the case of iSimangaliso, refuge behavior also could exacerbate the damage to commercially valuable trees in forest plantations.

Accounting for refuge behavior has important implications to our understanding of elephant space use. Seasonal variation in the spatial distribution of resources, primarily forage and water availability (Loarie et al. 2009), as well as social interactions (Mutinda et al. 2010) and the shape of fenced reserves (Vanak et al. 2010), are known to be key drivers of elephant space use. In addition to these factors, physiological state could influence space use and resource selection patterns. For example, in Pilanesberg and Phinda, elephants generally used resources in proportion to their availability (Figure 5). In contrast, elephants in iSimangaliso exhibited restricted spaces use patterns and selected forest plantations on the Western Shores in favor of native habitats. This does not rule out the possibility that elephants in Pilanesberg and Phinda exhibited refuge behavior over shorter periods of time or that they identified areas as refugia. It is likely that elephants in Phinda and Pilanesberg identified refugia that allowed them to recover following exposure to a stressor. For example, Woolley et al. (2008) documented that, following a catastrophic fire event in Pilanesberg, elephants exhibited a short-term elevation in stress hormone levels and moved to the northern portion of the reserve, which is designated as a “wilderness zone” closed to tourists. Thus, the availability of refugia, when needed, is likely critical to successfully avoiding development of a chronic physiological state.

The identification of refugia is particularly important in South Africa, where elephants are increasingly restricted to fenced reserves (Hayward and Kerley 2009). The use of fences in South Africa generally has been effective at limiting elephant movements and potential human-elephant conflict (Grant et al. 2008, Slotow 2012). However, our findings suggest that issues of human-elephant conflict and refuge behavior within fenced reserves need to be addressed. One potential solution to this problem is to identify areas that can serve as refugia for elephants (such as wilderness zones as in Pilanesberg) and limit human disturbance in those areas. Alternatively, managers might identify specific areas and periods when refugia are needed, similar to the current concept of virtual fences used to mitigate human-elephant conflict (Slotow 2012). For example, in iSimangaliso, where an individual female in each family group is monitored with a Global Positioning System collar linked to a cellular phone network, managers are using real-time elephant movement data in combination with computer technology based on geospatial maps, to send a notification message to one or more cell phones any time a collared elephant moves into a pre-determined zone, such as across a reserve border (Slotow 2012). Similarly, if reserve managers are able to identify refugia spatially or predict via movement patterns when elephants are exhibiting refuge behavior, they could limit human disturbance to that area for a period of time, and potentially provide corridors into or among refugia to mitigate the risk of chronic stress and potentially dangerous human-elephant interactions.

In summary, managers considering the translocation or reintroduction of wildlife should consider the possibility of chronic stress and potential consequences of refuge behavior. Chronic stress is common following wildlife translocation, and has been associated with reproductive failure, increased predation risk, disease risk, and movement away from the release site (Teixeira et al. 2007, Dickens et al. 2010). Our results suggest that chronic stress is associated with refuge behavior in translocated elephants, and we predict that it is likely to occur as a common facultative response in other species following translocation. Thus, future efforts to predict when, where, and to what extent wildlife populations will exhibit refuge behavior could likely be improved by an understanding of their physiological response.

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Table 1. Matrices and habitat rankings of African elephant seasonal resource selection in iSimangaliso Wetland Park (A), Phinda Private Game Reserve (B), and Pilanesberg National Park (C), South Africa. The + or – sign values within habitat comparisons indicate direction of selection based on positive or negative *t*-values; and +++ or – – – indicate both the direction of selection and if significant differences occurred at *P*<0.05. A rank of 1 indicates the highest level of selection.

(A)

iSimangaliso: dry season

	Plantation	Lowland forest	Dry forest	Grassland	Marsh and Swamp	Open beach	Freshwater lake	Human settlement	**Rank**
Plantation	.	+++	+	+++	+++	+++	+++	+++	1
Lowland forest	– – –	.	+	+	+++	+++	+++	+++	2
Dry forest	–	–	.	+	+++	+++	+++	+++	3
Grassland	– – –	–	–	.	+++	+++	+++	+++	4
Marsh and Swamp	– – –	– – –	– – –	– – –	.	+	+	+++	5
Open beach	– – –	– – –	– – –	– – –	–	.	+	+	6
Freshwater lake	– – –	– – –	– – –	– – –	–	–	.	+	7
Human settlement	– – –	– – –	– – –	– – –	– – –	–	–	.	8

iSimangaliso: wet season

Plantation	.	+++	+	+++	+++	+++	+++	+++	1
Dry forest	–	+++	.	+++	+++	+++	+++	+++	2
Lowland forest	– – –	.	– – –	+	+	+++	+	+++	3
Grassland	– – –	–	– – –	.	+	+++	+++	+++	4
Marsh and Swamp	– – –	–	– – –	–	.	+++	+	+++	5
Freshwater lake	– – –	–	– – –	– – –	–	+++	.	+++	6
Open beach	– – –	– – –	– – –	– – –	– – –	.	– – –	+++	7
Human settlement	– – –	– – –	– – –	– – –	– – –	– – –	– – –	.	8

(B)

Phinda: dry season

	Sand forest	Closed woodland	Palmveld	Riverine and wetland	Human habitation	Open grassland	*Acacia* woodland	*Lebombo* thicket	**Rank**
Sand forest	.	+++	+++	+++	+++	+++	+++	+++	1
Closed woodland	– – –	.	+++	+++	+++	+++	+++	+++	2
Palmveld	– – –	– – –	.	+	+	+	+	+++	3
Riverine and wetland	– – –	– – –	–	.	+	+	+++	+++	4
Human habitation	– – –	– – –	–	–	.	+	+++	+++	5
Open grassland	– – –	– – –	–	–	–	.	+	+++	6
Acacia woodland	– – –	– – –	–	– – –	– – –	–	.	+++	7
Lebombo thicket	– – –	– – –	– – –	– – –	– – –	– – –	– – –	.	8

Phinda: wet season

Acacia woodland	+	+++	+	+	+++	+++	.	+++	1
Closed woodland	+	.	+++	– – –	–	+++	– – –	+	2
Riverine and	+	+++	+++	.	+	+	–	+++	3

wetland									
Sand forest	.	–	+++	–	–	+	–	+	4
Lebombo thicket	–	–	+	– – –	– – –	+	– – –	.	5
Palmveld	– – –	– – –	.	– – –	– – –	–	– – –	–	6
Open grassland	–	---	+	– – –	– – –	.	– – –	–	7
Human habitation	+	+	+++	–	.	+++	–	+++	8

(C)

Pilanesberg: dry season	*Combretum* woodland	*Faurea* woodland	*Acacia caffra* woodland	Mixed *Acacia* woodland	*Acacia karoo* woodland	Grassland	*Acacia melifera* woodland	**Rank**
Combretum woodland	.	+	+	+++	+++	+++	+++	1
Faurea woodland	–	.	+	+	+	+++	+++	2
Acacia caffra woodland	–	–	.	+	+++	+++	+++	3
Mixed *Acacia* woodland	– – –	–	–	.	+	+++	+++	4
Acacia karoo woodland	– – –	–	– – –	–	.	+++	+++	5
Grassland	– – –	– – –	– – –	– – –	– – –	.	+++	6
Acacia melifera woodland	– – –	– – –	– – –	– – –	– – –	– – –	.	7
Pilanesberg: wet season								
Combretum woodland	.	+	+++	+++	+++	+	+++	1
Faurea woodland	–	.	+	+++	+++	+	+++	2

Acacia caffra woodland	– – –	–	.	+++	+++	+	+++	3
Grassland	–	–	–	+	+	.	+++	4
Acacia karoo woodland	– – –	– – –	– – –	+	.	–	+	5
Mixed *Acacia* woodland	– – –	– – –	– – –	.	–	–	+	6
Acacia melifera woodland	– – –	– – –	– – –	–	–	– – –	.	7

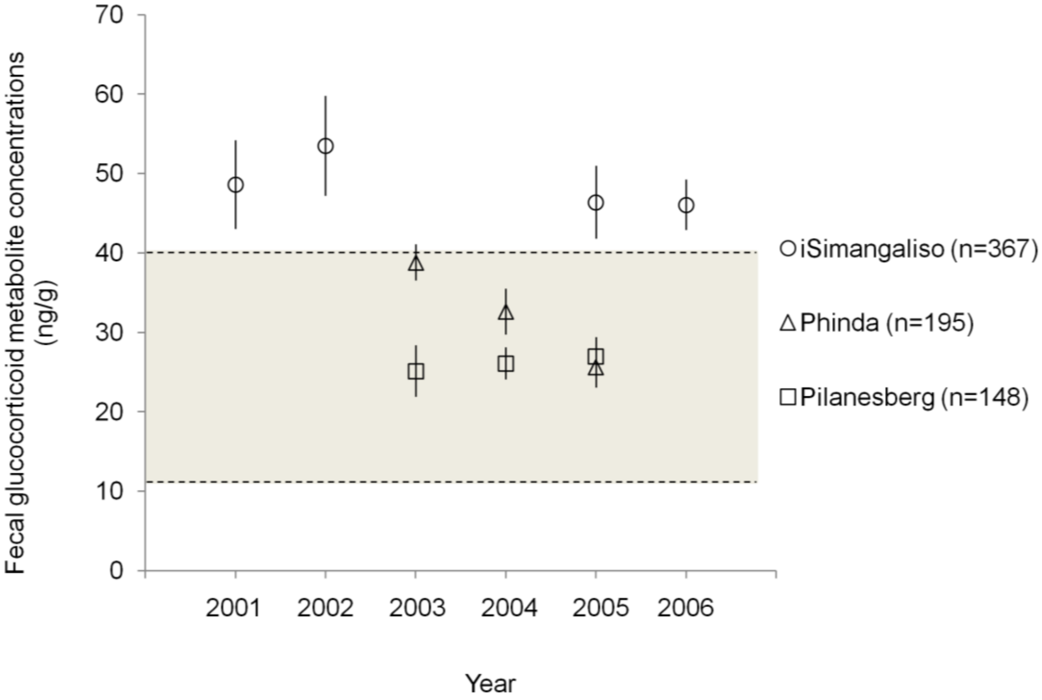

Figure 1. Fecal glucocorticoid metabolite values of elephants in each reserve. Average (with 95% confidence intervals) fecal glucocorticoid metabolite (FGM) concentrations (in dry weight ng/g) for each year samples were collected. Basal FGM concentrations for elephants (15-40 ng/g) are shaded grey.

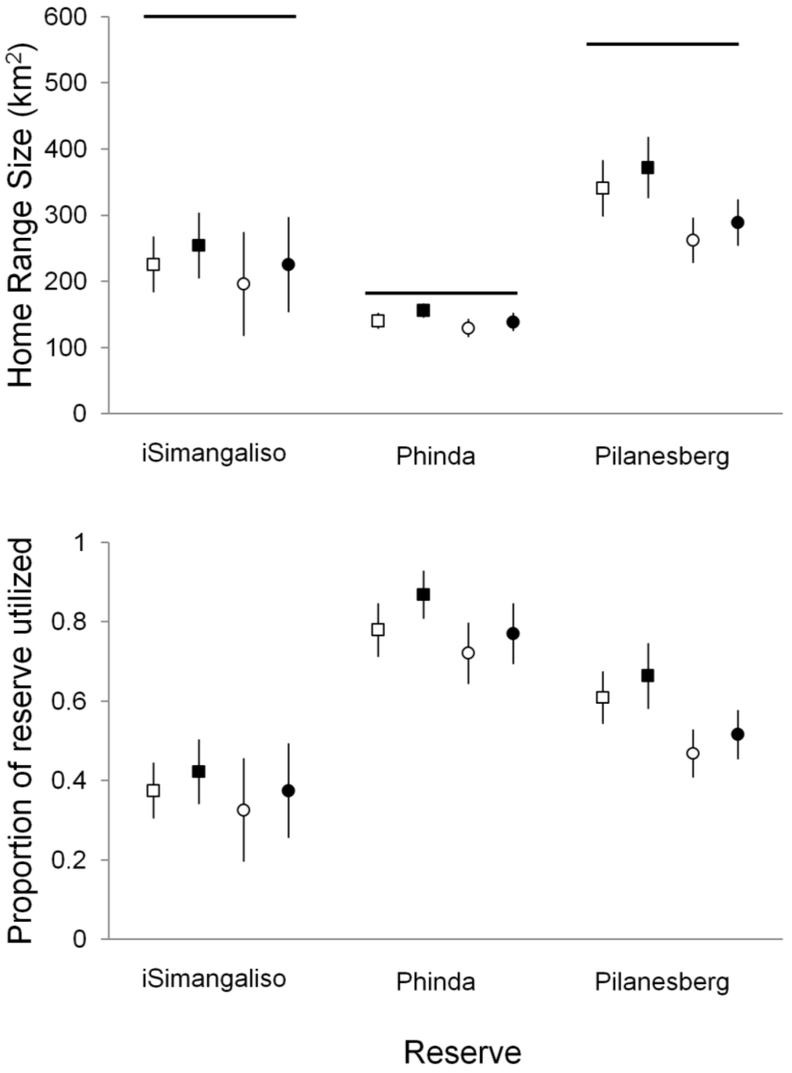

Figure 2. Home range size of elephants in each reserve. Average (with 95% confidence interval) home range size (km^2) during the wet (squares) and dry (circles) seasons (top graph). Horizontal lines indicate the size of each reserve. The bottom graph depicts the average (with 95% confidence interval) proportion of each reserve occupied by elephant home ranges. Solid symbols represent mean average home range sized based on utilization distributions (UDs) calculated from

nighttime locations and hollow symbols represent UDs calculated from daytime locations.

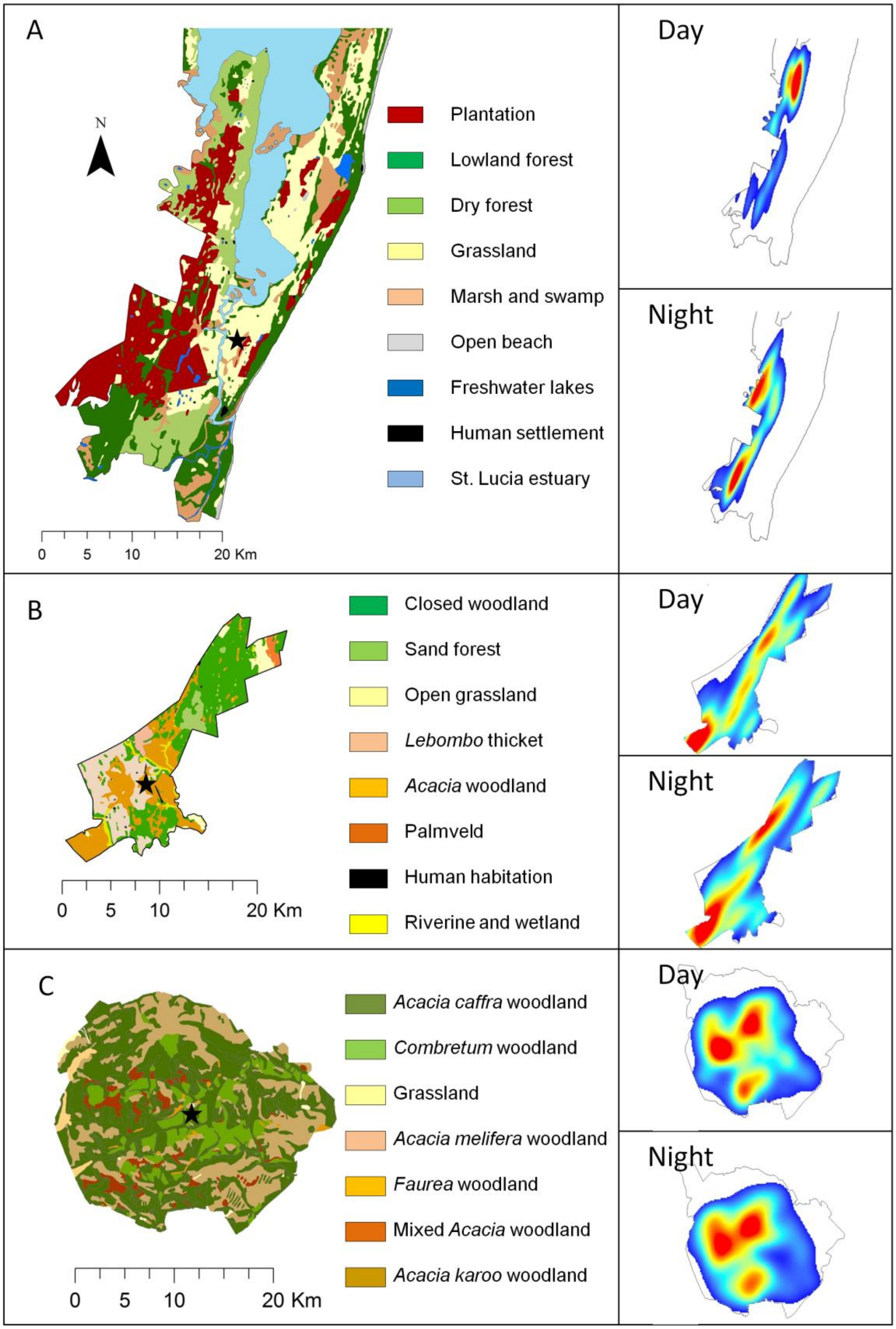
A
N
Plantation
Lowland forest
Dry forest
Grassland
Marsh and swamp
Open beach
Freshwater lakes
Human settlement
St. Lucia estuary
0 5 10 20 Km
Day
Night
B
Closed woodland
Sand forest
Open grassland
Lebombo thicket
Acacia woodland
Palmveld
Human habitation
Riverine and wetland
0 5 10 20 Km
Day
Night
C
Acacia caffra woodland
Combretum woodland
Grassland
Acacia melifera woodland
Faurea woodland
Mixed *Acacia* woodland
Acacia karoo woodland
0 5 10 20 Km
Day
Night

Figure 3. Space use patterns by elephants in each reserve. The distribution of habitat types within iSimangaliso Wetland Park (A), Phinda Private Game Reserve (B), and Pilanesberg National Park (C). The star within each reserve represents the location of the boma (or preconditioning enclosure) that was also the initial release site of elephants. Inset on the right are 95%fixed kernel seasonal utilization distributions (UDs) for a select adult female elephant in each of our study areas based on daytime (top) and nighttime (bottom) locations. Areas in red within the UD represent areas of high intensity use, which fade to blue in areas of low use, and reserve boundaries are demarcated by solid lines. Space use was restricted and differed between day and night at iSimangaliso Wetland Park (A), compared to Phinda Private Game Reserve (B) and Pilanesberg National Park (C).

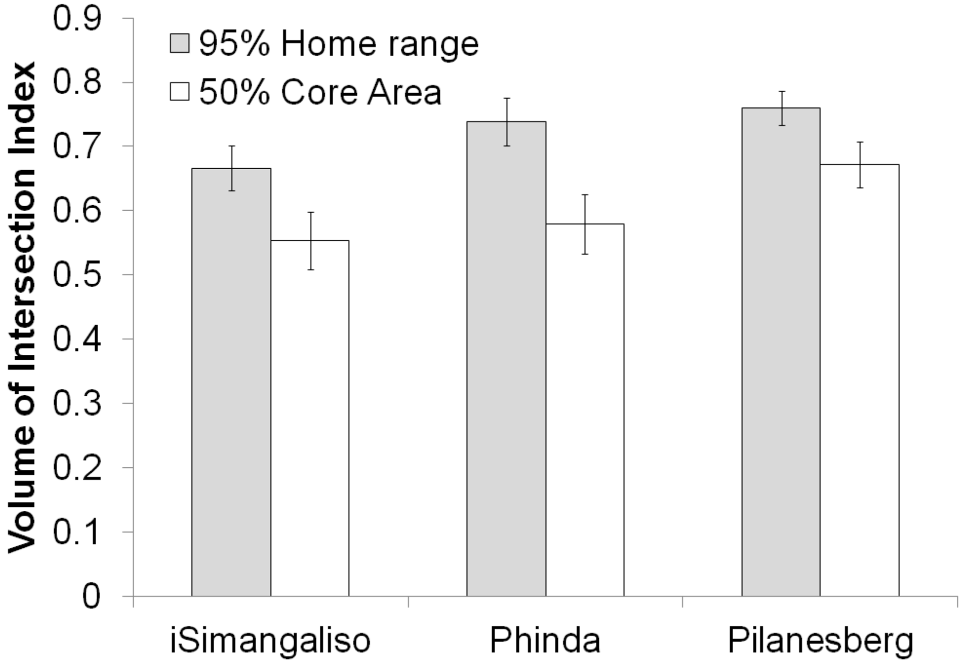

Figure 4. Day vs. night space use overlap by elephants in each reserve. Mean (with 95% confidence interval) volume of intersection index scores for elephant based on comparisons between day and night home range (grey) and core area (white) space use patterns. Volume of intersection index statistic measures the amount of overlap between two utilization distributions. Index values range from 0 to 1, where higher scores indicate a higher degree of overlap.

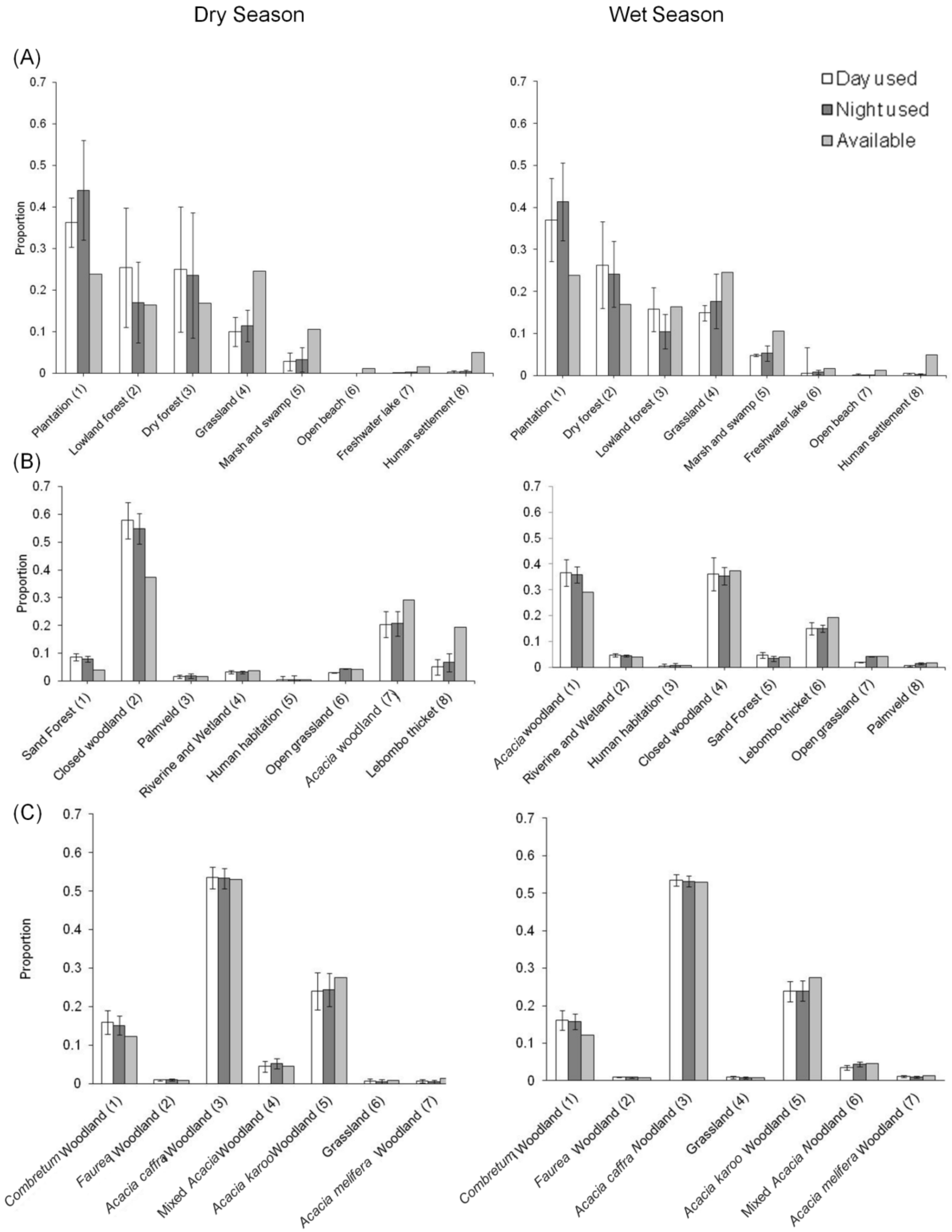

Figure 5. Compositional analysis of habitat use by elephants between day and night among reserves. Mean (with 95% confidence intervals) weighted day and night time use (calculated by summing UD fixed kernel scores by habitat type),

compared to availability of habitat types at the reserve level. Habitat names are followed by their compositional analysis rank (Aebischer et al. 1993). Graphs are separated by dry (left column) and wet (right column) season as well as by reserve in rows: iSimangaliso Wetland Park (A), Phinda Private Game Reserve (B), and Pilanesberg National Park (C).

CHAPTER 5

INFLUENCE OF INTERNAL STATE, ENVIRONMENTAL CONDITIONS, AND MEMORY ON MOVEMENT BY AFRICAN ELEPHANTS

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ABSTRACT

Despite the identification of internal state as a fundamental component of animal movement, the effect of an individual's internal physiological state on movement remains poorly understood. African elephants (*Loxodonta africana*) alter their behavior in response to their physiological state, and elevated stress hormone concentrations have been associated with reclusive behavior and aggression towards humans. Thus a better understanding of the link between elephant internal state and movement ecology is important to the ecology and management of elephants. We compared movement paths of African elephants in two physiological states (basal and elevated stress hormone levels) to understand variation in the use of space in relation to the proximity of environmental features and refugia. We documented differences in elephant use of space along movement paths by physiological state. Elephant family groups in a basal physiological state tended to venture away from refugia and commercial tree plantations, and use areas in closer proximity to fresh water. In contrast, elephant family groups in an elevated physiological state tended to use areas near refugia and commercial tree plantations. The use of commercial tree plantations during elevated states highlights an important concern for human safety as a result of human-elephant conflict. Our findings show that changes in elephant physiological state affect animal movement and the use of specific environmental features. Therefore, incorporating elephant physiological state into models built to describe animal movement could enhance the predictive ability of these models. Given that elephants are more prone to habitat disturbance and aggression when in an elevated physiological state, information about their movement tendencies could be used in combination with real-time tracking data to predict when and where elephants are potentially in elevated physiological states, and limit human access to these areas so as to mitigate human-elephant conflict.

INTRODUCTION

Responses of wildlife to environmental conditions typically are measured through physical movement (Schick et al. 2008). Thus an understanding of animal movement is fundamental to the ecology of a species. In addition to improving our understanding of ecology, monitoring and assessment of animal movements can provide insight into how species respond to environmental changes (including weather, land use, and anthropogenic disturbance) (Loarie et al. 2009, Sawyer et al. 2009). Movement ecology can also support the conservation of rare and declining species (Woodroffe and Ginsberg 1998, Biggins et al. 1999), and the mitigation of human-wildlife conflict (Sitati et al. 2003, Roever et al. 2010). However, correctly interpreting movement behavior is challenging given the complex interaction of external factors with the internal state, and locomotory and navigation capacities of the individual animal (Nathan et al. 2008).

Despite the identification of internal state as a basic component in the investigation of animal movement (Nathan et al. 2008), direct studies of an individual's internal state on movement behavior have been lacking. Technological and analytical advances in movement ecology have resulted in a variety of movement models inferring behavioral responses to internal state based on the location of successive data points (Morales et al. 2004, Schick et al. 2008). However, such inferential models are indirect, and the causal mechanisms, such as navigational capacity and internal state, remain relatively unknown in movement ecology (Holyoak et al. 2008, Getz & Saltz 2008). To better reflect the influence of internal state on animal movement, models could be improved by including information on physiological state (Patterson et al. 2008). For instance, it is widely appreciated that the release of stress hormones enables animals to respond to stressors (McEwen and Wingfield 2003, Romero and Butler 2007). Such responses include long-distance dispersal and restricted movements indicative of refuge behavior (Wingfield and Romenofsky 1997). However, existing efforts to link stress hormone production with animal movement have focused on laboratory settings (Wingfield and Romenofsky 1997, Breuner et al. 1998) or large-scale patterns in avian migration (Wingfield 2003) and animal space use (Breuner and Hahn 2003, Addis et al. 2011, Jachowski et al. 2012). We are aware of no examination of the role that stress hormones play in the fine-scale movement behavior of wild animals.

Existing research identifies that physiological state likely plays a key role in the movement behavior of African elephants (*Loxodonta africana*). Elevated stress hormone responses in free-ranging elephants have been associated with large-scale, unidirectional movement (Viljoen et al. 2008). Elephants in enclosed reserves respond to elevations in stress hormones by restricting their movement and seeking out sources of refuge away from disturbance events (Woolley et al. 2008, Jachowski et al. 2012). These circumstances indicate that knowledge of the responses of elephants to their physiological state can likely contribute to understanding their movement ecology at finer spatial and temporal scales.

A better understanding of the link between elephant internal state and movement ecology is important to the management of elephants and mitigation of human-elephant conflict. Many wild elephants increasingly occur in fragmented, human-altered landscapes, and are forced to utilize corridors between protected areas (Douglas-Hamilton et al. 2005, Epps et al. 2011). Given that interactions with humans (Pretorius 2004, Burke 2005, Gobush et al. 2008) and human-altered habitats (Ahlering et al. 2010) are known to elicit an elevated physiological stress response in elephants, if elevated physiological states result in restricted movement patterns in human-altered landscapes, then increases in human disturbance could further restrict habitat use and connectivity of elephant populations. Furthermore, elevated physiological states in elephants have been linked to aggressive behavior towards humans (Jachowski et al. 2012), posing a major human safety concern. Routine use of physiological measurement as a management tool is not always practical because the process of collecting and analyzing fecal samples for stress hormone concentrations is time and labor intensive. If patterns in elephant movement reflective of physiological states can be identified, real-time tracking data could be used to predict potential areas or periods of human-elephant conflict similar to computer programs used to monitor virtual fences (Slotow 2012), and allow managers to alter human access to those areas until elephants return to basal physiological states.

In this study we assessed the fine-scale movement decisions of elephants in two physiological states (basal and elevated stress hormone levels). We specifically compared the influence of an elephant family groups' proximity to environmental factors and refugia. Our state-dependent modeling approach was designed to reveal the influence of internal physiological state on fine-scale movement ecology. If physiological state plays a key role in movement behavior, future movement models could be improved and better indicate ways of prioritizing management actions and mitigating human-wildlife conflicts that result from stress responses in animals.

STUDY AREA

We evaluated movement of elephants in a reintroduced population in iSimangaliso Wetland Park (28°49’-27°55’S, 32°68’-32°22’E), (Fig. 1). iSimangaliso is located on the eastern coast of KwaZulu-Natal Province in South Africa. It is 602 km^2 in size and is composed of the Eastern Shores section (273 km^2) bordered by fencing to the north and south, the Indian Ocean to the east and the estuary of Lake St. Lucia to the west, and the Western Shores section (329 km^2) bordered by Lake St. Lucia to the east and electrified fence along its other boundaries. Reintroduction of elephants to iSimangaliso was initiated in 2001 with the translocation of 24 elephants (15 females and 9 males) from Hluhluwe-Umfolozi Park (originally from Kruger National Park), and in 2002 and 2003 with two additional family groups directly from Kruger National Park. We previously determined that these elephants persisted in a chronic physiological state and exhibited refuge behavior, where they occupied only the Western Shores section of the reserve (Jachowski et al. 2012). This area is closed to tourists and composed of commercial tree plantations (either *Eucalyptus globulus* or *Casuarina equisetifolia*) intermixed with native dry forest, lowland forest, grassland, marsh and swamp habitat types. Between 2005 and 2006 we monitored three elephant family groups using Global Positioning System (GPS) telemetry. At least one adult female elephant per group was fitted with a GPS collar (African Wildlife Tracking, Pretoria). Collars were programmed to record fixes at 30-min intervals and transmitted coordinates by Global System for Mobile Communications (GSM) cell phone signal or satellites to a ground station where they were stored on a master computer.

METHODS

Elephant physiology

To document elephant physiological state, we measured fecal glucocorticoid metabolite (FGM) hormones. FGMs provide a non-invasive measure of the internal physiological state of animals, and have received wide-scale use in a variety of vertebrate taxa (Millspaugh and Washburn 2004), including African elephants (Burke et al. 2008, Gobush et al. 2008, Woolley et al. 2009, Viljoen et al. 2008). On a daily basis, we tracked the GPS collars to locate elephant groups and searched the immediate vicinity for fecal samples. For each fecal sample we recorded the approximate age (based on desiccation (Burke et al. 2008)), the location of collection, and the identity of the individual or family group that deposited the sample based on visual observation or real-time tracking of family groups. We only retained samples for analysis that were < 72 hrs old. Samples for laboratory analysis were collected by opening the bolus and taking a portion from its center (Millspaugh et al. 2007, Burke et al. 2008). After collection, samples were treated with a 2% acetic acid solution and frozen for shipment (Millspaugh et al. 2003). In the laboratory, samples were stored at -80° C, freeze-dried, ground, and sifted through a stainless steel mesh. We extracted FGMs from feces using corticosterone I^{125} radioimmunoassay kits (MP Biomedicals, Costa Mesa, CA) following validated and established protocols (see

Wasser et al. 2000). To obtain replicate samples within a family group, we attempted to collect fecal samples from as many individuals in the family group as possible. We differentiated the animals from which these samples came based on bolus circumference (Burke et al. 2008). Two family groups exhibited correlated movements from December 2005-January 2006. As we were not able to differentiate between their fecal samples, we treated this period as a fourth temporary "grouping" of elephant families.

Unlike blood-based glucocorticoid assays, fecal glucocorticoid assays (due to metabolic differences and gut passage time) reflect the physiological state of an individual prior to sample collection and over a longer period of time (Wasser et al. 2000, Millspaugh et al. 2002). As a consequence, there is a need to consider when, and for how long, observed FGM concentrations correspond with the physiological state of an individual. The lag time between exposure to a stressor(s) and the occurrence of elevated FGMs in fecal samples varies among species (Wasser et al. 2000), and even among individuals within a species, depending on suite of factors including the temporal effect of time of day (Sherriff et al. 2009) or season (Millspaugh et al. 2002), as well as individual-specific factors such as diet (Wasser et al. 1993), metabolic rate and gut passage time (Millspaugh et al. 2002). Therefore, we evaluated two different techniques for determining time periods when FGM samples might accurately estimate the physiological status of an elephant (and subsequent movement path analyses) or what we hereafter refer to as windows of inference; 1) Fixed windows of inference were conservatively based on previous laboratory experiments and 2)

Flexible windows of inference were based on longer-term observed patterns in FGM concentrations during our study.

Fixed windows of inference

The fixed window of inference was based on laboratory studies, where individuals are experimentally injected with adrenocorticotropic hormone (ACTH) and fecal samples are collected to assess the lag time between injection and representation of the injection in the animal's feces (Wasser et al. 2000). FGM concentrations remain elevated in African elephants for a period of 24-96 hrs following ACTH injection (Wasser et al. 2000, Ganswindt et al. 2003). For our fixed window of inference we conservatively estimated that FGM samples represented the physiological state of an individual for a 72-hr period prior to defecation. Thus, we back-calculated the deposition time based on the time of collection and age of sample (based on visually inspecting its moisture content and texture (see Burke 2005)). When the 72-hr fixed window of inference overlapped samples from the same family group, we averaged FGM values and extended windows of inference to combine overlapping samples. Using these criteria, we identified 30 fixed, 72-hr windows of inference to assess movement characteristics of elephant family groups. We categorized each fixed window of inference as related to either a basal or elevated physiological state according to whether the averaged FGM values were below or above the overall mean FGM value for that family group over the duration of the study.

Flexible windows of inference

In the wild, FGM concentrations of animals typically represent a cumulative physiological state over an extended period of time (Millspaugh and Washburn 2004). Therefore, our flexible window of inference was based on longer-term trends in FGMs based on the consecutive samples taken from the same family group. We first backdated fecal samples based on bolus age and 24-hr gut passage time (Wasser et al. 2000, Ganswindt et al. 2003). We then averaged FGMs across individuals of the same family group when samples were representative of the same day based on back-dating of fecal samples (see above). We plotted values over time for each family group, and beginning with the first average FGM value, sequentially identified periods when consecutive average FGMs of a family group were either above or below the overall mean FGM value for that family group over the duration of the study (Fig. 2). All flexible windows of inference relied on two or more consecutive samples containing FGMs in the same physiological state for that family group. We ended flexible windows of inference when either of two conditions was met: >1 week (504 hrs) passed between consecutive samples, or FGM concentrations of consecutive samples differed above or below the mean compared to the previous sample (Fig. 2). Using these criteria, we identified seven flexible windows of inference to assess movements of elephant family groups, where we categorized the physiological state of family groups as either elevated (n = 5) or basal (n = 2) for periods of time that ranged in duration from 17.8 to 313.0 hrs ($\bar{x}$ = 116.6, SE = 44.1).

Movement Analysis

We used a Brownian bridge movement model (BBMM; Horne et al. 2007) to estimate the probability of habitat use along a movement path associated with our two windows of inference. A BBMM produces a utilization distribution (UD) based on the probability of an individual or family group being at a location along the movement path conditioned on the distance and elapsed time between successive locations, as well as on the Brownian motion variance that is a function of individual mobility (Horne et al. 2007, Sawyer et al. 2009). We selected BBMM over other movement models because of our relatively narrow windows of inference (72 hr – 2 week), and the ability of BBMM to account for time spent in an area as well as rate of movement (Sawyer et al. 2009). We calculated BBMM for each window of inference using the "BBMM" package (Nielson et al. 2011) in Program R version 2.11.1 (R Development Core Team 2010). We collected locational fixes at 30-min intervals with a success rate of > 99%. While locational data were not validated, based on previous studies utilizing GPS collars on elephants, mean locational error was < 50 m (Loarie et al. 2009, Vanak et al. 2010), and more likely < 25 m based on direct evaluations of similar GPS collars (Di Orio et al. 2003). Therefore, we selected 25 m as the locational error in our model. We used a resolution of 30 m for each of our UDs and trimmed the output so that each UD was represented by 99% of its volume (Fig. 3). Prior to analysis, we re-standardized the value of each UD so that each BBMM summed to 1 and then rescaled the UD to convert the probability into 99 UD percentiles on a scale of 1 to 99 based on equal interval distribution. The 1%

UD percentile corresponds to the highest probability of use while the 99th percentile corresponds to the lowest probability of use along a movement path.

Movement covariates

We developed *a priori* ten models to evaluate elephant movement behavior (Table 1). We hypothesized that the various covariates that could influence elephant movement would fit into two broad categories: environmental factors and sources of refugia (based on probability of prior habitat use).

Environmental factors

Elephants are habitat generalists (Laws 1970; Owen-Smith 1988), but movement is influenced by the availability and distribution of water sources, particularly during the dry season (Wittemyer et al. 2007, Loarie et al. 2009). To account for the potential negative effect of increasing distance from water on elephant movement, we calculated the Euclidean distance (in m) from elephant movement paths to fixed water sources. In iSimangaliso, these fixed water sources included freshwater lakes and pans.

Within fenced reserves, elephants also alter movement pathways in response to the location of boundary fences (Druce et al. 2008, Vanak et al. 2010). Therefore, we accounted for the negative influence of proximity to reserve boundaries by calculating the Euclidean distance (in m) from elephant movement paths to fixed fence or ocean boundaries (the eastern side of iSimangaliso is bounded by the Indian Ocean).

Human disturbance can have a major influence on elephant movement, disrupting movement behavior in response to natural landscape features (i.e. water and forage availability) (Boettiger et al. 2011). In iSimangaliso, elephants almost exclusively occupied the Western Shores portion of the reserve, which was closed to tourist activity but was composed primarily of commercial tree plantations (Jachowski et al. 2012). Because these tree plantations were more likely than native forest to be frequented by workers, we hypothesized that elephants in an elevated physiological state would exhibit movement patterns that avoided forest plantations, so as to limit potential interaction with humans. We measured the Euclidean distance (in m) from elephant locations to the nearest forest plantation, where plantation edge values correspond to 0 m, distance values increase positively farther from the plantation edge, and distance values increase negatively the farther the elephant location is inside of the plantation edge within a given patch.

Sources of refugia

The advanced cognitive abilities of elephants includes a highly developed navigational capacity (Foley 2002, Leggett et al. 2006, Hart et al. 2007), that is likely influenced by the physiological state of an individual. Woolley et al. (2008) observed that elephant FGMs were elevated following a catastrophic fire in Pilanesberg National Park, and that elephants subsequently moved toward wilderness areas away from disturbance by tourists. Previously, we found that elephants in iSimangaliso generally were in a chronic physiological state and that they exhibited long-term patterns of refuge behavior, where they repeatedly used discrete areas on the Western Shores over a 6-yr period (Jachowski et al. 2012). Therefore, we hypothesized that elephants in an elevated physiological state would be more likely to utilize areas they frequented in the past that served as refugia. We quantified the probability of using refugia by calculating seasonal (wet or dry of year in question) UDs for each family group based on GPS collar locations following methodology outlined in Jachowski et al. (2012). Similar to our treatment of BBMM UDs above, we rescaled refugia UD values into 100 categories from 1 to 100 based on equal interval distribution of original UD values, where a 1% UD percentile indicates the highest probability of refugia space use. To obtain covariate values, we extracted cell values from the refugia UD corresponding to the family group and season for each movement path location of interest.

Model evaluation

We fitted models individually to each window of inference (n = 37), and summarized results into fixed and flexible categories. We fit these models separately for elephants in basal and elevated physiological state. We used spatial mixed linear regression because of the autocorrelation in elephant movement behavior and repeated measures of locations within a day. We fitted models as:

$$Y_i = X\beta_i + Zu_i + e$$

where Y_i is the response variable (i.e. UD percentile along a Brownian-bridge movement path) at the i^{th} elephant family group GPS collar location, $X\beta_i$ represents the value of predictor covariates at the i^{th} elephant family group GPS collar location, Zu_i is the random effects term to account for correlated movements among family group on a given day, and e is the error term that was spatial autocorrelated based on distances between elephant family GPS collar locations during each window of inference. We standardized all continuous variables prior to analysis and tested our response variable for normality. We fitted models in SAS PROC MIXED (version 9.2, Cary, NC) using maximum likelihood estimation and a spherical covariance structure (Montgomery et al. unpublished). We evaluated model support based on Akaike Information Criteria (AIC) and AIC weights (Burnham and Anderson 2002). When model uncertainty existed, we model-averaged to calculate weighted parameter and unconditional standard error estimates (Royall 1997, Burnham and Anderson 2002).

We evaluated model fit for the most supported model within each window of inference by calculating the percent of variation explained, calculated as:

$$\%\text{ variation explained} = \left(\frac{\sigma^2_{\text{process}} - \sigma^2_{\text{residual}}}{\sigma^2_{\text{process}}} \right) x\ 100$$

where $\sigma^2_{process}$ = variance component estimate for the intercept-only model, and the $\sigma^2_{residual}$ = variance component estimate for the most supported model (Doherty et al. 2010).

RESULTS

Between 2005 and 2006 we collected 42,931 GPS locations and 171 fecal samples from the three elephant family groups. For both our fixed and flexible windows of inference, we observed a relatively high amount of model uncertainty and low support for a single most-supported model, where our most supported model on average explained 23.2% (SE = 4.5%, min = 1.6%, max = 92.0%) of variation in fixed windows of inference and 18.3% (SE = 4.8%, min = 1.4%, max = 41.0%) of variation in flexible windows of inference. Following model averaging, our predictive model of movement for elephant family groups in basal and elevated physiological states for each type of movement window of inference included nearly all covariates (Table 1). However, the relative influence of external factors and source of refugia covariates within our most supported (or model averaged) model varied depending on the type of window of inference we used, and on the physiological state of the elephant family group (Tables 1 and 2).

Fixed window of inference

Applying the 72-hr fixed window of inference, the probability of use along movement paths differed between elephant family groups in basal (*n* = 15) and elevated (*n* = 15) physiological states relative to their proximity to forest plantations and water sources. We failed to find support for our hypothesis that elephants in an elevated physiological state avoided tree plantations (Table 1). In contrast, 11 of the 15 individual fixed windows of inference for elephant family groups in elevated physiological states had positive parameter estimates (Table 2), suggesting lower probability of space use away from plantations when elephants were in an elevated physiological state (Fig. 3). For both elevated and basal physiological states, coefficient values were positive for use along elephant movement paths near refugia (Tables 1 and 2). However, in support of our original refugia hypothesis, parameter coefficient values were 70% higher during an elevated physiological state (Table 1), suggesting greater use of refugia when elephants were in an elevated physiological state (higher UD percentiles indicate lower probability of use) (Fig. 3). Elephants in a basal physiological state were more likely to use areas near freshwater, compared to elephants in an elevated physiological state (Tables 1 and 2). The effect of distance to boundary was uncertain, with eight positive and seven negative coefficient values for individual fixed windows on inference for elephant family groups in both basal and elevated physiological states (Table 1).

Flexible window of inference

Evidence from flexible windows of inference showed clearer distinctions in movement patterns between basal and elevated physiological states compared to the fixed windows of inference (Fig. 3). Contrary to our hypothesis that elephants in elevated physiological states would avoid commercial tree plantations, elephant groups in an elevated physiological state were more likely to use areas in close proximity to tree plantations, and elephant groups in a basal physiological state were more likely to use areas away from commercial forest plantations (Table 2). Based on our top-ranked model, for every 400-m increase in distance away from plantations, elephant UD percentiles increased by a relatively high 1.9% for elephant groups in an elevated physiological state and decreased 3.3% for elephant groups in a basal physiological state (Fig. 3). Elephant groups in an elevated physiological state used areas in closer proximity to sources of refugia (Table 1), where UD percentile increased by 1.5% for every 10 unit increase in refugia percentile (Fig. 3). By contrast, elephant groups in a basal physiological state utilized areas further away from refugia (Fig. 4), where UD percentiles decreased by 1.5% for every 10 unit increase in refugia percentile (Fig. 3). In contrast to results using a fixed window of inference, those using flexible windows of inference showed that elephant family groups in both elevated and basal physiological states were likely to use areas in closer proximity to freshwater (Tables 1 and 2). Elephant family groups in an elevated physiological state were generally more likely to utilize areas away from reserve boundaries (Fig. 3), although population-level standard error values overlapped 0 (Table 1).

DISCUSSION

Our study provides evidence that an understanding of physiological state can help explain patterns in complex, fine-scale movement behaviors of elephants. We demonstrated that depending on the physiological state of the family group, elephant movement behavior differed in relation to environmental conditions and spatial memory. Physiological status was particularly important in discerning movement responses to factors associated with human disturbance, where elephants in a basal physiological state were more likely to utilize areas away from commercial forest plantations and refugia. Given that elephants and many other vertebrate taxa increasingly come into contact with human-altered landscapes (Blanc et al. 2007, Epps et al. 2011), the predictive ability of movement models could likely be improved by incorporating a better understanding of the role of internal physiological state on movement behavior.

Differences in movement behavior revealed by applying our two windows of inference suggest that the amount of time an individual is in an elevated or basal physiological state can affect animal movement. The relatively short (72 hr) fixed window of inference typically was based on 1-4 fecal samples collected on a given day, and provided a short-term estimate of physiological state. By contrast, the longer-term flexible windows of inference were based on multiple samples over a span of nearly consecutive days, and likely reflected trends in physiological status over longer periods of time. Understanding the duration of a physiological state is important because the amount of time an individual is in an elevated physiological state is likely to have consequences for the intensity and duration of a behavioral response (Romero 2004), where longer-term elevations in stress responses can result in longer-term alterations in behavioral patterns (McEwen and Wingfield 2003). Given that elephants in iSimangaliso are generally in a chronic physiological state and exhibit restricted space use patterns indicative of refuge behavior compared to other elephant populations (Jachowski et al. 2012), it is likely that restricted space use patterns will only be relaxed when elephants in a chronic physiological state temporarily enter a basal physiological state. Our findings support this hypothesis, where elephant family groups in a basal physiological state were more likely to use areas away from refugia, particularly during our longer, flexible windows of inference. Thus, it is likely that elephants in a basal physiological state for sustained, longer-term periods of time would make more frequent exploratory movements and be less restricted in their space use. Overall, we encourage future researchers to similarly evaluate both the long-term physiological status of an individual or population, and use flexible windows of inference to evaluate the effects of physiological state on animal behavior.

The greater use of areas away from refugia during periods of basal FGMs suggests that physiological state influences the degree to which spatial memory influences elephant movement patterns. Various approaches have been developed for incorporating memory into movement models (Dalziel et al. 2008, Smouse et al. 2010). However, there still is a great deal of uncertainty in determining when and to what extent memory influences animal movement (Smouse et al. 2010). Memory-based movement towards sources of refugia is hypothesized to be a facultative response to elevated physiological state for a variety of vertebrates (Wingfield and Romenofsky 1997), including elephants (Jachowski et al. 2012). Further, our findings show that the establishment and duration of refuge behavior varies depending on the type (either elevated or basal) and duration of a family group's physiological state. Therefore, it is likely that the relative influence of spatial memory on elephant movement patterns (such as movement toward or away from a refuge) is associated with the physiological status of an individual or family group, and future attempts to incorporate memory into movement models should incorporate a measure of physiological condition.

The alteration of movement patterns in response to elevated physiological state could have a number of important implications to the conservation of wild elephant populations. Our findings suggest that elephants in an elevated physiological state are more likely to use refugia, and less likely to make exploratory movements. In wild elephant populations, such exploratory movements between protected areas are likely to be critical to dispersal and population connectivity (Douglas-Hamilton et al. 2005, Cushman et al. 2010, Epps et al. 2011). Thus, future attempts to create corridors between protected areas to facilitate connectivity among populations should account for the influence of changing physiological status on elephant movement. In addition, attempts to predict the impact of additional human disturbance on existing elephant populations should consider the possibility that perturbations leading to an elevated physiological state in elephants will likely result in their avoidance of certain areas and limited use of corridors.

Evidence that elephants in a prolonged basal physiological state are more likely to use areas away from commercial tree plantations suggests that either elephants are selecting plantations as a consequence of experiencing elevated FGMs, or that their use of plantations is causing elevations in FGMs. Elephants in an elevated physiological state might have been selecting the extensive matrix of plantation lands on the Eastern Shores for cover and refugia in preference to the available native forest and grasslands in iSimangaliso (Jachowski et al. 2012). Alternatively, elephants might have acquired an elevated physiological state through their sustained use of plantations, similar to the elevated FGM levels in crop-raiding compared to non-crop-raiding male elephants (Ahlering et al. 2010). However, similar to crop-raiding, it remains to be seen if elevated FGMs cause, or are in response to, use of human-altered landscapes.

Regardless of whether the relationship between plantation use and elevated stress hormone levels is reactionary or causative, managers could identify when elephants are in an elevated physiological state based on real-time patterns of plantation use, and thereby limit human-elephant conflicts. In iSimangaliso, given that elephants in an elevated physiological state are more likely to restrict their movement patterns within tree plantations, and that elephants in an elevated physiological state can be prone to aggressive behavior toward humans (Jachowski et al. 2012), we suggest that managers limit human access to these areas when they are occupied by elephants. Further, given that we observed elephants typically utilizing the same corridors or movement paths among refugia (Fig. 4), managers should identify key bottlenecks and points of potential human disturbance on such paths so as to facilitate movement among refugia. Using technology similar to that currently employed to detect when elephants cross reserve boundaries (Slotow 2012), it would be possible for managers to be notified in real time via global position system telemetry data and computer programs that identify when elephants utilize refugia and corridors. In iSimangaliso and other reserves, once these areas of likely human-elephant conflict are identified, such real-time tracking and computer alert systems could be used to avoid potentially dangerous human-elephant interactions.

Elephants have complex behaviors and they adapt their movement in patterns unique to their surroundings, so it would be naïve to assume our model results fully describe movement patterns for all elephant populations. Although we failed to observe differences in the effect of distance to reserve boundary between elephants in basal and elevated physiological states in iSimangaliso, fences generally play a key role in elephant movements when comparing fenced to unfenced populations (Druce et al. 2008; Loarie et al. 2009). Elevated stress hormone concentrations in unfenced elephant populations are associated with long, unidirectional movement or streaking behavior (Viljoen et al. 2008) compared to fenced populations, where they are associated with restricted space use or refuge behavior (Jachowski et al. 2012). Further, differences in the effect of boundary fences on elephant movement likely exist among reserves based on longer-term trends in elephant physiological status and space use. Within fenced reserves containing elephants in a long-term basal physiological state, such as Pilanesberg National Park (Jachowski et al. 2012), elephants less commonly utilize areas near boundary fences (Vanak et al. 2010). By contrast, fence-related edge effects are likely counteracted in iSimangaliso, where elephant populations are in a long-term (up to 6 yr) chronic physiological state, by the greater relative importance of the availability of forest cover (both closed native forests and commercial tree plantations) near boundaries on the Western Shores (Jachowski et al. 2012). In iSimangaliso, the availability of cover when elephant family groups were in an elevated physiological state likely, also explains differing use patterns in relation to proximity to water, where water sources typically occurred in open areas near the center of the reserve and were less likely to be utilized during periods of time when elephants had elevated stress hormone concentrations. Thus, the availability of cover that restricts visibility is likely to be a key component of space use when elephants are in an elevated physiological state, resulting in movement patterns that are unique to the habitat conditions of the reserve.

Collectively, our findings show that by incorporating measures of physiological state into models of animal movement, we can begin to better understand patterns in typically complex movement behaviors. State-dependent modeling approaches commonly focus on long-term or persistent states of hunger

(McNamara and Houston1987, Berger-Tal et al. 2010), individual development (McNamara and Houston 1996) or age (Montgomery et al. unpublished). Our study, by contrast, showed that changes in relatively short-term physiological state were associated with fine-scale changes in elephant movement. Given the role that these short-term physiological states play in conjunction with other factors commonly thought to influence animal movement, we predict that incorporation of physiological state as a variable will enable the creation of more effective movement models.

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Table 1. Summary of the number of times parameter coefficients for most supported (or model averaged) models were positive or negative. Model coefficients reflect hypotheses used to predict space use along movement paths when elephant family groups in iSimangaliso Wetland Park were in a basal or elevated physiological state. Periods of investigation into elephant movement were assessed based on fixed and flexible time periods that represented the measured physiological status of each family group.

Covariates	Basal				Elevated			
	Fixed		Flexible		Fixed		Flexible	
	+	-	+	-	+	-	+	-
Refugia use	14	1	0	2	11	4	3	2
Distance from plantation	6	9	0	2	11	4	2	3
Distance from water [a]	11	3	1	0	6	9	4	1
Distance from boundary	8	7	2	0	8	7	2	3

[a] Distance from nearest freshwater source was not retained in the top model for one movement window in both fixed and flexible windows of inference.

Table 2. Population-level average parameter utilization coefficients (with standard error) for the most supported (or model averaged) model used to predict space use along movement paths when elephant family groups in iSimangaliso Wetland Park were in a basal or elevated physiological state. Periods of investigation into elephant movement were assessed based on fixed and flexible time periods that represented the measured physiological status of each family group.

Covariates	Basal		Elevated	
	Fixed	Flexible	Fixed	Flexible
Intercept	63.22 (7.11)	71.53 (2.37)	67.24 (5.25)	75.35 (1.42)
Refugia use	1.09 (0.78)	-1.23 (0.90)	3.61 (1.51)	2.71 (1.74)
Distance from plantation	-0.36 (1.29)	-9.83 (7.73)	-0.41 (0.85)	2.35 (2.02)
Distance from water	2.95 (1.48)	10.14 (-)[a]	-0.81 (0.63)	0.89 (0.82)
Distance from boundary	0.04 (0.51)	0.48 (0.43)	0.82 (0.58)	-2.71 (2.79)

[a] Distance from nearest freshwater source was only retained within supported model for a single flexible window of inference when that family group was in a basal physiological state, so no standard error estimate was calculated.

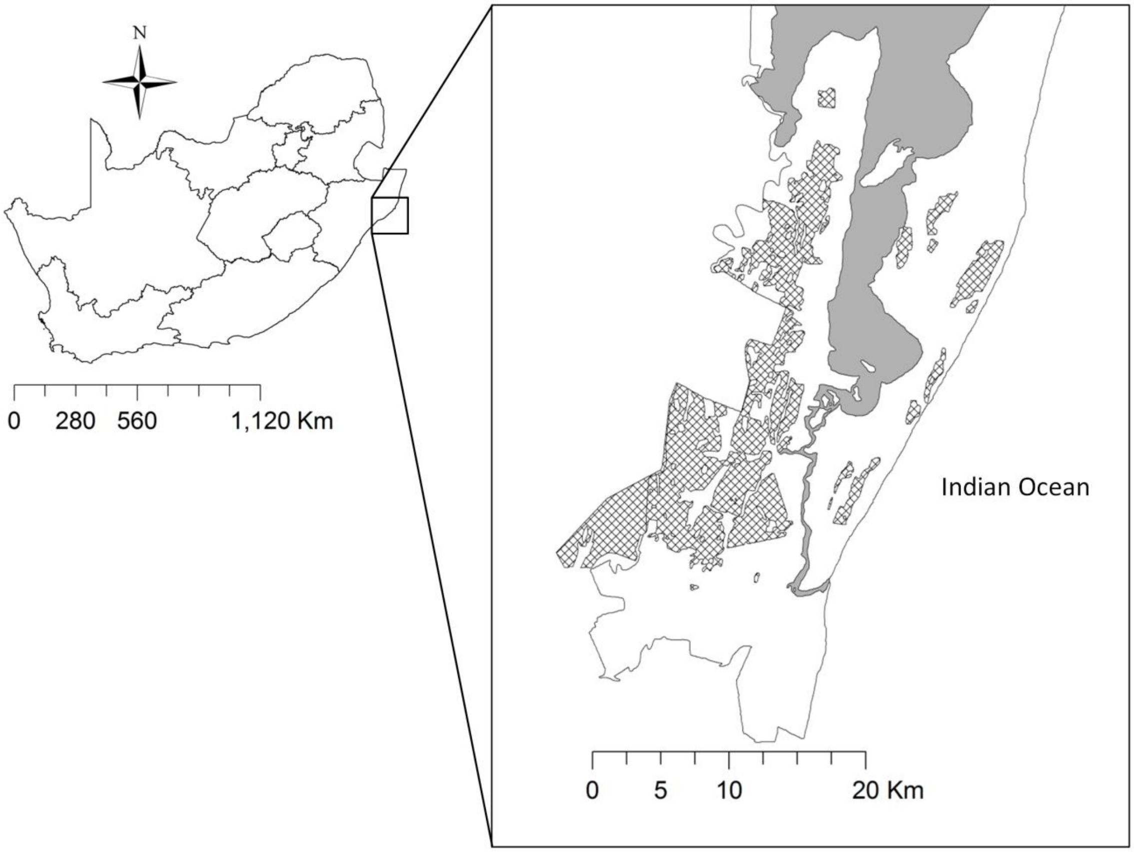

Figure 1. Location of iSimangaliso Wetland Park within South Africa. Solid black line in inset depicts the park boundary, areas cross-hatched depict commercial forest plantations, and the area in grey is estuarine Lake St. Lucia, which divides the Western from Eastern Shores region of the park.

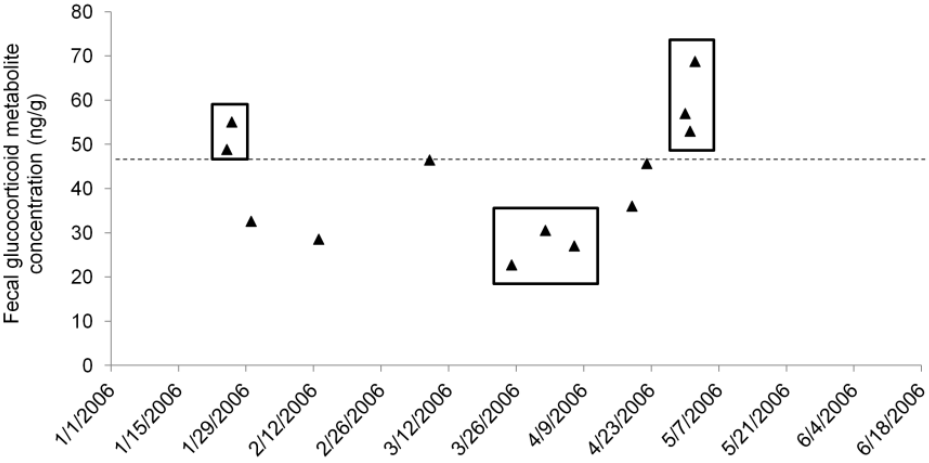

Figure 2. Mean fecal glucocorticoid metabolite (FGM) concentration values by day (corrected for deposition and gut passage time) for family group two in iSimangaliso Wetland Park between January and April 2006. The dashed line represents the average FGM concentration across all samples for family group two ($\bar{x}$=48.31, SE=2.30) during this study (2005-2006). Boxes indicate maximum flexible windows of inference that we identified for subsequent movement analysis.

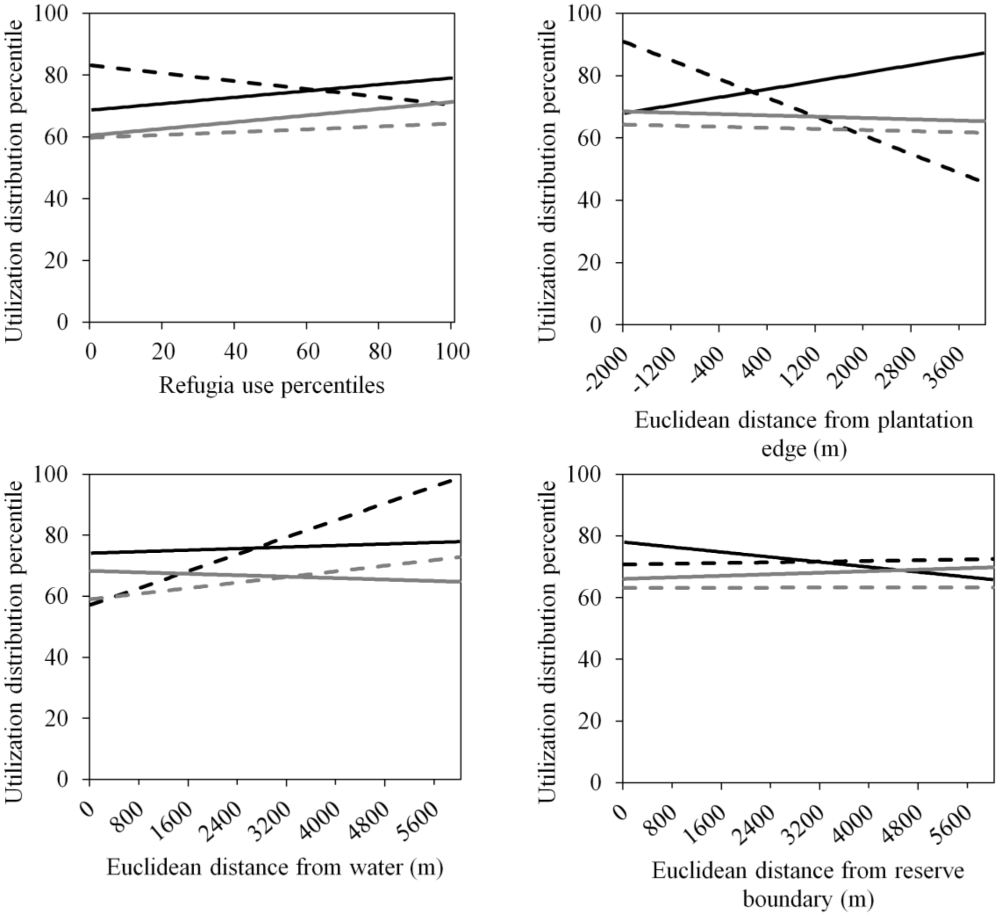

Figure 3. Predicted relationships between Brownian Bridge Movement Model utilization distribution utilization distribution (UD) percentile values in relation to proximity to refugia, as well as distance to commercial tree plantations, fresh water, and reserve boundaries. Predictive values were based on parameter coefficients from top-ranked models for flexible (black lines) and fixed windows of inference (grey lines) for elephant family groups in basal (dashed lines) and elevated (solid lines) physiological states in iSimangaliso Wetland Park. Lower UD percentile values indicate higher probability of use.

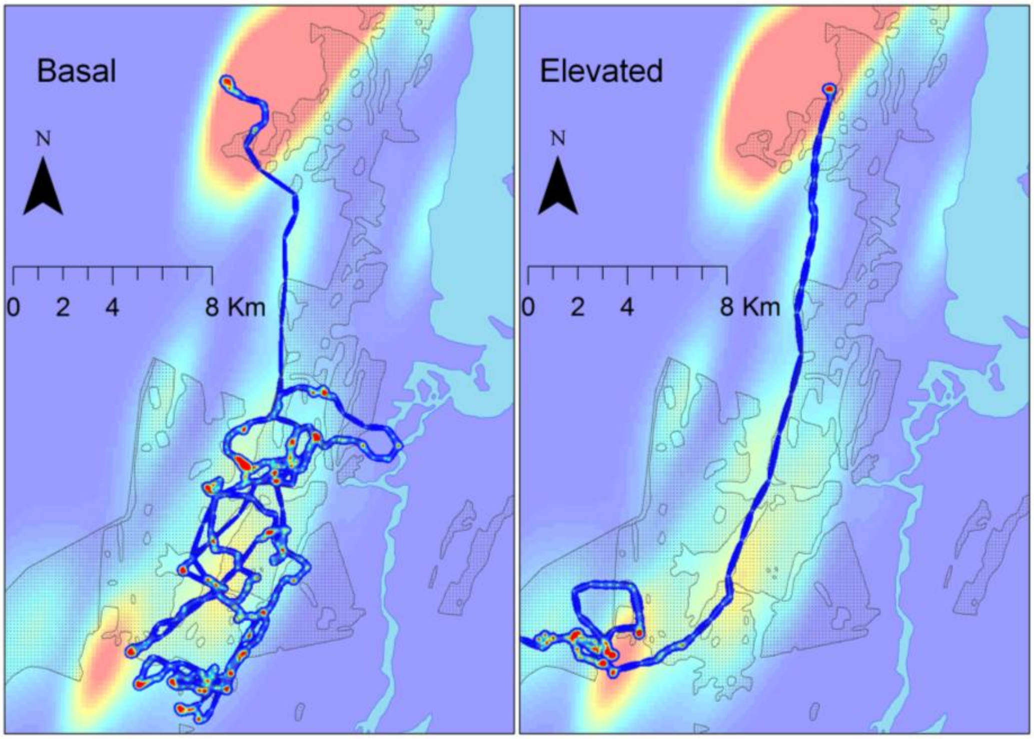

Figure 4. Brownian bridge movement model (BBMM) 99% utilization distribution (UD) percentile for an elephant family group during flexible windows of inference corresponding to a basal (left) and elevated (right) physiological state in iSimangaliso Wetland Park (2006). BBMM was based on GPS collar locational fixes at 30 min intervals, and areas in red within the BBMM indicate areas of high probability of use. Background colors indicate refugia UD percentile values, where areas in red indicate highest percentile of refugia.

CHAPTER 6

IMPLICATIONS OF PHYSIOLOGICAL AND BEHAVIORAL FINDINGS CONCERNING AFRICAN ELEPHANTS TO REINTRODUCTION BIOLOGY

Findings from our research on African elephants emphasize the importance of incorporating measures of physiology and behavior in the practice of wildlife reintroduction and translocation. By measuring levels of stress hormones in elephant droppings, we were able to monitor the physiological condition of animals post-release and to determine how long it took them to acclimatize to their new surroundings (Chapter 3). In addition, by integrating physiological and behavioral data, we gained insight into patterns of refuge behavior (Chapter 4) and found that refugia are likely to be a critical component of the acclimatization process for elephants following release. We also found that refuge behavior could occur during relatively short-term elevations in physiological state (Chapter 5), suggesting that refuge behavior is a facultative behavioral response to elevated stress hormone concentrations, and likely occurs in other elephant populations (Woolley et al. 2008).

Given that elephants, as generalist megaherbivores, exhibit refuge behavior post-release, such patterns also are likely to be evident in other species following reintroduction. Species at lower trophic levels are known to be subject to predation risk effects that have been linked to refuge behavior (Kauffman et al.

2007). Thus, refuge behavior for many reintroduced species could be intensified when the released animals confront predators or dominant competitors. Refuge behavior by elephants illustrates that limited resources (in this case refugia associated with forest cover (Chapter 4)) are important even to species that are considered habitat generalists (Owen-Smith 1988). This suggests that for those species of wildlife with more specialized habitat requirements that restrict their use of space (such as the black-footed ferret discussed in Chapter 2), the stress of reintroduction is likely to lead to fine-scale refuge behavior within those habitats.

Collectively, our findings suggest that in addition to monitoring physiological and behavioral conditions of wildlife following reintroduction, managers need to account for refuge behavior and provide suitable refugia post-release. In elephants, and likely other reintroduced wildlife species, an understanding of the spatial and temporal patterns of refuge behavior is critical in mitigating behavioral problems that influence reintroduction success. The now common pre-release practices of preconditioning or soft release strategies for wildlife translocation and reintroduction help to limit dispersal and enhance survival (Armstrong and Seddon 2008). Our results suggest that the provision of refugia post-release is important for facilitating the behavioral and physiological acclimatization of wildlife to their release areas. Thus, managers might improve the likelihood of success in reintroducing wildlife by incorporating both pre-release and post-release steps, including the provision of areas that serve as refugia for wildlife to acclimatize for an extended period of time post-release.

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VITA

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