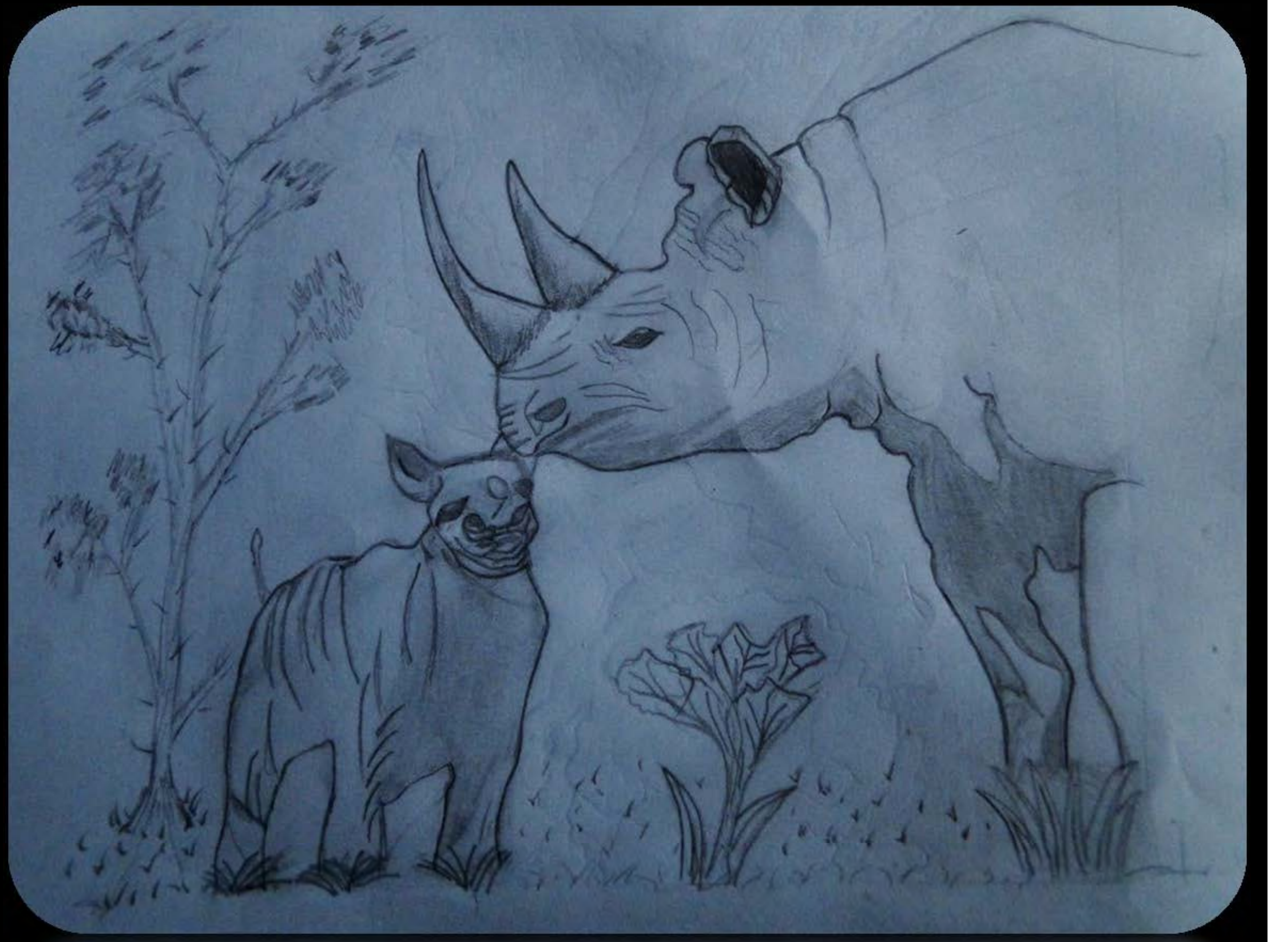
Population densities of eastern black rhinoceros

Unravelling the controls



Benson Okita Ouma

Population densities of eastern black rhinoceros – Unravelling the controls B. Okita Ouma 2014

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Thesis

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Dedication

To the rhinoceroses and the people who care for them
To my grandparents and parents (James and Jane) for their love for education
To my family; you are a source of inspiration

Photo credit: B. Okita-Ouma

Abstract

Understanding the forces that cause variability in population sizes is a central theme in ecology. The limiting factor in populations of large mammals which are not controlled top-down by predation is food, *i.e.*, such populations are controlled by bottom-up processes. However, there is little evidence of density dependence in large- to mega-herbivores. Yet, conservationists have managed Critically Endangered mega-herbivores like rhinoceros as if their population growth were density dependent, *i.e.,* following a logistic growth curve, focusing on large growth at population densities presumed to be at half-carrying capacity (K/2). This would enable them to translocate animals at presumed half-carrying capacity to retain local population densities and to create new populations in areas of suitable habitat, where animals are considered safe against poaching. This study focussed on one such mega-herbivore, the eastern black rhinoceros (*Diceros bicornis michaeli*) to re-consider the density dependence population regulation in a mega-herbivore and use the findings to contribute to possible solutions towards conservation challenges facing this species. The expectations were that increases in population density would result in a decrease in reproductive performance, and that physiological stress levels in animals in populations of high density would be higher than in animals in populations of low densities. Nine populations of black rhinoceros across Kenya were studied, with variation in their respective densities, Plant Available Moisture ('PAM' *i.e.*, 'soil moisture') and Plant Available Nutrients ('PAN' *i.e.*, 'soil fertility'). Data from available records (1993-2010) were used to assess reproductive performance. Dietary quality and levels of corticosterone were estimated through faecal analysis from animals sampled in the field and from data on feeding trials of black rhinoceroses in zoos (dietary analysis only). Woody cover estimates were used to assess available browse for black rhinoceros. Two measures of density were used, *i.e.*, absolute density (animals/km^2) and relative density, *i.e.*, absolute density as a ratio to the estimated maximum stocking density or 'carrying capacity'. The effects of PAM and PAN, and subsets of PAM (rainfall and temperature) were incorporated and controlled in testing expectations. No evidence for density dependence was found. Reproductive performances tended to be better where PAM was high, PAN was low and woody cover was sparse. PAM was found to be directly correlated with quality of dietary browse. Black rhinoceros populations appeared controlled more by bottom-up processes through key resources, even though their densities were perhaps too low to fully support this alternative view. It was apparent that the density dependence concept still requires more investigation. Deliberate efforts should be made to secure high PAM – low PAN – sparse woody cover areas for conservation of black rhinoceros. Conservation managers are advised to consider set percentage translocations, as opposed to the current translocation of black rhinoceros on the basis of an imaginary 'carrying capacity' and density dependence.

Key words: black rhinoceros, browser, corticosterone, diet, density dependence, minerals, moisture, physiological stress, savanna, soil nutrients, woody cover.

Photo credit: Dauglas Kimanzi

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Chapter 1

General Introduction

Benson Okita Ouma

Background to the concept of density dependence

Populations cannot grow infinitely within the confines of finite resources of food or space. Populations also show variability in abundance. Understanding the forces that cause this variability is a central theme in population ecology (Krebs 2002, Turchin 2003, Krebs 2014). Limiting factors are apparently operating, and the sources of these comprise to a large extent deterministic and stochastic processes. Deterministic processes are feedbacks from population density (density dependence) to population growth rate. Density dependence results from competitive interactions within a population. These processes interact to generate complex patterns of animal abundance over time and space (*e.g.*, Coulson and Hudson 2003, Boyce *et al*. 2006, Owen-Smith 2006, Owen-Smith and Mills 2006). The limiting factor in populations of large mammals which are not controlled top-down by predation is food; in other words, such populations are controlled by bottom-up processes (*e.g.*, Mduma *et al*. 1999, Sinclair *et al*. 2003). Food limitation as a result of high density results in decreased birth rates and increased mortality rates (*e.g.*, McCullough 1999, Pastor *et al*. 2006). Food limitation can also lead to chronic physiological stress that in turn aggravates susceptibility to disease, immunosuppression, tissue atrophy, and lowered reproductive performance (*see review in* Li *et al.* 2007). These effects manifest themselves differently in different age classes of large mammals. This type of density dependence is a key factor in stabilizing and regulating large mammal populations, especially those occurring in more homogeneous environments (Fowler 1987, Gaillard *et al.* 2000, Sinclair *et al.* 2003).

Apart from resource limitation, there is also a growing recognition that processes such as environmental stochasticity interplay with population density and have significant effects on populations (*e.g.*, Saether 1997, Sinclair *et al.* 2003, Owen-Smith and Mills 2006, Gaidet and Gaillard 2008, Wang *et al.* 2011). Low environmental stochasticity leads to small population fluctuations and result in a rather stable long-term average population size, whereas very high environmental stochasticity affects populations much more than density dependence processes (Chamaillé-Jammes *et al*. 2008). These limitations suppress the initial growth from what would otherwise been described by the J-shaped exponential growth curve $dN_t/dt = r_{max}N_t$, to a S-shaped logistic growth curve described by the function $dN_t/dt = r_{max}N_t[(K-N_t)/N_t]$; where r_{max} = maximum difference between birth and death rate; K is the ‘carrying capacity’, or the situation when $dN_t/dt = 0$ (*e.g.*, Verhulst 1838, Gabriel *et al*. 2005, Ross 2009). Over time in the logistic growth, the population enters a fluctuation phase with points of ‘equilibrium’ when N_t = K, and a stability phase when fluctuations in N_t are within a constant range depending on the strengths of density dependence and environmental stochasticity (Fig. 1.1).

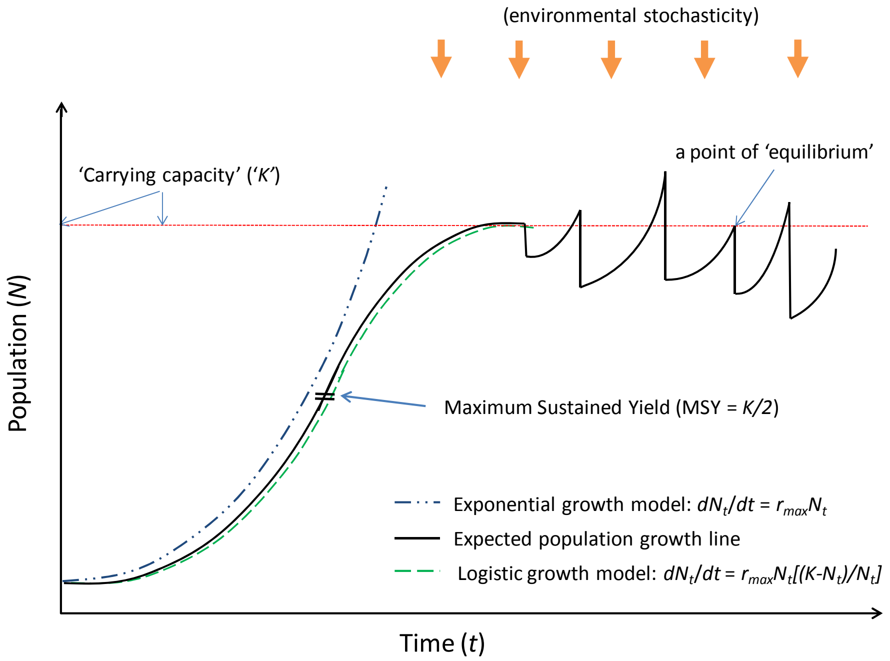

Figure 1.1: A hypothetical illustration of growth of population (N) over time (t) (*bold line*). Initially there is a convex exponential population growth rate. Maximum growth (MSY) is achieved between K/2 and K. The growth later slows down then fluctuates around a 'carrying capacity (K)' when $dN_t/dt = 0$. A point of 'equilibrium' is reached when N = K. The fluctuations depend on strengths of density dependence and environmental stochasticity. This growth process is described by the classic logistic growth model.

In applying the concept of density dependence and mathematical models associated with it, conservation managers aim to maintain animal numbers at around a putative maximum sustained yield (MSY). In the classic logistic model MSY is at K/2 (half the carrying capacity – 'K') (Fig. 1.1). However, it has become clear that this model does not fit the mega-herbivores (Fowler 1987) and in some instances its application has been questioned (*e.g.*, Sibly *et al*. 2005, Doncaster 2006, Peacock and Garshelis 2006, Ross 2006). Recently, Peacock and Garshelis (2006) concluded from many long term studies of population changes in large mammals, that these species exhibit strong density dependence only near K while MSY occurs between K/2 and K. If this would also be the case in mega-herbivores, then MSY would be greater than K/2 *i.e.*, around 70 - 80% K (*e.g.*, Owen-Smith 1988, McCullough 1992, McCullough 1999).

Density dependence in mega-herbivores

Not many studies are known to have analysed density dependence in mega-herbivores living under natural conditions over a long time span. This is mainly caused by the current unnaturally low densities of these animals.

The best data come from some African elephant (*Loxodonta africana*) populations which have had the opportunity to grow virtually unmanaged for a considerable period. Recruitment of elephants in Addo National Park increased despite being at population densities expected to exhibit density dependence (Gough and Kerley 2006). Similarly, Morgan *et al*. (2009) reported an optimal population growth of black rhinoceros in Mun-ya-Wana Game Reserve in South Africa, despite them exceeding the estimated K. On the contrary density dependence has been suggested to show in some elephant populations (Armbruster and Lande 1993, Van Aarde *et al*. 1999, Sinclair *et al*. 2003, Chamaillé-Jammes *et al*. 2008, Junker *et al*. 2008). It can therefore be assumed that in elephant populations, where density dependence does not seem to play a large role, populations are regulated by environmental stochasticity (*e.g.*, Armbruster and Lande 1993). In the semi-arid tropics, it is generally accepted that rainfall is the main driver of many large and mega-herbivores, through its effects on food resource (*e.g.*, Illius and O'Connor 2000). Nevertheless, the extent to which density dependence plays a role in regulating populations of mega-herbivores living in highly varied environments is not known, eighty years since Nicholson (1933) first advanced the concept in population regulation.

Despite the uncertainties, and lack of evidence concerning the occurrence of density dependence, especially in large to mega-herbivores, the concept of density dependence has been applied in many instances by conservation managers to manage and conserve mega-herbivores (Owen-Smith 1988, Law *et al.* 2013). This involves removing "surplus" animals through translocations or culling, to start new populations or for conservation and economic reasons. Under the concept of density dependence, it is argued that removal of surplus animals at MSY results in maximal reproductive performance of the remaining animals in a population (Owen-Smith 1988). Sometimes, such animals include emblematic, or rare or Critically Endangered species suffering from population declines due to anthropogenic factors such as poaching or habitat loss (*e.g.*, Baudron and Giller 2014). Because of the need to increase the numbers of particularly rare or endangered, slow breeding and long-living species, some institutions have therefore embedded this concept into their policy guidelines for the conservation and management of those species (*e.g.*, Okita-Ouma *et al.* 2007b, Goodman *et al.* 2008, KWS 2012, Litoroh *et al.* 2012, MET 2012, KWS 2013).

Problem statement

It is evident that there still exist many uncertainties surrounding the concept of density dependence in mega-herbivores. The need to understand how populations of mega-herbivores are controlled is critical, especially when their conservation management is guided by concepts that are not yet fully proven. It is even more urgent to unravel what controls populations of mega-herbivores especially if they are Critically Endangered. I chose to study the eastern black rhinoceros (*Diceros bicornis michaeli*), a Critically Endangered (IUCN 2013b) mega-herbivore, to understand the application of this concept in this species' conservation and management. This species, on the one hand, like the other rhinoceros species, faces a risk of extinction from an upsurge in poaching to supply illegal demand for its horn (*e.g.*, Milliken and Shaw 2012, Prins and Okita-Ouma 2013) and therefore requires high protection. On the other hand, the species needs to be dispersed more widely if negative density feedbacks are to be avoided in order to sustain maximal population growth. These two situations conflict in two ways and pose a conservation dilemma to wildlife managers: Firstly, the lack of sufficient resources for high protection in open, unmanaged areas makes it difficult to disperse the animals. Secondly, the choice between accommodating negative density feedbacks (if any) and exposing the "surplus" animals to a higher poaching threat by moving them to open areas that are expensive to protect, is challenging. These conflicts are further compounded when available land for dispersing the animals is also competing for other land uses such as agriculture or human settlements.

Research questions

So, can a better understanding of the concept of density dependence help to solve these complex problems? How much time can we buy for Critically Endangered mega-herbivores at high densities while resources for protection are sourced, and while illegal demand for rhino horn is curtailed? What alternative approaches can we find for longer term conservation of this species? The central theme of my study was guided by these questions. I explored whether there was density dependence in the eastern black rhinoceros in Kenya so that they can be managed and conserved accordingly. My study is also important in further investigating the applicability of the carrying capacity models and their derived MSY models, since selecting mathematical models as the basic premise for conservation decisions may yield very undesirable results. A case in point is the management of Pilanesberg National Park in South Africa where removal of zebra (*Equus quagga*, formerly *Equus burchellii*) from a high stocking density to a density in line with a predicted MSY, resulted in a vegetation that was no longer controlled by grazing but by fire, thus jeopardizing tourist experience and financial income (Prins H.H.T. *pers. comm.*).

The study animal

Plate 1.1: A picture of the study animal, the eastern black rhinoceros *D. b. michaeli,* taken in Masai Mara National Reserve in Kenya. The picture shows the distinctive slender curved horn and distinctive skin ridges that give this subspecies a corrugated appearance on its sides that are lacking in other subspecies of black rhinoceros. The birds perching on its back are normally red-billed oxpecker (*Buphagus erythrorhynchus*) or yellow-billed oxpecker (*Buphagus africanus*) that feed on insects and ticks on its body. These birds alert the rhinoceros to danger by flying away and making alarm calls. Kenyan rhinoceros monitoring rangers refer to these birds in Swahili as "*askari wa faru*" meaning "guard for rhinoceros". Further detailed descriptions about this species can be found elsewhere (*e.g.*, Kingdon and Hoffmann 2013).

History and conservation status of rhinoceroses and the study species

The study animal, eastern black rhinoceros on Plate 1.1, belongs to the Order of Rhinoceroses. This Order has been in existence for at least 50 million years, with complex evolutionary paths from the late Palaeocene (65 million years ago) to early Pleistocene (2 million years ago) (Prothero *et al*. 1989). At one period between these evolutionary epochs, rhinoceroses were the most abundant and diverse Order of mammals, far outnumbering other Orders in Eurasia, Africa, and North America (Prothero 2013). However, the number of species in this Order has declined since the late Miocene (23 million years ago), possibly due to climate changes, and many went extinct first in America about 4 million years ago and then in Europe about 12,000 years ago (Prothero 1998, 2013). Today**,** five extant species of rhinoceros survive in low numbers in Asia and Africa. In Asia, the Indian rhinoceros (*Rhinoceros unicornis*) numbers about 3,000 animals, the Sumatran rhinoceros (*Dicerorhinus sumatrensis*) about 200 animals, and the Javan rhinoceros (*Rhinoceros sondaicus*) about 50 animals (IUCN 2013b). In Africa, the white rhinoceros (*Ceratotherium simum*) comprises two subspecies; the southern white (*C. s. simum*) numbering about 20,000 animals with >90% in South Africa and the northern white (*C. s. cottoni*) of only 8 known animals (IUCN

2013a). The black rhinoceros (*Diceros bicornis*), numbers about 5,100 animals comprising four subspecies (Emslie and Brooks 1999); the south-western (*D. b. bicornis*), the southern-central (*D. b. minor*), the eastern (*D. b. michaeli*), and the western (*D. b. longipes*) which was declared extinct in the wild in 2006 (IUCN 2013b, Kingdon and Hoffmann 2013). The current distribution of black rhinoceroses by country is shown in Figure 1.2. The IUCN (2013b) Red List of Threatened Species lists white rhinoceros as Near Threatened, the Indian rhinoceros as Vulnerable, and the Black, Javan and Sumatran rhinoceroses as Critically Endangered.

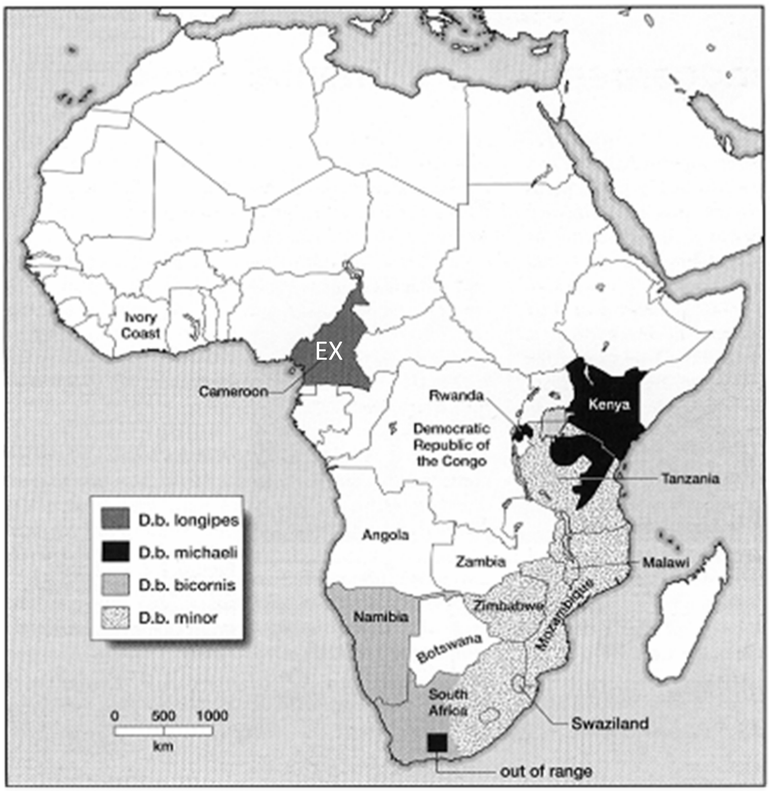

Figure 1.2: Distribution of black rhinoceros subspecies by country in Africa as at the end of 2013. (*Adapted from* Emslie and Brooks 1999). There may be 1 or 2 black rhinoceros(es) in Rwanda, whereas the subspecies *D. b. longipes* was declared extinct (EX) in the wild in Cameroon in 2006 (IUCN 2013a).

In Kenya, where I conducted this study, the plague rinderpest killed many Artiodactyla ungulates in the1880s (WOAH 2011) but black rhinoceros, like many other hind-gut Perissodactyla ungulates, was not susceptible to this disease and its numbers continued increasing. Hunting diaries of large game hunters refer to endless hunting and shooting of this species (*e.g.*, Meinertzhagen 1957, Underwood 2003). Sometimes as many as 1,000 animals would be hunted within a month to clear land and nuisance animals for agriculture and human settlements. Reliable historical population estimates of this species have not yet been published. In the 1960s Kenya had more than 20,000 black rhinoceroses (*e.g.*, Goddard 1969, Brett and Wanjohi 1990, KWS 1993). The indiscriminate hunting and poaching for the illegal trade in rhinoceros horn in the 1970s (Leader-Williams *et al*. 1990, Leader-Williams 1992) reduced numbers to less than 350 by the mid-1980s (KWS 1993). By 2013, following huge investment in protection and biological management, Kenya had about 630 eastern black rhinoceros (Kariuki *et al*. 2014) constituting 75% of the globally estimated 800 animals (Emslie and Adcock 2013, IUCN-SSC-AfRSG 2013)**.** A majority of these animals are in fragmented populations of less than 100 animals, mostly in fenced areas (Amin *et al.* 2006a, Okita-Ouma *et al.* 2007b, KWS 2013). Some of these populations were reported to have exceeded their estimated maximum stocking densities (Adcock *et al.* 2007) from predictions made using the only available carrying capacity model for black rhinoceros (Adcock 2001).

Reasons for studying black rhinoceros

I chose the black rhinoceros as the theme of this study because apart from its Critically Endangered status and the need for significant efforts in protection and evidence based biological management, this mega- browser is significantly different from other mega-herbivores such as the elephant and white rhinoceros. It can be argued that the black rhinoceros lives in a more stable environment than the elephant or white rhinoceros, because it is a browser and the browse food supply shows less temporal fluctuation than that of the mixed feeders or grazers (*e.g.*, Owen-Smith 2008). Furthermore, black rhinoceroses have a less roaming behaviour (*review in* Kingdon and Hoffmann 2013) than elephants and thus have fewer possibilities to mediate environmental fluctuations by moving large distances. Its twig-removing feeding behaviour (*see review in* Emslie and Adcock 2013) suggests that the black rhinoceros may have a potentially large impact on its habitat heterogeneity and nutrient recycling (Owen-Smith 1988, Hansen and Galetti 2009), especially if enclosed at high density (Luske *et al*. 2009). By virtue of its large body size, the black rhinoceros like other mega-herbivores, does not have effective predators, apart from man, to regulate or control its population top-down, hence its population is mainly controlled by food resources through bottom-up processes (*e.g.*, Fritz *et al*. 2002). Its hind-gut digestive system can process plants of low nutritional quality such as *Euphorbia* spp. with high concentrations of secondary compounds (*e.g.*, Goddard 1968, 1970, Mukinya 1977, Hitchins 1979, Hall-Martin *et al*. 1982, Luske *et al*. 2009). These characteristics make this species suitable for a study on consumer-resource density feedbacks and 'carrying capacity' estimates.

Approach to the study and expectations

I attempted to answer my research questions by evaluating density dependence in the long term (1993-2010) measures of reproductive performance; in the dietary quality; and in the endocrinal changes (mainly physiological stress through measures of levels of hormone corticosterone) in the eastern black rhinoceros. I used two measures of density, *i.e*., absolute density (animals/km^2) and relative density, *i.e*., absolute density as a ratio to the estimated maximum stocking density or 'carrying capacity' (Adcock *et al.* 2007). I expected that increases in population density would result in a decrease in reproductive performance, and that physiological stress levels in animals in populations of high density would be higher than in animals in populations of low densities.

To test these expectations, I incorporated, explored and controlled the effects of edaphic factors. These factors were mainly soil nutrients (Plant Available Nutrients, 'PAN') which I estimated from different literature sources and soil databases, and soil moisture (Plant Available Moisture, 'PAM'). I calculated PAM from satellite (http://www.cpc.ncep.noaa.gov) derived rainfall and temperature estimates (LSASAF 2010, Tchuenté *et al.* 2011) and compared these calculations with actual and reliable field estimates. PAN and PAM have been used as standard indices to characterise habitats and the heterogeneity in forage quality and quantity (*e.g.*, Walker and Langridge 1997, Olff *et al*. 2002, Ahrestani *et al*. 2011). I used woody cover estimates (Hansen *et al*. 2002) as a measure for available browse for black rhinoceros.

I extracted data for different measures of reproductive performance from the Kenyan rhino information system (Amin *et al*. 2001) and verified these with field records derived from standard data collection procedures (Mulama *et al*. 2005). I used faecal contents of 77 animals sampled in the field between July 2010 and November 2011 for the evaluation of physiological stress corticosterone (*e.g.*, Möstl and Palme 2002, Millspaugh and Washburn 2004). I used the faecal mineral concentration to evaluate changes in dietary quality (*e.g.*, Prins and Beekman 1989, Van der Waal *et al*. 2003). I confirmed the premise that dietary quality is reflected in faecal mineral nutrients in two ways: firstly by analysing data from feeding trials of 15 black rhinoceroses in zoos (Clauss *et al*. 2007), and secondly by correlating the faecal mineral nutrients to PAN and PAM.

In this thesis I interchangeably use 'maximum stocking density', 'safe stocking density' and 'carrying capacity' to mean the maximum number of rhinoceroses that key resources can sustain without the animals showing signs of density dependence.

The study areas

This study was carried out in nine conservation areas for the black rhinoceros in Kenya. They included Aberdare National Park (Aberdare), Lake Nakuru National Park (Nakuru), Masai Mara National Reserve (Mara), Nairobi National Park (Nairobi), Ngulia Rhino Sanctuary (Ngulia), Ol Jogi Pyramid (Ol Jogi), Ol Pejeta Conservancy (Ol Pejeta), Tsavo East National Park (Tsavo East), and Tsavo West National Park Intensive Protection Zone (Tsavo West IPZ) (Fig. 1.3). Selection of these areas was based on their varying PAM and PAN (Fig. 1.4) and their low and high population densities (Table 1.1).

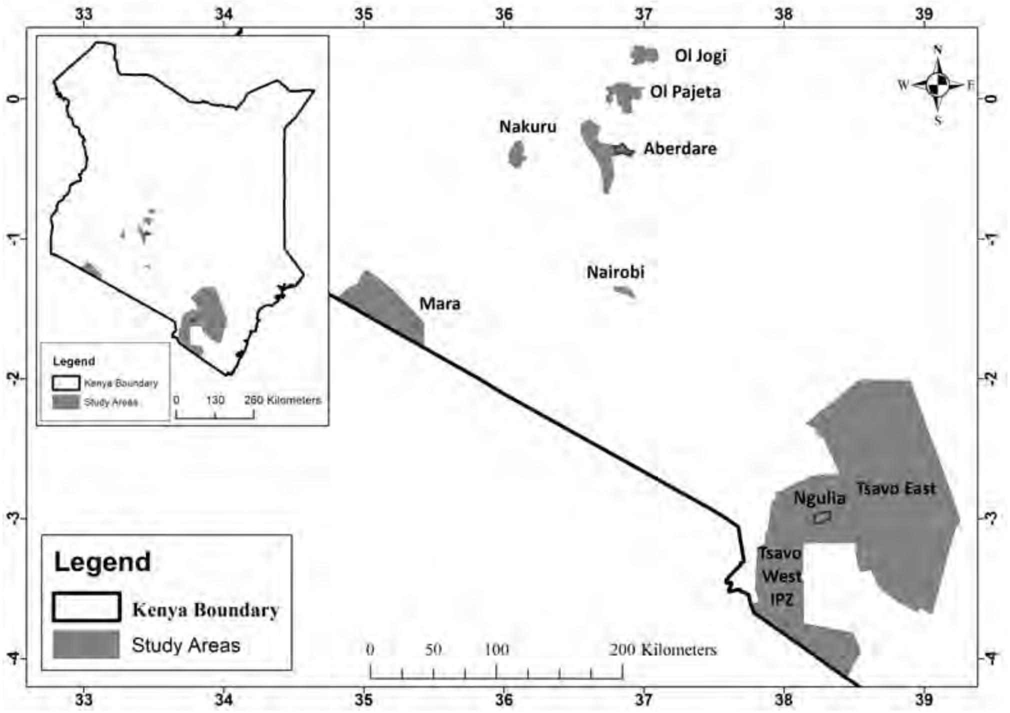

Figure 1.3: Geographic locations of the nine study areas within Kenya. Nakuru, Ol Pejeta, Ol Jogi, Nairobi and Ngulia are fenced populations but the fences are designed to allow migration of other wildlife species apart from rhinoceroses. The Aberdare population is partially fenced within the eastern section of the larger, completely fenced Aberdare National Park. Mara, Tsavo East and Tsavo West IPZ are not fenced except for boundaries that are prone to human-wildlife conflicts.

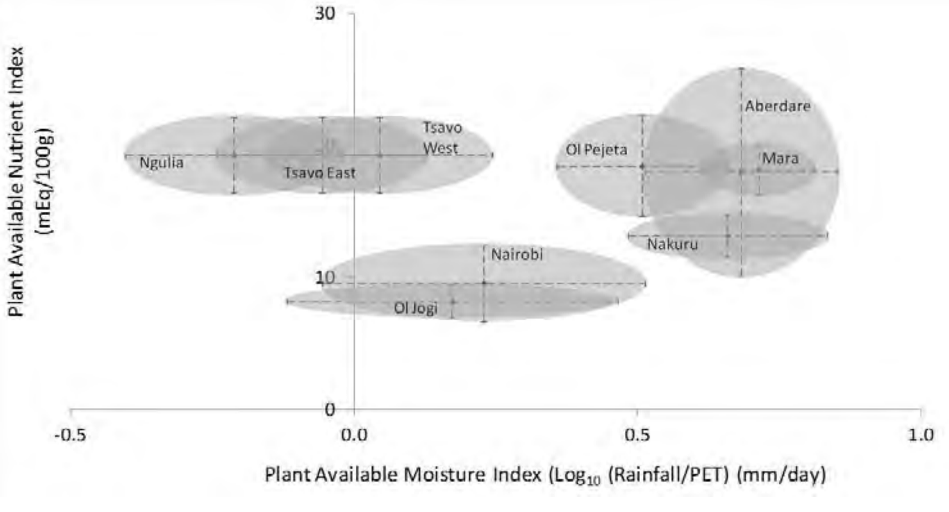

Figure 1.4: Nine black rhinoceros conservation areas plotted in the plane of Plant Available Moisture (PAM) and Plant Available Nutrient (PAN). The dotted lines give the 95% Confidence Intervals. For all values of PAM; *n* = 10. For values of PAN; *n* = 179 for Ngulia, Tsavo East & Tsavo West-IPZ, *n* = 17 for Ol Jogi, *n* =28 for Nairobi, *n* = 19 for Ol Pejeta, *n* = 47 for Nakuru, *n* = 23 for Aberdare, and *n* = 43 for Mara. PAN-values are from the literature and soil databases, PET = Potential evapotranspiration (*see* the methods in the chapters).

Table 1.1: Study areas listed in descending order of relative densities. Absolute and relative density estimates are shown with 95% confidence intervals (CI) with minimum (Min) and maximum (Max) ranges between 1993 and 2010. The size of the area utilized by black rhinos is indicated in brackets next to the name of each study area.

Name of study area & size (Km^2)	Absolute density (*n* = 18) (animals/km^2)			Max. stocking density (Adcock et. al. 2007)	Relative^a density (*n* = 18) (-)		
		Range				Range	
	Mean ±95% CI	Min.	Max.	(animals/km^2)	Mean ±95% CI	Min.	Max.
Ngulia (92)	0.69 ± 0.11	0.27	1.06	0.405	1.69 ± 0.25	0.67	2.63
Nairobi (117)	0.54 ± 0.02	0.46	0.68	0.339	1.60 ± 0.08	1.36	2.02
Ol Jogi (50)	0.35 ± 0.06	0.18	0.64	0.299	1.17 ± 0.19	0.60	2.14
Ol Pejeta (300)	0.28 ± 0.04	0.16	0.52	0.307	0.93 ± 0.15	0.53	1.68
Nakuru (144)	0.38 ± 0.06	0.22	0.58	0.514	0.74 ± 0.11	0.43	1.13
Aberdare (70)	0.45 ± 0.15	0.10	0.93	1.002	0.45 ± 0.15	0.10	0.93
Mara (1,510)	0.02 ± 0.00	0.02	0.03	0.069	0.35 ± 0.02	0.27	0.44
Tsavo East (1,800♣)	0.02 ± 0.01	0.02	0.03	No Est.	No Est.	No Est.	No Est.
Tsavo West IPZ^b (1,200♣)	0.01 ± 0.00	0.01	0.01	No Est.	No Est.	No Est.	No Est.

[a] The ratio of absolute density to the recommended maximum safe stocking density (no units).

[b] Newly established in 2008. Its absolute density averaged over 3 years (*n* = 3).

♣This is the area that is used by black rhino (on basis of field reports by rangers) within the entire Tsavo East NP of *ca.* 11,000km^2 and Tsavo West NP *ca.* 9,000km^2.

No Est. = No estimates available for maximum stocking densities

Outline of the thesis

Density dependence provides insights into the population dynamics of large herbivores and their interactions with their environment. **In Chapter 2, 'density dependence and population dynamics of black rhinoceros in Kenyan rhinoceros sanctuaries'**, I present results on the interrelationships between population density, sex ratio and underlying population growth rate (*r*) using four population growth models. The results do not show density dependence in black rhinoceros. In **chapter 3, 'yearly percentage of females calving in black rhinoceros (*D. b. michaeli*): is it density dependent?'**, I use longer term data on life history traits and focus on one measure of reproductive performance, *i.e*., yearly percentage of females calving (%FC) because of its longitudinal nature and its direct relationship with inter-calving interval. This measure inherently has higher sample size and statistical power than other measures of reproductive performance because it does not require very detailed information such as exact dates of birth for females as other measures such as age at first calving would require. Here I use two measures of density, *i.e*., absolute and relative densities, to test if populations that have exceeded these safe stocking densities indeed exhibit negative density feedback. The results are interpreted in consideration of soil nutrients (PAN) and soil moisture (PAM). I conclude that %FC is not density dependent but that PAM tends to play a significant role. In other words, both Chapters 2 and chapter 3 point in the same direction of lack of density dependence in the populations I studied. This leads me to further test the expectation that high population density may lead to population limitation due to low quality of forage. I control for effects of PAN and PAM which are known to influence quality of forage in **Chapter 4, 'the relationships between dietary and faecal mineral concentrations in black rhinoceros (*D. b. michaeli*) and the influence of population density on diet quality',** here I first show that faecal and dietary mineral concentrations are linked using data from feeding trials of black rhinoceroses in zoos. PAM comes out as the strongest explanatory variable for the observed changes in dietary mineral concentration. In this chapter too, I did not find density dependency, but instead, that bottom-up control processes seem to play a stronger role even though the densities were perhaps too low to fully support my alternative view**.** In **Chapter 5, 'reproductive performance in black rhinoceros (*D. b. michaeli*) as a function of Plant Available Moisture and Plant Available Nutrients'**, I test for the bottom-up control processes suggested in Chapter 4. Here, I incorporate woody cover as an indicator for available browse. I use three reproductive performance measures, *i.e*., age at first calving, Inter-calving intervals, and yearly percentage of females calving. I find reproductive performance measures to be better in areas of high PAM, sparse woody cover and low PAN. Lastly, because high population densities may result in elevated chronic physiological stress, which can lower reproductive performance, In **Chapter 6, 'physiological stress levels in black rhinoceros (*D. b. michaeli*) in relation to population density and extrinsic factors'**, I check for changes in corticosterone levels in relation to population density and browse quality as measured through faecal content. Here, I also consider the influence PAM and PAN on plant quality. I report a positive but not significant correlation between population density and levels of corticosterone. Physiological stress levels are found to increase as constituents of PAM (*i.e*., rainfall and temperature) increase. Corticosterone levels are also found to be inversely related to concentration of dietary phosphorous. Based on the consistency in findings of Chapters 2, 3, 4 & 6 that directly evaluate density dependence, I conclude that the populations of black rhinoceroses studied are not exhibiting negative density feedbacks. Bottom-up control processes through browse quality and quantity influenced mainly by PAM are the main factors controlling these populations. PAN does not seem to play a significant role. The bottom-up control processes are consistently shown in Chapters 4, 5 & 6. Finally In **Chapter 7, 'density dependence and conservation of a mega-herbivore: a synthesis'**, I attempt to answer my research questions by offering some short and long term solutions based on my results and a better understanding of the concept of density dependence. In this chapter, I also provide a critique of my study and suggest areas for further research.


Photo credit: B. Okita-Ouma

Chapter 2

Density dependence and population dynamics of black rhinoceros (*D. b. michaeli*) in Kenya's rhinoceros sanctuaries

Benson Okita-Ouma, Rajan Amin, Frank van Langevelde, and Nigel Leader-Williams

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Abstract

Density dependent feedback mechanisms provide insights into the population dynamics and interactions of large herbivores with their ecosystem. Sex ratio also has particularly important implications for the growth rates of many large mammal populations *through its influence on* reproductive potential. Therefore, the interrelationships between density dependent factors, comprising density, sex ratio and underlying growth rate (r) were examined for the eastern black rhinoceros (*Diceros bicornis michaeli*) living in three rhinoceros sanctuaries in Kenya, using four population models. The exponential and logistic models gave similar results and the former were accepted because they better portrayed the actual situation on the ground. Sex ratios in all sanctuary populations were positively correlated with *r* but interpreted with realization of other factors also affecting r. We caution that the results of population models should be interpreted alongside ground-truthed observations. We recommend that future translocation strategies should take into account sex and age structures of the donor population, while future studies of density dependence should take into account both biotic and abiotic factors.

Key words: *black rhinoceros, density dependence, population models, sanctuary, sex ratio, underlying growth rate*

Introduction

Density dependence can play an important role in regulating population dynamics of many species of mammals (Krebs 2002). Thus, increasing population densities can result in a situation where birth rates decline, while mortality rates and emigration increase. As a result, population density can rise to a level where net growth rate equals zero when food resources are limiting (Primack 2002). Likewise, changes in adult sex ratios can have important implications for the population dynamics of many large mammals (Verme and Ozoga 1981, Saltz and Rubenstein 1995), particularly through their influence on reproductive potential (*e.g.*, Owen-Smith 1988). Skewed sex ratios in large mammals may be indicative of chance in demographic factors in small populations and/or may be influenced by parental condition (Trivers and Willard 1973). Populations with adult sex ratios skewed in favour of females in adequate food supplies would be expected to achieve a higher growth rate(Owen-Smith 1988, Emslie 1999). Biased adult sex ratio can also influence the effective population size and levels of genetic heterozygosity (Kimura and Crow 1963, Lande and Barrowclough 1987, Nunney and Elam 1994). It is particularly important to understand how factors such as density dependence and skewed adult sex ratios can influence the population dynamics of endangered species like the black rhinoceros (*Diceros bicornis*) managed intensively *in situ*.

Severe poaching significantly reduced many black rhinoceros populations across Africa in the 1970s and 1980s (Emslie and Brooks 1999). In response, black rhinoceros Range States adopted measures to conserve them *in-situ*. These measures included creation of Rhinoceros Conservation Areas, Rhinoceros Conservancies, Rhinoceros Sanctuaries and Intensive Protection Zones (IPZs) (Brett 1990, Nduku and Martin 1993, Leader-Williams *et al.* 1997). In Kenya, small, fenced sanctuaries with adequate protection were established on State and private lands from the mid-1980s onwards. This approach led to a significant reduction in poaching, resulting in the gradual recovery of black rhinoceros numbers from 380 in 1987 to 458 in 2003 and 570 in 2007, thereby maintaining Kenya as the stronghold of 84% of the eastern black rhinoceroses *D. b. michaeli* in the wild (Emslie and Brooks 1999, Okita-Ouma and Kariuki 2008). Because Kenyan rhinoceros sanctuaries are small and mostly fenced, they require sound biological management. For the established sanctuaries, a growth rate of ≥6% per annum has been set by Kenya as its policy for the recovery of its black rhinoceros population (Okita-Ouma *et al.* 2007).

Black rhinoceros populations can grow with a long term maximum intrinsic growth rate of approximately 9% per annum, although young growing populations with very low adult mortality rates may grow even faster (Owen-Smith 1988, Emslie 1999). Metapopulation management through translocations has been the key feature of Kenya's rhinoceros conservation strategy to achieve higher growth rates. For example, translocation records for 1992 to 2003 show that Nairobi National Park (Nairobi) was primarily a source population that supported a total of 41 outward translocations and received one inward translocation. In contrast, Ngulia Rhinoceros Sanctuary (Ngulia) was primarily a recipient population that received 16 inward translocations and supported one outward translocation. Meanwhile, Lake Nakuru National Park (Nakuru) did not receive or support any translocations during this period.

Given the intensive programme of managing Kenya's black rhinoceroses within sanctuaries, it is important to understand relationships between rhinoceros density, sex ratio and growth rates in order to improve population performance and minimise genetic problems (Avise and Hamrick 1996, Woodruff 2001). This study analyses historical black rhinoceros monitoring data collected over 12 years in the three State land rhinoceros sanctuaries of Nairobi, Nakuru and Ngulia. These data encompassed basic demographic parameters including densities, births, deaths, translocations and sex and age structures. We seek to examine how changes in population density and adult sex ratios correlate with underlying intrinsic growth rates over time by using the most appropriate density dependence model and testing the hypothesis that increasing adult sex ratios in favour of females are positively correlated with intrinsic growth rates hereinafter also referred to as underlying growth rate. We conclude by recommending the best model for predicting underlying growth rates of intensively managed black rhinoceros populations, and the interventions necessary for improving underlying growth rates for these populations.

Methodology

Study areas

Lake Nakuru National Park (Nakuru)

Nakuru (0°50'S to 1°00'S and 36°20'E to 36°25'E) was designated as a bird sanctuary in 1960, upgraded into a national park in 1968, established as a RAMSAR site in 1974 and became a rhinoceros sanctuary in 1986. The completely fenced rhinoceros sanctuary covers 188 km^2 of which an area of 44 km^2 is a shallow highly alkaline soda lake. Nakuru ranges in altitude from 1200 m to 1750 m and has an average annual rainfall of 850 mm. Its vegetation comprises a mixture of open grassland, *Acacia*, *Tarchonanthus* bush land, deciduous and *Euphorbia* forests and riverine bush land (Mwasi 2002, Adcock *et al.* 2007).

Nairobi National Park (Nairobi)

Nairobi (2°18'S to 2°20'S and 36°23'E to 36°28'E) was established as the first national park in East Africa in 1945, and became a rhinoceros sanctuary in 1963. It is partially fenced covering 117 km^2. The unfenced 20km stretching along the southern boundary is intended to allow wild ungulate migration. Nairobi ranges in altitude from 1508 m to 1790 m and has an average annual rainfall of 800 mm. Its vegetation comprises deciduous forest, riverine thorn forests, shrubs and grasslands (Muya and Oguge 2000, Adcock *et al.* 2007).

Ngulia Rhinoceros Sanctuary (Ngulia)

Ngulia (3°01'S to 3°06'S and 38°06'E to 38°10'E) was established in 1985, and lies inside Tsavo West National Park. The completely fenced Ngulia was expanded twice before the study period, during which it covered 62 km^2. Ngulia was expanded for a third time in 2007 to reach 92 km^2 (Okita-Ouma *et al.* 2008), and was conceived of as a way to re-establish a large free-ranging population within a much larger national park. This objective was initiated in 2008 when 10 black rhinoceroses were translocated out of Ngulia into the larger Tsavo West. Ngulia ranges in altitude from 600 m to 1800 m, and has an average annual rainfall of 600 mm. Its vegetation is composed of mixed species bush land thickets, grasslands, shrubs, low tree and herbs (Adcock *et al.* 2007).

Data collection

Black rhinoceros populations have been monitored using an individual ID-based approach in each of the sanctuaries since their establishment. Rhinoceros monitoring staff patrol each sanctuary on a daily basis, on foot or by vehicle, mainly in early morning and late afternoon hours as it is the best time to sight mainly nocturnal black rhinoceroses. Monitoring staff record a set of standardised parameters for each rhinoceros sighting including identity (if known), location, group composition, behaviour, age and sex. Individual rhinoceroses are recognised by a combination of features including ear notches, distinctive body marks, horn shapes, age, and sex (Okita-Ouma *et al.* 2008, Okita-Ouma *et al.* 2011). Rhinoceroses that could not be recognised by any combination of these features were referred to as "clean". The ageing and sexing of rhinoceroses followed standard categories established by the IUCN's SSC-African Rhino Specialist Group (AfRSG) (Adcock and Emslie 2000). Birth and mortality data were also recorded using standardised data collection forms (Okita-Ouma *et al.* 2008). To ensure good quality data, the Kenyan Rhino Programme has implemented data quality control procedures. The sighting information is checked by experienced accredited observers using 'ID' master files which contain details of potential identification features such as horn shape, ear tears, deformities, body scars or tail shape. The data are then stored and analysed using a comprehensive geographic information database management system (Amin *et al.* 2001) in each rhinoceros area. Automatic retrieval of the data for further analysis was completed through computer scripting in MS-Access™ version 97 and Matlab™ version R12 software packages. These were also counter-checked by random manual calculations.

Data analysis

In order to better examine underlying population trends and to reduce the possible impact of year-to-year variations in the data, we based calculations of underlying growth rates on cumulative rhinoceros numbers, which is the growth of a population after allowing for removals and introductions and man-induced deaths such as poaching. Data analysis was restricted to the period 1992 and 2003 because historical information was more readily available and analysable from 1992 onwards. Underlying growth rate was calculated for the sum of rhinoceroses sighted in current year t and in the previous year t-1.

Different models were evaluated to test for density dependence in Kenya's black rhinoceros populations, following the approach of Cromsigt *et al.* (2002). This approach assumes the following relationship between the modelled population number, N(*t*), and the observed census population number, P(*t*), as;

$$P(t) = N(t) + \varepsilon(t)$$

where ε(*t*) is an error term that shows the difference between N(*t*) and P(*t*). The estimates for modelling N(*t*) are computed by minimizing the sum of squares of the error term. The models were evaluated based on the explained variance, R^2, as well as on the correlation between the predicted values and the observed values. This approach was applied to different models with a different structure of density dependence. We started with a simple exponential growth model, *i.e.*, the black rhinoceros population grows with a constant specific growth rate r:

$$N(t+1) = N(t) + r \times N(t)$$

The *ln*-transformed version of this model was also applied to test for the density dependence feedback. The exponential model was used as null model against which to compare the logistic model and the so-called Fowler model (Fowler 1981). This logistic model is formulated as:

$$N(t+1) = N(t) + N(t) \times r \times \left(1 - \frac{N(t)}{K}\right)$$

where K is the so-called carrying capacity. Whereas this logistic equation assumes a linear relationship between N*(t)* and the factor determines the strength of the density dependence, (1-N*(t)*/K). For large animals, Fowler (1981) suggested that it is more realistic to assume that density dependent factors play a more important role closer to K. Using parameter n, the relation between N*(t)* and $(1-(N(t)/K)^n)$ is curvilinear and makes the contribution of this factor relatively large close to K.

Effective rhinoceros density was expressed as numbers of rhinoceroses per km^2 of the entire sanctuary. In the case of the partially fenced Nairobi NP it was assumed that the number of rhinos straying out through the unfenced southern boundary was insignificant. This assumption was confirmed by the seasonal geographic locations of all rhinos as recorded in rhino information system. However, in the case of Nakuru, the 44 km^2 lake was excluded in density calculations. Adult sex ratio was derived by dividing the number of adult females by the number of adult males in the population at the end of year *t*.

Linear and non-linear regression analysis was used to investigate the density dependence models and the correlations between the underlying growth rate and sex ratio by use of Microsoft Office Excel 2003 and SPSS packages.

Results

The estimates of the regression coefficients for the different density dependence models are shown in Table 2.1. The results of the Fowler model are not reported as the standard errors of the estimates for the regression coefficients were much larger than the values of the estimates. We define the best fit as the model with the highest correlation coefficient (C) and the lowest standard error. For Nakuru NP, exponential and logistic models performed more or less the same but exponential model was selected because logistic model had very large standard error. The same reasoning of large standard error led to selection exponential model over the better logistic model for Nairobi NP. For Ngulia sanctuary, the logistic model was the best in describing the density dependence but it gave unrealistic high values for the growth rate hence exponential model was selected over it. The results for the selected models in Table 2.1 are shown as predicted population numbers for each sanctuary in Fig. 2.1.

Table 2.1: Results for three density dependence models for black rhinoceros in each rhinoceros sanctuary. The selected models are indicated in italic.

Nakuru	*r*	K	R^2	C
Exponential model	*0.068 (0.024)*	-	*0.91*	*0.954 (p<0.001)*
Exponential model with *ln*	0.017 (0.006)	-	0.89	0.954 (p<0.001)
Logistic model	0.118 (0.115)	122.4 (160.7)	0.91	0.955 (p<0.001)
Nairobi				
Exponential model	*0.030 (0.026)*	-	*0.30*	*0.660 (p=0.027)*
Exponential model with *ln*	0.008 (0.006)	-	0.26	0.657 (p=0.028)
Logistic model	0.048 (0.392)	160.4 (2094.6)	0.43	0.656 (p=0.028)
Ngulia				
Exponential model	*0.086 (0.022)*	-	*0.943*	*0.986 (p<0.001)*
Exponential model with *ln*	0.030 (0.01)	-	0.859	0.963 (p<0.001)
Logistic model	0.287 (0.084)	63.2 (8.7)	0.965	0.983 (p<0.001)

The symbols are as follows: *r* is the estimated underlying growth rate (standard error between brackets), K the estimated 'carrying capacity' (standard error between brackets), R^2 is the explained variance by the model, and C is the correlation coefficient between the observed and predicted population numbers.

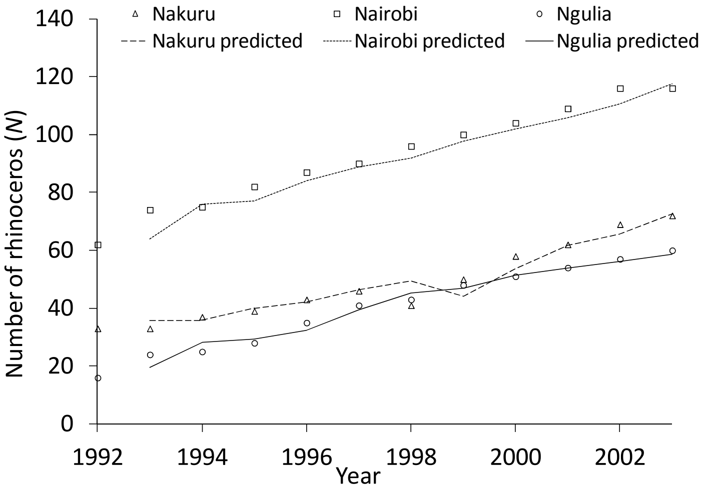

Figure 2.1: Observed (shapes) and predicted (lines) population numbers of black rhinoceros in each rhinoceros sanctuary. The predicted population numbers are based on the selected models given in Table. 2.1.

Densities of black rhinoceroses increased in each sanctuary between 1992 and 2003. Densities rose from 0.23 to 0.48 rhinoceroses/km^2 in Nakuru; from 0.44 to 0.64 rhinoceroses/km^2 in Nairobi; and from 0.26 to 0.94 rhinoceroses/km^2 in Ngulia (Fig. 2.2a). The underlying growth rates over the same period increased in Nakuru but decreased in Nairobi and Ngulia (Fig. 2.2b). Adult sex ratio in Nakuru remained in favour of males while in Nairobi and Ngulia it remained in favour of females (Fig. 2.2c).

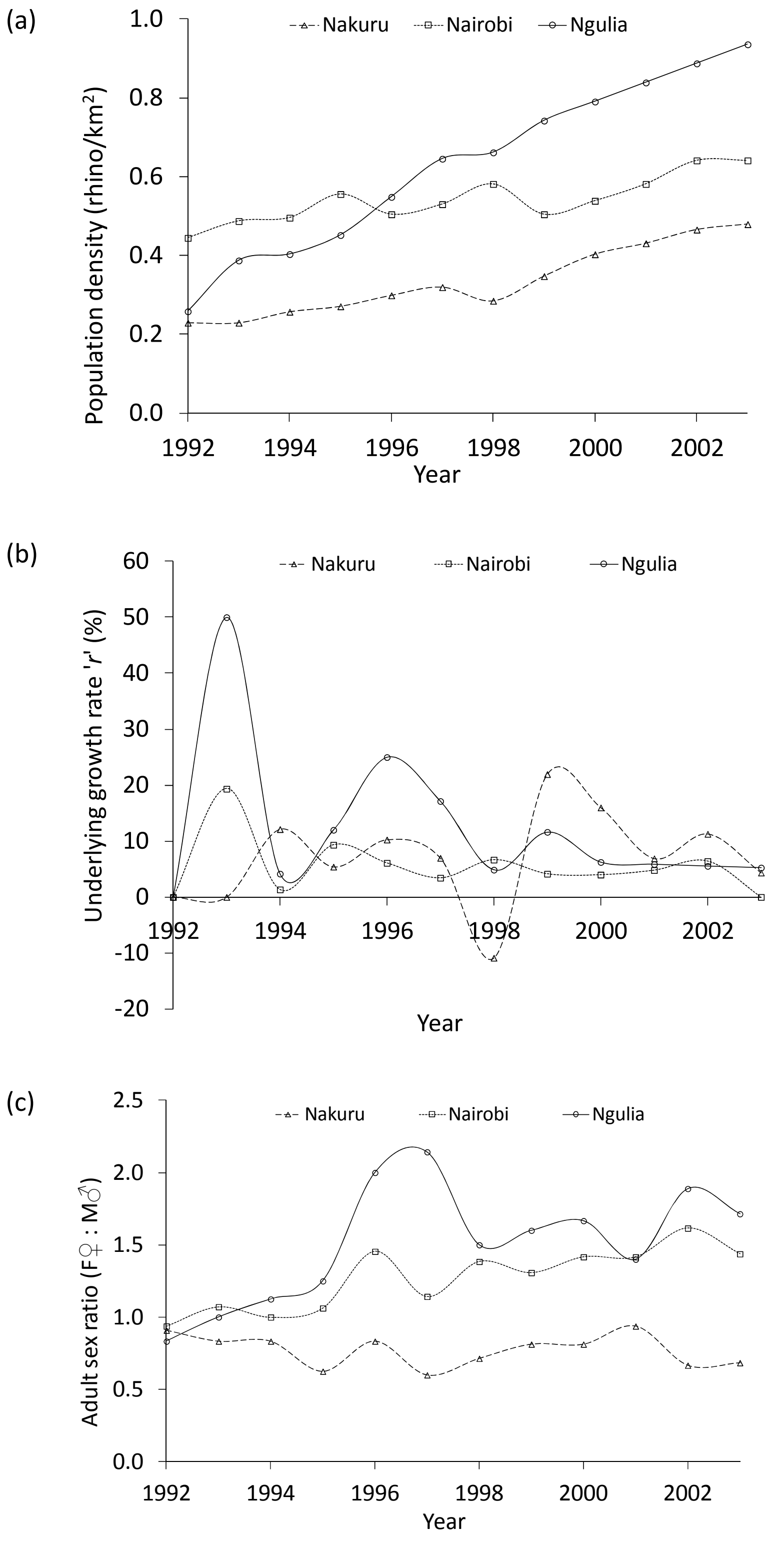

Figure 2.2: A comparison of annual trends in black rhino (a) density; (b) underlying growth rate '*r*'; and, (c) sex ratio in the three rhinoceros sanctuaries between 1992 and 2003.

All the three sanctuaries showed a positive relationship between underlying growth rate and sex ratio. In all cases, the intercept was not significant (Table 2.2, Fig. 2.3).

Table 2.2: Results of the regression analysis with underlying growth rate as the dependent variable and sex ratio as the independent variable.

	b_1	t	P	R^2
Nakuru	10.290 (3.303)	3.116	0.011	0.49
Nairobi	4.329 (1.255)	3.449	0.006	0.54
Ngulia	7.750 (2.755)	2.813	0.018	0.39

The symbols are as follows: b_1 is the estimated regression coefficient for the growth rate (standard error between brackets), t is the t-value of the regression coefficient and p is the corresponding p-value, R^2 is the explained variance by the model.

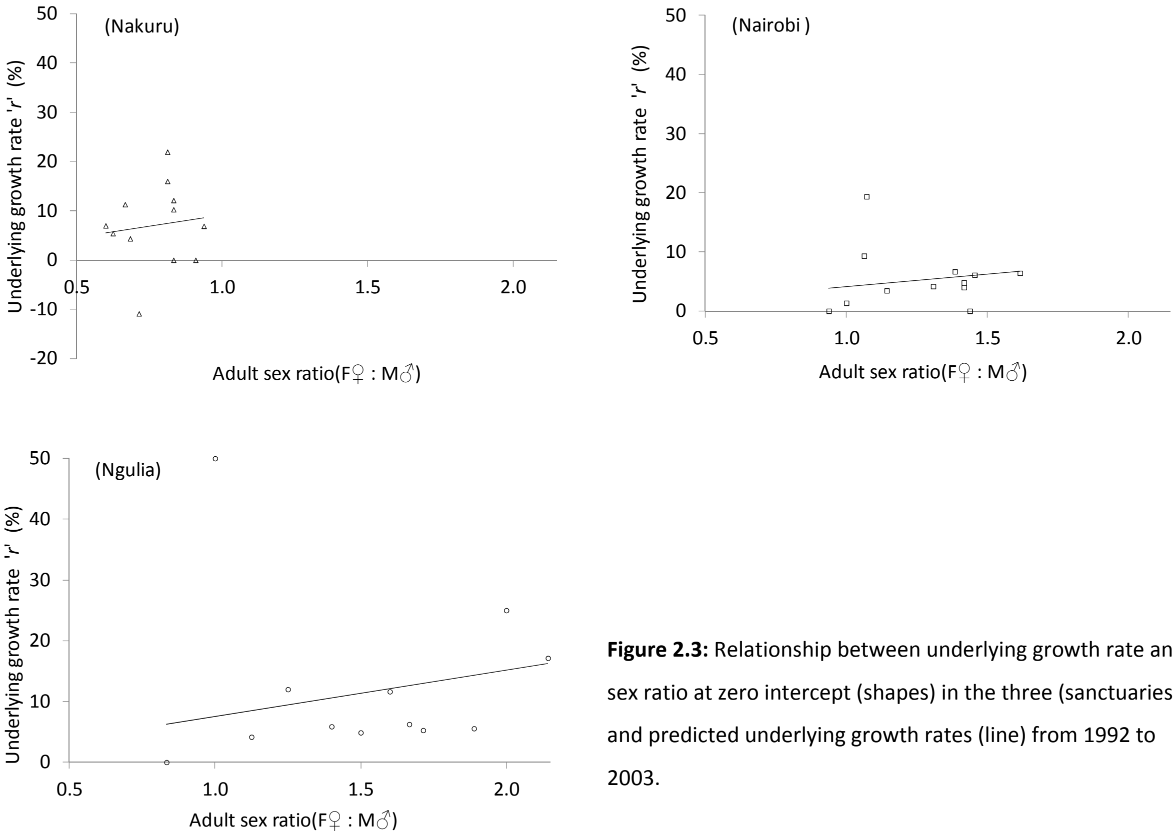

Figure 2.3: Relationship between underlying growth rate and sex ratio at zero intercept (shapes) in the three (sanctuaries) and predicted underlying growth rates (line) from 1992 to 2003.

Discussion

Density dependence models

Even though the logistic model provided the best fit to describing the trends in black rhinoceros numbers in the three sanctuaries it provided large standard errors and unrealistic values for *r* and K in some populations. For these reasons the exponential model which assumes black rhinoceros grows endlessly at a constant r, was selected since it gave realistic *r* but with similar significance levels as logistic model (Table 2.1, Fig. 2.1). Since the logistic model failed to give realistic *r* and K its assumption that black rhino populations will become limited at high densities by a lack of resources and that populations will possibly stabilize near an equilibrium density yet did not hold for these populations. This implied that there were no density dependent effects on *r* in any population. For the selected exponential model, we found maximum *r* to be between 0.6% and 10.8%. Other studies on rhinoceros found comparable growth rates varying between 4.7% and 11% (Hitchins and Anderson 1983, Moehlman *et al.* 1996, Loon and Polakow 1997, Cromsigt *et al.* 2002). The reason exponential models gave the best fit compared with other models, could have several explanations: i) overall, the degrees of freedom were only eleven per population hence the possibility of masking the occurrence of density dependence. ii) In Nakuru, the results could have actually confirmed that indeed the population had not reached its maximum stocking density for density dependence effects to start playing important role. Recent studies (Adcock *et al.* 2007) estimated highest stocking density for Nakuru as 0.51 rhinoceros/km^2 (74 animals) while the actual population size at the end of the study period was 69 animals. iii) In Nairobi, despite the translocation of 41 animals out of the national park over the study period the exponential growth ranged between 3.0% (± 2.6%). Population density averaged 0.54 rhinoceros/km^2 (63 animals) well above the expert maximum estimated stocking density of 0.34 rhinoceros/km^2 (40 animals) (Adcock *et al.* 2007). Given a relatively low *r*, translocations may not have fully achieved the primary objective of improving *r*. iv) In Ngulia, even though the exponential model gave the best fit, the relatively high *r* could be misleading partly because of the few degrees of freedom and the initial high growth rates recorded as a result of small population size. The reality on the ground was a declining *r* that was attributed to deteriorating habitat due to high density of other browsers (Okita-Ouma *et al.* 2008). Because of the highly degraded habitat by inter-specific competition from elephants (*Loxodonta africana*) and giraffes (*Giraffa camelopardalis*) (Brett and Adcock 2002), Ngulia's maximum stocking densities reduced from estimated 0.81 rhinoceros/km^2 (50 animals) (Foose *et al.* 1992) to 0.41 rhinoceros/km^2 (25 animals) (Adcock *et al.* 2007). Inter-specific competition itself may have been playing an important role on *r*. It is thus important to ground-truth results of any density dependent model especially for long lived slow growing herbivores as other factors could be playing a greater role.

Sex ratio and underlying growth rates

Based on our hypothesis we cautiously deduce from the model results that female biased sex ratios can contribute to the increase in r. The *r* in Nakuru increased despite a male biased sex ratio, in Nairobi the *r* remained very low despite the high female biased sex ratio, whilst in Ngulia ground-truthing indicated a declining *r* despite female biased sex ratio. Three factors, including low R^2, translocation strategies and density dependent effects underpin our cautionary deduction and also help in explaining these counter-intuitive predictions. The positive correlation between *r* and sex ratio in Nakuru could have meant that other factors for example females' ages at first calving and inter-calving intervals were excellent. The phenomenon of male-biased adult sex ratios has been observed in some enclosed black rhinoceros populations in South Africa (Adcock 2000) and in other wildlife species such as the re-introduced Asiatic wild ass (*Equus hemonius*) in Negev Desert, Israel (Saltz and Rubenstein 1995). The phenomenon arises from unexplained phenotypic or genotypic factors or through chance in small populations (Clutton-Brock *et al.* 1984). Whatever the underlying explanation of male-biased sex ratios in small populations of medium to large body sized herbivores, it is imperative to adopt translocation strategies that enhance *r*. In Nairobi, translocation records showed that translocation strategies targeted sub-adults, leaving behind females with calves, and very old or reproductively immature males unable to immediately continue with breeding. Previous discussion on the density dependent models reveal that Nairobi and Ngulia were mostly above their estimated maximum stocking densities hence the possibility of density dependence however the translocation strategy that targeted breeding females in Nairobi and the inter-specific competition in Ngulia could have played a more important role in limiting r than density of black rhinoceros. We therefore recommend that surplus males should be removed when the population is increasing in size. In addition, it is important that females with calves at >2 years old constitute the priority candidates for translocation (Brett *et al.* 2001). This mixed translocation strategy ensures a balanced sex and age structure for both the donor and recipient populations as successfully practiced in Zimbabwe(Brett *et al.* 2001) and currently being tried in Kenya.

Conclusion

We conclude by recommending that in evaluating density dependent models, it is important to interpret results alongside ground truthing observations. We suggest that future density dependence studies should evaluate densities relative to expert derived maximum stocking densities that take into account biotic and abiotic factors rather than literally using animal density of number of animals per unit area. Doing so would provide opportunity to assess accuracy of the estimated maximum stocking densities. We recommend that sex ratios must be continuously monitored and manipulated to achieve maximum productivity in intensively managed populations, such as those in Kenya's rhinoceros sanctuaries. We also recommend timely translocations and strategies that take into account age and sex structures of donor population. When carefully managed, Kenya's rhinoceros sanctuaries have great potential to provide a substantial and continuous surplus of rhinoceroses for re-stocking other areas. Our results have important implications for future management of black rhinoceroses, both in Kenya and elsewhere.

Acknowledgements

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Chapter 3

Yearly percentage of females calving in black rhinoceros (*D. b. michaeli*): is it density dependent?

Benson Okita-Ouma, Ignas M.A. Heitkönig, Sip E. van Wieren, Frank van Langevelde, Rajan Amin, and Herbert H.T. Prins

Abstract

The concept of density dependence is not well understood in its application to the conservation and management of mega-herbivores. This lack of understanding is partly due to a lack of reliable long-term data on life history traits of mega-herbivores and partly because of inaccuracies associated with the estimation of maximum stocking densities ('carrying capacities'). Browse plant also respond differently to Plant Available Nutrient (PAN) and Plant Available Moisture (PAM) in the semi-arid tropics. In an attempt to improve our understanding of density dependence in mega-herbivores, we studied long-term (1993 to 2010) variability of yearly percentage of females calving (%FC) in the browsing black rhinoceros (*D. b. michaeli*) in seven populations. The populations were of different densities and of varying PAN and PAM in Kenya savanna ecosystems. We used two indices of population densities of black rhinoceros: absolute density (animals/km^2) and relative density *i.e.*, the ratio of absolute density to a priori estimated maximum stocking density. We hypothesised a decrease in the %FC in black rhinoceros with increasing population density. We also hypothesised that the rate of decrease in the %FC due to density feedbacks (if any) should be constant and identical between populations where they occur. Generalized Estimating Equations (GEEs) and t-test revealed that %FC decreased with increase in population density in 1 (Ol Pejeta) out of 5 populations of high relative densities (0.5 and above). The %FC of 4 out 5 populations of high densities remained optimal. Generally these high density populations were located in areas of PAN and PAM favourable for the production of abundant browse plants. Interestingly, the %FC in 2 populations (Aberdare and Mara) of low relative densities (less than 0.5) decreased with increasing density. The slope of %FC against density was identical between Ol Pejeta and Mara. Apparently in a population where density dependence seemed to occur, that population was either in an area of PAN and PAM that is not favourable for production of abundant browse plants, or that population was affected by inter-specific competition, or that population was significantly affected by poaching. We therefore concluded that the %FC in the populations of black rhinoceros studied were not density dependent but seemed to be controlled by PAN and PAM.

Key words: black rhinoceros, calving, density dependence, populations, soil moisture, soil nutrients, reproductive performance

Introduction

Density dependence is an important concept in the conservation and management of wildlife, yet it is not well understood. Negative density dependence occurs when per-capita population growth decreases with increases in density, whereas a positive dependence - also known as the Allee effect (Allee 1931) - occurs when per-capita population growth rate increases with increasing density (Fig. 3.1).

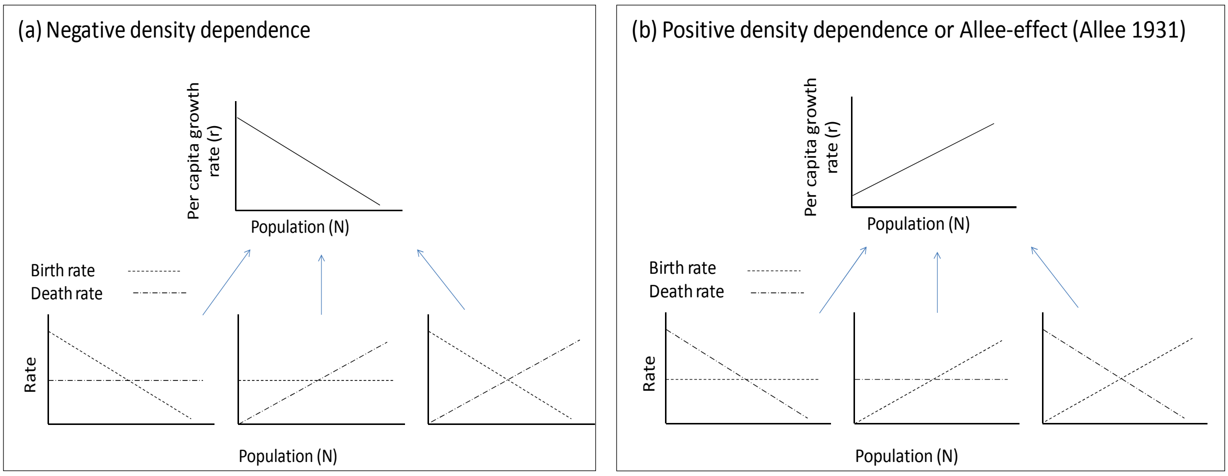

Figure 3.1: Schematic illustration of negative and positive dependence of reproductive performance on population density. a) Per capita growth rate decreases with increasing population size. b) Per capita growth rate increases with increasing population size, also known as Allee effect (Allee 1931).

In applying the concept of density dependence and mathematical models associated with it, conservation managers maintain animal numbers at around a putative maximum sustained yield (MSY). In the classic logistic model (Verhulst 1838, Gabriel *et al.* 2005), MSY is at K/2 (*i.e.*, halfway the ‘carrying capacity’ – ‘K’). However, it has become clear that this model does not fit mega-herbivores (Fowler 1987) and in some instances its application in other species of animals has been questioned (*e.g.*, Sibly *et al.* 2005, Doncaster 2006, Peacock and Garshelis 2006, Ross 2006). Recently, Peacock and Garshelis (2006) concluded from many long term studies of population changes in large mammals that these species exhibit strong density dependence only near K. If this would also be the case in mega-herbivores then density dependence would occur at a point greater than K/2, such as around 70 - 80% of K (*e.g.*, Owen-Smith 1988, McCullough 1992, McCullough 1999). Once density dependence sets in, it can be assumed that the intensity should be constant, *i.e.*, the slopes of reproductive performance against population density should be constant and identical between populations (Fig. 3.2).

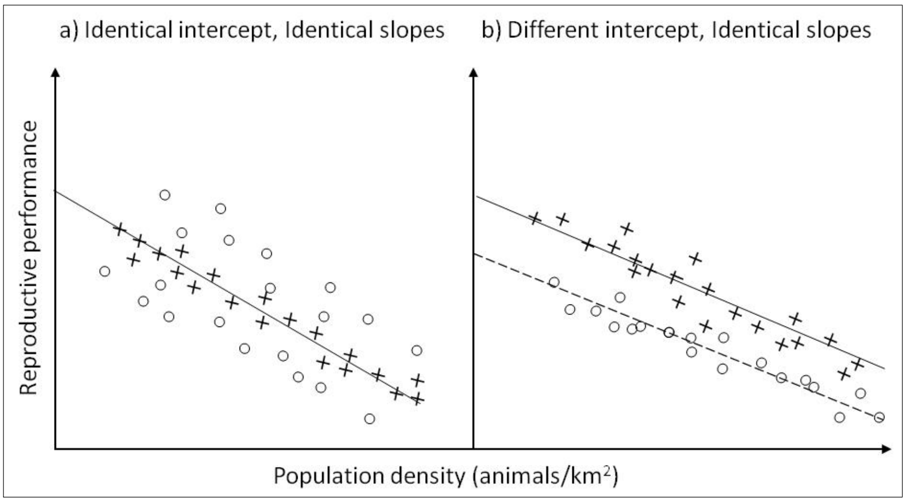

Figure 3.2: Hypothetical illustration of the expected response of reproductive performance to population density between two populations. Density dependence may start at the same or different densities (intercepts) but the intensities (slopes) between populations where density dependence occurs should be identical if other factors are constant.

It is also known that per-capita population growth rate of a species subject to sufficient environmental variability is governed by key resources that are largely independent of population density (*e.g.*, Behnke and Scoones 1993, Scoones 1994, Illius and O'Connor 2000). Recent studies of mega-herbivores indicated that the elephants (*Loxodonta africana*) in Addo National Park maximally reproduce despite their numbers and overshoot what was estimated as K for elephants in that Park (Gough and Kerley 2006). Morgan *et al.* (2009) also reported maximal reproductive performance in black rhinoceros (*Diceros bicornis*) in Mun-ya-Wana Game Reserve, South Africa despite having exceeded the a priori estimated maximum stocking densities ('carrying capacity') for the Reserve. These findings could mean that there was no density dependence, or that the carrying capacity estimates were incorrect, or that the carrying capacity of the area kept changing with environmental stochasticity. These populations were therefore controlled bottom-up by key resources (*e.g.*, Illius and O'Connor 2000).

Notably, most studies on density dependence in long-living mega-herbivores are largely based on short-term as opposed to long-term reliable data on life history traits (Messier 1994, Turchin 1995, Saether *et al.* 1996, Coulson *et al.* 2004, Simard *et al.* 2008). One long-term and often cited study of density dependence in black rhinoceros was conducted in Hluhluwe-Umfolozi Game Reserve, South Africa. Fecundity in black rhinoceros was reported to decrease with increasing population density (Hitchins and Anderson 1983). Emslie (1999) attributed this decline in fecundity between 1960s and mid-1980s to habitat changes resulting from over browsing. Hitchins and Anderson (1983) also reported mass die-offs with most deaths found where densities were highest (1.7 rhinoceros/km^2). Post-mortem examination of causes of these mass die-offs were not conclusive (Neitz 1962). Very similar mass die-offs of black rhinoceroses as reported by Hitchins and Anderson (1983) occurred at Ol Jogi Pyramid in Kenya. The Kenyan case was linked to clostridial enterotoxaemia (Ndeereh *et al.* 2012). Density of black rhinoceros prior to these mass die-offs was 0.3 animals/km^2 (Okita-Ouma *et al.* 2007b). The highest population density of black rhinoceros that was published (without investigating density dependence) was 4 rhinoceros/km^2 in approx. 540 km^2 within the larger the Tsavo East National Park in Kenya (Goddard 1969).

These uncertainties on density dependence in mega-herbivores are compounded further by estimates of 'carrying capacity', which are often subjective and can vary significantly over time. The population census data upon which 'carrying capacity' are derived also sometimes lack precision and accuracy. The point at which density dependence sets in mega-herbivores is therefore not known. However, despite these uncertainties, and because of the need to increase the numbers of especially Critically Endangered mega-herbivores, some conservation managers have resorted to maintaining populations of these species at a putative MSY of 70 - 80% of 'carrying capacity' (Owen-Smith 1988). This is a cautionary approach to avoid density dependence and maximize reproduction. Some institutions have consequently embedded this cautionary approach into their policy guidelines for the conservation of such endangered mega-herbivores (*e.g.*, Okita-Ouma *et al.* 2007b, Goodman *et al.* 2008, KWS 2012, Litoroh *et al.* 2012, MET 2012, KWS 2013). An alternative approach to circumvent these uncertainties is the set percentage translocation, where 'surplus' animals are periodically removed at a set percentage that is lower than the intrinsic population growth rate (Goodman 2001).

Here we choose to study density dependence in black rhinoceros (*D. b. michaeli*) in an attempt to improve our understanding of density dependence in mega-herbivores. Apart from its Critically Endangered status (IUCN 2013b), this mega browser is significantly different from other mega-herbivores like the elephant and white rhinoceros (*Ceratotherium simum*). We can argue that black rhinoceros live in a more stable environment than the elephants or white rhinoceros because it is a browser and the browse food supply shows less temporal fluctuation than that of mixed feeders or grazers (*e.g.*, Owen-Smith 2008). Furthermore, black rhinoceros have a less roaming behaviour (*review in* Kingdon and Hoffmann 2013) than elephants and have thus fewer possibilities to mediate environmental fluctuations by moving across large distances. For example, its twig-removing feeding behaviour (*see review in* Emslie and Adcock 2013) suggest that the black rhinoceros may have a potentially large impact on its habitat heterogeneity and nutrient recycling (Owen-Smith 1988, Hansen and Galetti 2009), especially if enclosed at high density (Luske *et al.* 2009). By virtue of its large body size, the black rhinoceros like other mega-herbivores does not have effective predators apart from man to regulate or control its population top-down hence its population is mainly controlled by food resources through bottom-up processes (*e.g.*, Fritz *et al.* 2002). Its hind-gut digestive system can process plants of low nutritional quality such as *Euphorbia* spp. with high concentrations of secondary compounds (*e.g.*, Goddard 1968, 1970, Mukinya 1977, Hitchins 1979, Hall-Martin *et al.* 1982, Luske *et al.* 2009). Long term and reliable monitoring data for this species is also available in Kenya (Amin *et al.* 2001, Mulama *et al.* 2005) providing an opportunity for an in-depth trend analysis. Currently this species, like other species of rhinoceroses, represents a major conservation challenge (*e.g.*, Milliken and Shaw 2012, Prins and Okita-

Ouma 2013) that requires high compensatory population growth rates. These reasons make this species suitable for a study on consumer-resource density feedbacks and carrying capacity estimates.

We therefore evaluated density dependence in the black rhinoceros by studying yearly percentage of females calving (%FC) between 1993 and 2010. The populations were of different densities and varied in Plant Available Nutrient (PAN) and Plant Available Moisture (PAM). PAN and PAM have been used as standard indices to characterise habitats and the heterogeneity in forage quality and quantity (*e.g.*, Walker and Langridge 1997, Olff *et al.* 2002, Ahrestani *et al.* 2011). We used two indices of population densities of black rhinoceros: absolute density (animals/km^2) and relative density *i.e.*, the ratio of absolute density to a priori estimated maximum stocking density ('carrying capacity' (Adcock *et al.* 2007)). We hypothesised a decrease in the %FC in black rhinoceros as its population density increased, and that this effect is stronger in populations of a generally high level of density. We also hypothesised that the rate of decrease in the %FC due to density feedbacks (if any) should be constant and identical between populations where they occur. We used relative density because it is derived from estimates of 'carrying capacity' which is integral in the concept of density dependence. Using relative density therefore offers an opportunity to further investigate the applicability of the 'carrying capacity, models and their derived MSY models.

Methods

Study areas

Aberdare National Park (Aberdare)

Aberdare lies between 36^0 25'E to 37^0 00'E and 0^0 05'S to 0^0 45'S (Fig. 3.3) at an altitude range of 1,829 m to 4,001m a.s.l. It was gazetted as a National Park in 1950 and in 1988 was listed as a Rhino Conservation Area. The Park is ring-fenced and covers an area of 766 km^2 with the rhino population concentrated in a section of the Park called Salient about 70 km^2. The rhino density ranged between 0.1 animals/km^2 and 0.9 animals/km^2 between 1993 and 2010. There was however a gradual decline of rhinoceros number during this period mainly attributed poaching. The average annual rainfall (± SD) over the period of study was 607 ± 149 mm (www.cpc.ncep.noaa.gov). The mean plant available nutrient index was 18.0 ±7.9 mEq/100g at 95% C.I., *n*=23 while plant available moisture index was 0.7 ±0.2 mm day^{-1} at 95% C.I., *n*=10 (*see* Chapters 4 and 5 this thesis). The vegetation is characterized by montane forest habitat with an undergrowth of mixed species of bush land thickets (Adcock *et al.*, 2007). Higher altitudes are dominated by bamboo forest and grass tussocks on the moorlands. Soil typology is described by Sombroek *et al.* (1982).

Lake Nakuru National Park (Nakuru)

Nakuru is located between 36^0 20'E to 36^0 25'E and 0^0 50'S to 1^0 00'S (Fig. 3.3) at an altitude range of 1,753m to 2,073m a.s.l. The Park was gazetted in 1961 and established as a Rhino Sanctuary in 1986. It is completely fenced, covering an area of 188 km^2, of which 44 km^2 lies in the shallow highly alkaline soda lake. The density of black rhino gradually increased from 0.2 animals km^{-2} in 1993 to 0.6 animals km^{-2} in 2010 with 35 rhinos translocated to other protected areas between 2004 and 2010. The average annual rainfall over the period of study was 755 ± 169 mm (www.cpc.ncep.noaa.gov). The mean plant available nutrient index was 13.2 ±1.5 mEq/100g at 95% C.I., n=47 while plant available moisture index was 0.7 ±0.2 mm day^{-1} at 95% C.I., n=10 (*see* Chapters 4 and 5). The vegetation is a mixture of open grassland, *Acacia*, *Tarchonanthus* bush land, deciduous and *Euphorbia* forests and riverine bush land (Maskall and Thornton 1989, Adcock *et al.* 2007, Okita-Ouma *et al.* 2009). Soil is dominated by lacustrine and riverine sediments and volcanic ash (Sombroek *et al.* 1982).

Masai Mara National Reserve (Mara)

Mara is located between $34^0$45' to $35^0$25'E and $1^0$13' to $1^0$45'S (Fig. 3.3) at an altitude range of 1,500m to 2,180m a.s.l. The reserve has been a black rhino conservation area since it was gazetted as a protected area in 1958. Mara is not fenced and covers an area of 1510 km^2 in the south-west of Kenya and is part of Tanzania's Serengeti National Park ecosystem. Density of black rhino for the entire reserve has remained at less than 0.1 animals km^{-2} between 1993 and 2010. The average annual rainfall over the period of study was 911 ± 147 mm (www.cpc.ncep.noaa.gov). The mean plant available nutrient index was 18.2 ±1.9 mEq/100g at 95% C.I., n=43 while plant available moisture index was 0.7 ±0.1 mm day^{-1} at 95% C.I., n=10 (*see* Chapters 4 and 5). The situation was managed by removing 255 elephant (Okita-Ouma *et al.* 2008a, Okita-Ouma *et al.* 2009), 200 buffalo and 12 black rhinoceroses. The vegetation comprises mainly of grassland with isolated scrublands and woodlands especially along drainage lines (Walpole 2002, Adcock *et al.* 2007, Kanga *et al.* 2011). Soil typology is described by Sombroek *et al.* (1982).

Ngulia Rhino Sanctuary (Ngulia)

Ngulia is located between $38^0$06'E to $38^0$10'E and $3^0$01'S to $3^0$06'S (Fig. 3.3) within Tsavo West National Park at an altitude range of 1,500 m to 1,800 m a.s.l. It was formed in 1985 as part of a longer term strategy to re-establish a large free-ranging black rhino population within the Park. The fenced sanctuary has progressively expanded from 3 km^2 in 1985 to 20 km^2 in 1987 to 63 km^2 in 1990 and to the current 92 km^2 in 2007. Density of black rhino ranged between 0.3 and 1.1 animals km^{-2} between 1993 and 2010. The expansion of the sanctuary in 2007 and the translocation of 12 rhinoceroses in 2008 and 2009 into the surrounding unfenced Intensive Protection Zone decreased the density from 1.1 animals km^{-2} to 0.7 animal km^{-2} in 2010. The average annual rainfall over the period of study was 385 ± 146 mm (www.cpc.ncep.noaa.gov). The mean plant available nutrient index was 19.3 ±2.8 mEq/100g at 95% C.I., n=179 while plant available moisture index was -0.2 ±0.2 mm day^{-1} at 95% C.I., n=10 (*see* Chapters 4 and 5). The habitat was significantly impacted by the high density of herbivores mainly elephant (*Loxodonta africana*), buffalo (*Syncerus caffer*) and the black rhino in the mid 2000's (Brett and Adcock 2002). The vegetation is predominantly *Commiphora* and *Acacia* woodland with scattered baobab trees (Leader-Williams *et al.* 1993, Brett and Adcock 2002, Adcock *et al.* 2007). Soil typology is described by Sombroek *et al.* (1982).

Nairobi National Park (Nairobi)

Nairobi is located between 36^0 23'E to 36^0 28'E and 2^0 18'S to 2^0 20'S (Fig. 3.3). It covers an area of 117 km^2 at an altitude range of 1,533m to 1,760m a.s.l. It was gazetted as a protected area in 1946 and made a rhino sanctuary in 1963. The Park is ring-fenced but approximately 20 km perimeter on its southern boundary remains unfenced as part of wildlife migratory corridor (Gichohi 2000). Density of black rhino ranged between 0.5 and 0.7 animals km^{-2} with 67 black rhinos translocated to other reserves between 1993 and 2010. The average rainfall over the period of study was 540 ±151 mm (www.cpc.ncep.noaa.gov). The mean plant available nutrient index was 9.6 ±2.9 mEq/100g at 95% C.I., *n*=28 while plant available moisture index was 0.2 ±0.2 mm day^{-1} at 95% C.I., *n*=10 (*see* Chapters 4 and 5). Major vegetation communities comprises deciduous forest, riverine thorn forests, shrubs and grasslands (Muya and Oguge 2000, Adcock *et al.* 2007). Soil typology is described by Sombroek *et al.* (1982).

Ol Jogi Pyramid (Ol Jogi)

Ol Jogi is located between 37^0 00'E to 37^0 05'E and 0^0 15'N to 0^0 20'N (Fig. 3.3) at an altitude range of 1,600m to 1,700m a.s.l. It is completely fenced covering an area of 50 km^2 and was established as a rhino sanctuary in 1979. Density of black rhino steadily increased from 0.2 animals km^{-2} in 1993 to 0.5 animal km^{-2} in 2010 with 30 animals translocated to other reserves between 1993 and 2010. Nine black rhinos died from clostridial enterotoxaemia in 2010 (Ndeereh *et al.* 2012). The average annual rainfall over the period of study was 459 ± 113 mm (www.cpc.ncep.noaa.gov). The mean plant available nutrient index was 8.2 ±1.3 mEq/100g at 95% C.I., *n*=17 while plant available moisture index was 0.2 ±0.3 mm day^{-1} at 95% C.I., *n*=10 (*see* Chapters 4 and 5). The vegetation comprises a mosaic of grassland, *Acacia* woodland and shrubs (Mizutani 1999, Adcock *et al.* 2007). Soil typology is described by Sombroek et al (1982).

Ol Pejeta Conservancy (Ol Pejeta)

Ol Pejeta is located between 36^0 40'E to 37^0 00'E and 00^0 02'S to 00^0 07'N (Fig. 3.3) at an altitude range of 1,670m to 1,820m a.s.l. It was designated as a black rhino sanctuary in 1988. It is completely fenced with a design allowing the migration of wildlife species except rhinoceros. The conservancy covers an area of 300 km^2 expanded from 93 km^2 in 2007. Density of black rhino gradually increased from 0.2 to 0.5 animals km^{-2} between 1993 and 2006. However with the expansion of the sanctuary in 2007, density decreased to 0.2 animals km^{-2} in 2007 and gradually increased to 0.3 animals km^{-2} by 2010 with the population supplemented by 27 animals in 2007. The average annual rainfall over the period of study was 557 ± 115 mm (www.cpc.ncep.noaa.gov). The mean plant available nutrient index was 18.4 ±3.8 mEq/100g at 95% C.I., *n*=19 while mean plant available moisture index was 0.5 ±0.1 mm day^{-1} at 95% C.I., *n*=10 (*see* Chapters 4 and 5). The vegetation is a mosaic of grassland, *Acacia* woodland, *Euclea* shrub and riverine woodland grassland (Birkett 2002, Adcock *et al.* 2007). Soil typology is described by Sombroek *et al.* (1982).

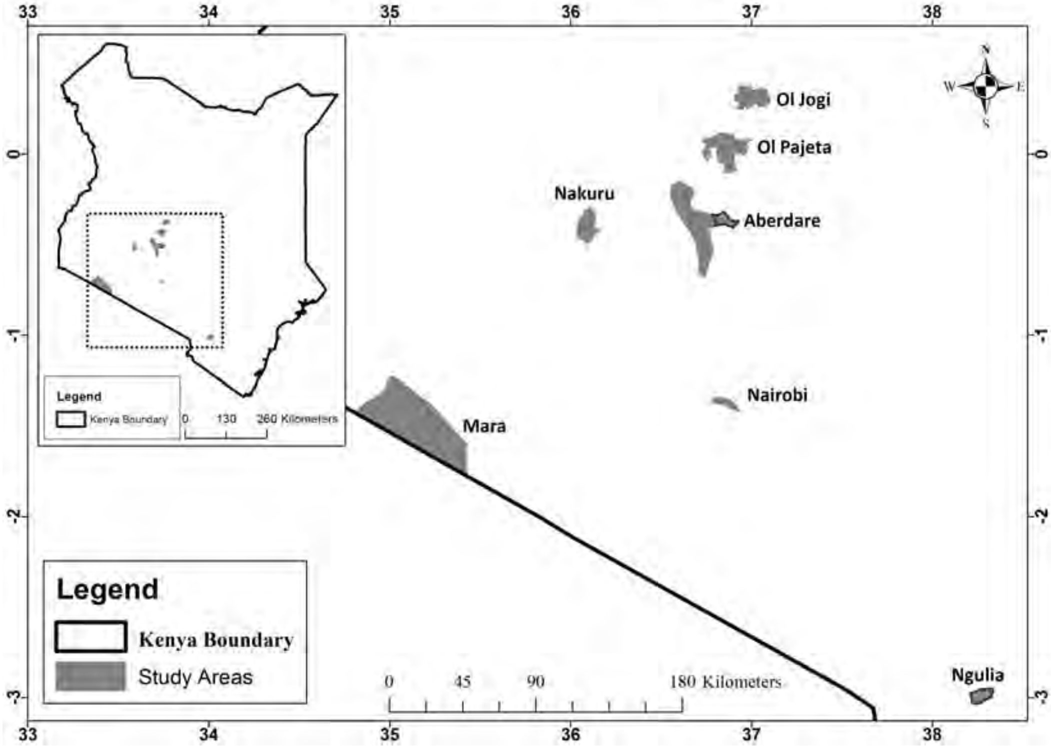

Figure 3.3: Locations of the seven study areas on the map of Kenya. The section of Aberdare where the study was carried is shown by bold border line.

Study design

The study was carried out in seven black rhino populations of contrasting densities and distinct plant available nutrient and moisture, namely Aberdare National Park (Aberdare), Lake Nakuru National Park (Nakuru), Masai Mara National Reserve (Mara), Nairobi National Park (Nairobi), Ngulia Rhino Sanctuary (Ngulia), Ol Jogi Pyramid (Ol Jogi) and Ol Pejeta Conservancy (Ol Pejeta). These populations were established before 1993 with monitoring data extending back to 1993 stored in the Kenya Rhino Information System (Amin *et al.* 2001). The monitoring is done by rangers through a standardized programme of patrols to obtain information on rhinoceros sightings. Rhinoceros are identified individually and registers of the features of individual animals are maintained. Where needed, recordings are also made of sightings of 'clean' rhinoceros (*i.e.,* those that are not individually recognizable) (Mulama *et al.* 2005)

Data analyses

Yearly percentage of females calving (%FC) was used as the measure for reproductive performance and was derived as the proportion of number of calves born in year ***t*** to the number of live adult females (≥ 7years) in year ***t***. The %FC is selected firstly as a measure of reproductive performance over other measures because of its longitudinal nature and its direct relationship with the inter-calving interval. Secondly, the %FC does not require very detailed individual animal information such as exact dates of birth for females as would be required for other reproductive performance measures such as inter-calving interval (ICI) or age at first calving (AFC) (duToit *et al.* 2001), which facilitates a higher sample size and greater statistical power.

Absolute density was estimated by first summing the total number of black rhinos, number of births and number of inward translocation in year ***t***, and then subtracting the sum total of number of deaths and number translocated outward in year ***t.*** The results were then divided by area (km^2) of study area that was assumed available to black rhinoceros. The available area for black rhinoceroses in vast study areas (>1,000km^2), especially Mara, was estimated from long-term home-ranges of rhinos as recorded in Kenya's rhino information system (Amin *et al.* 2001). In the case of Lake Nakuru National Park, approximately 44 km^2 occupied by the lake was excluded in the calculation of density. Absolute density relative to maximum stocking density (relative density) was derived as the ratio of absolute density to the estimated maximum stocking density (Adcock *et al.* 2007). The estimated maximum stocking density took into account factors such as browse availability, browse suitability, competing browsers, rainfall, soil nutrient, temperature and fire (Adcock *et al.* 2007).

Statistical analyses

We used IBM® SPSS® Statistics 19 for the statistical analyses (Norusis 2011). We applied the Generalized Estimating Equation (GEE) approach (Zeger *et al.* 1988), an extension of generalized linear models, to investigate the response of the longitudinal yearly percentage of females calving (%FC) to population density. The GEE approach with an identity link function based on a normal distribution was the appropriate statistical procedure for this type of data given that %FC has a between-year dependency arising from its annual measurements from the same animals. The dependent variable %FC was divided by 100 to obtain proportions and then arcsine transformed to achieve a normal distribution (we tested for a normal distribution of the residuals). We modelled this dependent variable separately against the absolute and relative population densities calculated at one- and two-year lags. We lagged the densities because possible delays in density feedbacks (Holyoak 1994). Lagging density would also cover the approximately 15 months gestation period for a black rhinoceros (*see review in* Emslie and Adcock 2013a). We applied 'Year' as subject variable. The GEE model with the smallest Corrected Quasi Likelihood under Independence Model Criterion (QICC) was selected in terms of their goodness of fit. Two-year lag was selected over one-year lag as the best fitted model (Appendix 3.1). The response of %FC to density for Ngulia was derived for two scenarios: the first scenario excluded the expansion of the sanctuary from 63 km^2 to 92 km^2 in 2007 in the calculation of population density, while the second scenario included this expansion.

We used the t-test to test for significant difference in the intensity of %FC as a function of absolute and relative density on reproductive performance between and within populations. We assumed equal variances for slopes between populations. We compared the slopes (a_1 and a_2) by computing t-statistic as the difference between the two slopes divided by the standard error (*S*) of the difference between the two slopes (Equation 1).

$t = \frac{a_1 - a_2}{s_{a_1 - a_2}}$, where $s_{a_1 - a_2} = \sqrt{s_{a_1}^2 + s_{a_2}^2}$ at $df = n_1 + n_2 - 2$ (Wuensch 2007); (Equation 1).

Results

Absolute density ranged from 0.02 animals/km^2 to 0.69 animals/km^2 and relative densities between 0.35 and 1.69 (Table 3.1).

Table 3.1: Absolute density estimates with 95% confidence intervals (C.I.) and their value relative to maximum stocking densities of black rhino in seven Kenyan conservation areas between 1993 and 2010.

	Absolute density (*n*=18 years) (rhinos/km^2)			Max. stocking density	Relative[1] density (*n*=18 years) (-)		
Study Area	Mean ±95% C.I.	Min.	Max.	(rhinos/km^2)	Mean ±95% C.I.	Min.	Max.
Aberdare	0.45 ± 0.15	0.10	0.93	1.002	0.45 ± 0.15	0.10	0.93
Nakuru	0.38 ± 0.06	0.22	0.58	0.514	0.74 ± 0.11	0.43	1.13
Mara	0.02 ± 0.00	0.02	0.03	0.069	0.35 ± 0.02	0.27	0.44
Ngulia	0.69 ± 0.11	0.27	1.06	0.405	1.69 ± 0.25	0.67	2.63
Nairobi	0.54 ± 0.02	0.46	0.68	0.339	1.60 ± 0.08	1.36	2.02
Ol Jogi	0.35 ± 0.06	0.18	0.64	0.299	1.17 ± 0.19	0.60	2.14
Ol Pejeta	0.28 ± 0.04	0.16	0.52	0.307	0.93 ± 0.15	0.53	1.68

[1] The ratio of absolute density to maximum stocking density (Adcock *et al.* 2007).

The yearly percentage of females calving (%FC) decreased with increasing absolute and relative density in all populations, except in Ol Jogi where %FC increased with increasing absolute and relative density (Fig. 3.4). These relationships were however only statistically significant in Aberdare, Mara and Ol Pejeta (Table 3.2a and 3.2b). These results were also consistent with the higher inter-calving intervals (ICI) of 3.4 ± 0.4 years at 95% C.I. (*n*=35) for Aberdare, Mara and Ol Pejeta compared to the lower ICI of 3.0 ± 0.2 years 95% C.I. (*n*=50) for Nakuru, Nairobi and Ol Jogi and of 3.1 ± 0.4 years 95% C.I. (*n*=15) for Ngulia (*see* Chapter 5 this thesis). Even though not significantly different (t <2.042, d.f. =30, P>0.05; Appendix 3.2a), the slopes of %FC against relative density in all populations were less steep compared to the slopes of %FC against absolute density (Fig. 3.4).

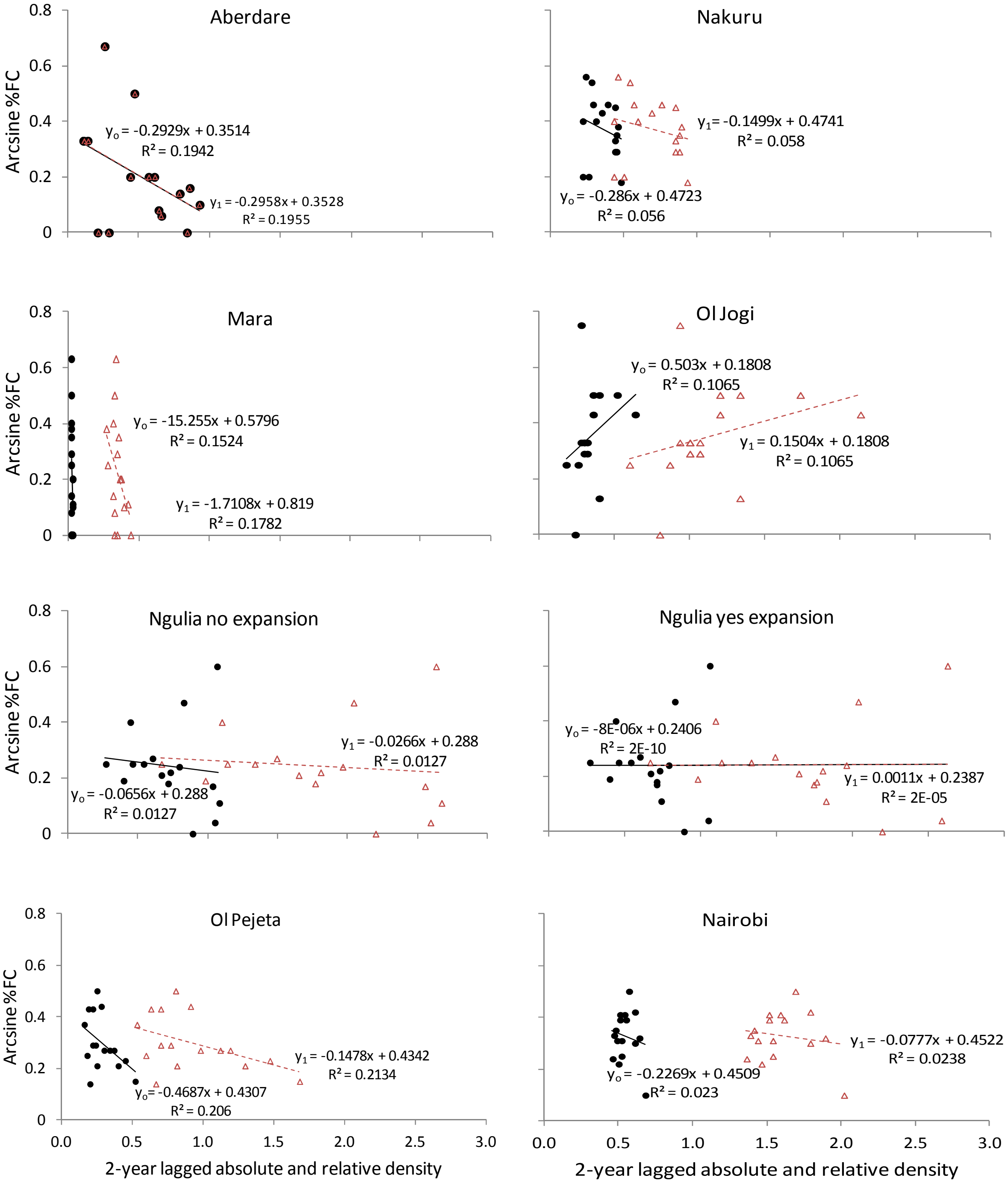

Figure 3.4: Relationships between arcsine of yearly percentage of females calving (%FC) and 2-year lagged absolute density (*bold line, y_o*) and relative density (*dotted line, y_1*) of black rhinoceros in seven populations in Kenya (1993 to 2010). Relative density is the ratio of absolute density to the estimated maximum stocking density (Adcock *et al.* 2007). Significant relationships between %FC and density were found in Aberdare, Mara and Ol Pejeta (P < 0.05, Table 3.2).

Table 3.2a: The results of Generalized Estimating Equations model with dependent variable arcsine-transformed yearly percentage of females calving and independent variable two-year lag of absolute density.

Area	Standard estimate	Std. Error	95% Wald Confidence Interval, 1 d.f. Lower	Upper	Wald χ²	Sig.
Aberdare	-.293	.1149	-.529	-.056	5.889	.015*
Nakuru	-.286	.3399	-.952	.380	.708	.400
Mara	-15.255	6.7496	-28.484	-2.026	5.108	.024*
Ngulia yes expansion (92 km^2)	-8.372E-6	.2303	-.451	.451	.000	1.000
Ngulia no expansion (63 km^2)	-.067	.1624	-.385	.251	.172	.679
Nairobi	-.234	.4962	-1.207	.738	.223	.637
Ol Jogi	.503	.2709	-.028	1.034	3.448	.063
Ol Pejeta	-.469	.1595	-.781	-.156	8.631	.003*

* Significant relationship (p<0.05)

Table 3.2b: The results of Generalized Estimating Equations model with dependent variable arcsine-transformed yearly percentage of females calving and independent variable two-year lag of absolute density relative to maximum stocking density.

Area	Standard estimate	Std. Error	95% Wald Confidence Interval, 1 d.f. Lower	Upper	Wald χ²	Sig.
Aberdare	-.293	.1149	-.529	-.056	5.889	.015*
Nakuru	-.147	.1747	-.490	.195	.712	.399
Mara	-1.753	.4423	-2.620	-.887	15.716	.000*
Ngulia yes expansion (92 km^2)	.002	.0937	-.182	.185	.000	.985
Ngulia no expansion (63 km^2)	-.026	.0660	-.156	.103	.157	.692
Nairobi	-.080	.1693	-.412	.252	.224	.636
Ol Jogi	.151	.0807	-.007	.309	3.501	.061
Ol Pejeta	-.148	.0479	-.242	-.054	9.584	.002*

* Significant relationship (p<0.05)

We found a significant negative relationship between yearly percentage of females calving and 2 year lagged absolute densities in Mara, Aberdare and Ol Pejeta and. The slopes of these relationships were significantly different between Mara and all populations (t = 2.042, d.f. = 30, P < 0.05) but identical between Aberdare and Ol Pejeta (t = 0.90, d.f. = 30, P > 0.05) (Fig. 3.5). We found similar results for 2-year lagged relative densities (Appendix 3.2b and 3.2c).

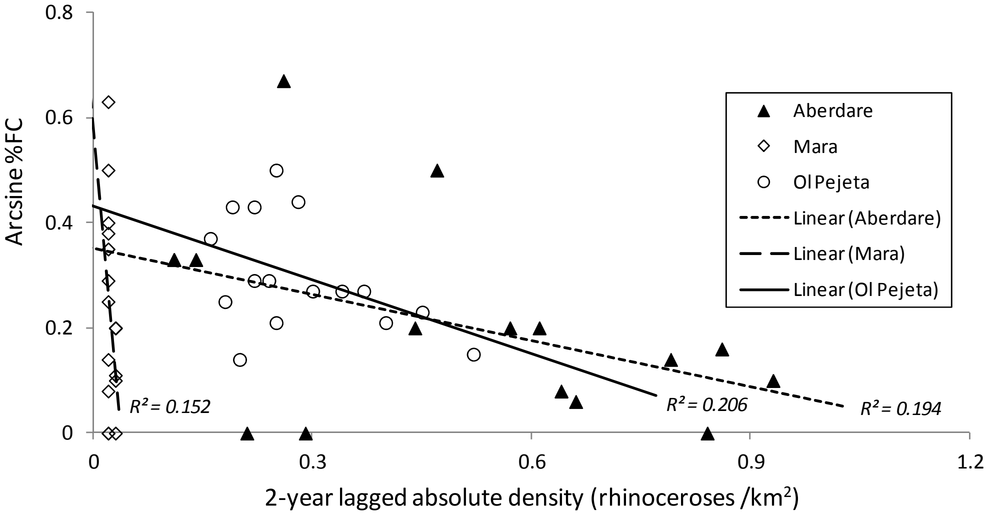

Figure 3.5: Relationships of yearly percentage of black rhino females calving (%FC) and 2-year lagged absolute density in Aberdare National Park (Aberdare), Masai Mara National Reserve (Mara) and Ol Pejeta Conservancy (Ol Pejeta) in Kenya (1993 to 2010). Slopes between Mara and Aberdare and between Mara and Ol Pejeta are significantly different. Similar results were obtained for absolute density relative to maximum stocking density (Appendix 3.2b and 3.2c).

Discussion

We expected to find a decrease in %FC with increase in population density particularly in populations of high densities. However, only the %FC for Ol Pejeta showed a decline with increase in density. Contrary to our expectations, %FC in Aberdare and Mara showed a significant negative relationship with density despite being of low density. The Aberdare population was significantly affected top-down through poaching (Okita-Ouma and Wandera 2006, Okita-Ouma *et al.* 2007b), which explains the observed negative relationship.

Mara is located in area of high plant available nutrient (PAN) and high plant available moisture (PAM) (Chapter 5, this thesis). A habitat of high PAN and high PAM tends to be more grassy with little production of woody cover (Sankaran *et al.* 2005) sufficient for dietary browse. Limited suitable browse (Adcock *et al.* 2007) due to browsing pressure has been reported previously for Mara (Walpole *et al.* 2004). Mara was the only unfenced population in this study. Its wild animals occasionally migrate into the bordering Serengeti National Park of *ca.* 15,000km^2 in Tanzania (Walpole 2002). This migration and leads to less precision in estimating population densities from acreage utilized by rhinoceroses. Other several forms of disturbances ranging from the annual wildebeest migration, cattle incursion, agricultural activities in dispersal areas, fires and tourism related activities (*e.g.*, Karanja 2002, Waithaka 2004, Walpole *et al.* 2004, Okita-Ouma *et al.* 2007a) could also be affecting reproductive behaviour and performance for black rhinoceros in the Mara. Disturbances could lead to chronic physiological stress which has been linked to low reproductive success (*see review in* Li *et al.* 2007). Therefore our observed negative relationship between %FC and density of black rhinoceros could have been driven more by these described confounding and difficult-to-measure factors.

In a slight contrast to the characteristics of PAN and PAM for Mara, Nairobi, Nakuru and Ol Jogi are located in areas of relatively lower PAN and moderate PAM. Their %FC remained optimal with increasing density and despite populations being of high density. A combination of low PAN and moderate PAM is favourable for growth of abundant woody plants in savanna ecosystems receiving annual rainfall of 200 mm to less than 1,000 mm (Bell 1982, Huntley 1982, Sankaran *et al.* 2008). The abundant woody plants in these three populations could have provided sufficient dietary browse required for optimal reproductive performance. Favourable ecological conditions give the females easy access to high quality foods that are important for their offspring development in early stages of life and are likely to greatly influence their lifetime reproductive success (Clutton-Brock *et al.* 1991, Saether 1997). Furthermore, components of the vegetation show different dynamics in quality and quantity in relation to plant available moisture; for example, the small amounts of browse material remaining after habitat deterioration due to drought show very little decline in mineral concentration compared to grasses for grazers (Owen-Smith 2008). Further, although herbivores at high density can impact negatively on the habitat (Prins and Van der Juegd 1993, Augustine and McNaughton 2004), mineral concentration in the dietary browse is not significantly affected (*See* Chapter 4 this thesis). The quality of leaves of woody plants especially from thorny *Acacia* spp., a favourable dietary browse species for black rhinoceros, tend to be consistent in the African Savannas (Owen-Smith 2008). This implies that the %FC in black rhinoceros in Nairobi, Nakuru and Ol Jogi was controlled bottom up by the availability of key resources as influenced by edaphic and climatic factors (*e.g.*, Lack 1947, 1948, 1966, Emslie 1999, Illius and O'Connor 2000, Bayliss and Choquenot 2002, Gandiwa 2013). Consequently, we did not find signs of density dependence in these high density populations.

Ol Pejeta was the only high density population whose %FC declined with increase in density. This can be attributed to several factors: Firstly, the combination of the high PAN and moderate PAM conditions of Ol Pejeta do not favour production of abundant dietary browse necessary for optimal reproductive performance (*see* Chapter 5 this thesis). Secondly, the woody plants in Ol Pejeta were reported to have been significantly damaged and reduced in quantity by high densities of giraffe (*Giraffa camelopardalis*) and elephant (Birkett 2002). This deterioration of habitat led to the expansion of the Conservancy from 93km^2 to 300km^2 in 2007 to provide more browse and forage for other species. However, the rather little roaming and territorial behaviour of the black rhinoceros (*see review in* Emslie and Adcock 2013) meant a slow colonization of newly expanded area. This movement behaviour could have resulted to even a much longer-term negative effect of deteriorated dietary browse on the reproductive performance. Emslie (1999) also reported long term negative effects of over-browsed habitat in the reproductive performance of black rhinoceros (*D. b. minor*) in Hluhluwe-Umfolozi Park. Similarly, Brett (2001) reported negative effects of a deteriorated habitat on reproductive performance of black rhinoceros (*D. b. michaeli*) in Solio Game

Reserve in Kenya. Intense inter-specific competition for dietary browse seems to play a significant role in influencing reproductive performance in black rhinoceros. In the cases for Ol Pejeta and Solio, outward translocations of buffaloes, elephants and giraffes were done between 2006 and 2010 (KWS Unpbl. reports).

Ngulia Rhinoceros Sanctuary had similar situation of intense inter-specific competition in the early to mid-2000s (Brett and Adcock 2002). Outward translocation of more than 75% of buffalos, elephants and giraffes from Ngulia was done in 2007 through to 2008 (Okita-Ouma *et al.* 2008a). This was followed immediately by expansion of the sanctuary from 62 km^2 to 92 km^2 in 2008. Brett and Adcock (2002), and Okita-Ouma *et al.* (2009) reported decreased intrinsic growth rate between 2000 and 2005 in black rhinoceros at Ngulia. However, our results showed an average optimal %FC over the period of study, indicating an improvement of the reproductive performance from that previously reported. This reduction of inter-specific competition through translocation and the consequent improvement in the reproductive performance further underscores the bottom-up control processes for this mega browser. The Ol Pejeta and Ngulia examples may imply that intense inter-specific competition for browse can have a stronger influence on vital rates of population growth than intra-specific competition. It may also mean that the impact of high density of the browsing black rhinoceros on its browse plants may not be as large as the impact of other mega-herbivores such as the elephant on woodlands (*e.g.*, Owen-Smith 1988).

The trends for %FC were identical in correlation with both absolute and relative densities (T-test, $P > 0.05$ in all cases). The slopes for relative density were less steep than the slopes for absolute density but not significantly different. However, we can reasonably speculate that the less steep slopes occurred because relative density as opposed to absolute density is associated to maximum stocking densities ("carrying capacity"). These findings indicate that actual densities have a higher impact in terms of density dependence than relative densities in this species.

Our findings were consistent with propositions that density feedbacks are extremely difficult to measure *in situ* in a variable environment and habitat (Stubbs 1977, McCullough 1992, Sibly *et al.* 2005, Doncaster 2006, Peacock and Garshelis 2006, Ross 2006), and may sometimes not exist. Red deer (*Cervus elaphus*), for example, had its fecundity influenced by population density in Scotland but by climate in Norway (Langvatn *et al.* 1996). We concur with observations by Goodman (2001) that the level at which density dependence occurs in black rhinoceros is not known. Identification for such a level would require precise quantification of factors including the strengths of the dynamic intrinsic and extrinsic factors into a population model - a task that is ecologically unrealistic for *in situ* populations (McCullough 1997; Forsyth 2006). Such a model - if at all achieved - would only be only applicable for a specific population at a specific point in time in a specific ecosystem.

We conclude that in black rhinoceros, the yearly percentage of females calving is not regulated by population density but appears to be controlled bottom-up by key resources in the populations we studied. Conservation managers should adopt alternative translocation strategies other than keeping black rhinoceros at a putative MSY of 70 - 80% of 'carrying capacity' where animals must be removed. Such alternative strategies include set percentage translocation. This strategy involves translocating rhinoceroses at fixed percentages below their intrinsic growth rate of 8 - 9% p.a. and monitoring population responses and adjusting the next percentage accordingly (Goodman 2001). Finally, we strongly recommend regular review of priori estimated maximum stocking densities ('carrying capacity') and models for calculating them where they must be used in managing the Critically Endangered black rhinoceros.

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Appendices

Appendix 3.1: Summary results Generalized Estimating Equations model with dependent variable arcsine-transformed yearly percentage of females calving and independent variable one and 2-year lag of absolute density and relative to maximum stocking density

			95% Wald Confidence Interval		Hypothesis Test				
Parameter	B	Std. Error	Lower	Upper	Wald χ^2	d.f.	Sig.	QIC	QICC
(Intercept)	.327	.0875	.155	.498	13.940	1	.000*	3.767	4.464
ANP_D_Lag1	-.280	.1149	-.506	-.055	5.960	1	.015*		
(Scale)	.031								
(Intercept)	.351	.0875	.167	.536	13.978	1	.000*	3.737	4.427
ANP_D_Lag2	-.293	.1149	-.529	-.056	5.889	1	.015*		
(Scale)	.031								
(Intercept)	*.327*	*.0875*	*.155*	*.498*	*13.940*	*1*	*.000**	*3.767*	*4.464*
ANP_D_Lag1	*-.280*	*.1149*	*-.506*	*-.055*	*5.960*	*1*	*.015**		
(Scale)	*.031*								
(Intercept)	*.351*	*.0875*	*.167*	*.536*	*13.978*	*1*	*.000**	*3.737*	*4.427*
ANP_D_Lag2	*-.293*	*.1149*	*-.529*	*-.056*	*5.889*	*1*	*.015**		
(Scale)	*.031*								
(Intercept)	.467	.1197	.233	.702	15.238	1	.000*	4.732	4.191
LNP_D_Lag1	-.260	.2934	-.835	.315	.787	1	.375		
(Scale)	.013								
(Intercept)	.472	.1368	.204	.740	11.910	1	.001*	4.870	4.191
LNP_D_Lag2	-.286	.3399	-.952	.380	.708	1	.400		
(Scale)	.014								
(Intercept)	*.472*	*.1186*	*.240*	*.705*	*15.869*	*1*	*.000**	*4.729*	*4.190*
LNP_D_Lag1	*-.141*	*.1495*	*-.434*	*.152*	*.889*	*1*	*.346*		
(Scale)	*.013*								
(Intercept)	*.472*	*.1366*	*.205*	*.740*	*11.953*	*1*	*.001**	*4.869*	*4.190*
LNP_D_Lag2	*-.147*	*.1747*	*-.490*	*.195*	*.712*	*1*	*.399*		
(Scale)	*.014*								
(Intercept)	.412	.1254	.2043	.812	4.065	1	.044*	4.032	4.519
MMR_D_Lag1	-8.227	4.8423	7.7115	6.887	1.138	1	.286		
(Scale)	.035								
(Intercept)	.580	.1883	.211	.949	9.473	1	.002*	3.571	4.445
MMR_D_Lag2	-15.255	6.7496	-28.484	-2.026	5.108	1	.024*		
(Scale)	.032								
(Intercept)	*.956*	*.2905*	*.387*	*1.526*	*10.834*	*1*	*.001**	*3.976*	*4.409*
MMR_D_Lag1	*-2.112*	*.7863*	*-3.653*	*-.571*	*7.219*	*1*	*.007**		
(Scale)	*.027*								
(Intercept)	*.836*	*.1789*	*.486*	*1.187*	*21.852*	*1*	*.000**	*2.860*	*4.434*
MMR_D_Lag2	*-1.753*	*.4423*	*-2.620*	*-.887*	*15.716*	*1*	*.000**		
(Scale)	*.031*								
(Intercept)	.360	.1138	.137	.583	10.033	1	.002*	4.318	4.326
NGL_D_Lag1	-.187	.1597	-.500	.126	1.364	1	.243		
(Scale)	.022								
(Intercept)	.241	.1303	-.015	.496	3.410	1	.065	6.106	4.336
NGL_D_Lag2	-8.372E-6	.2303	-.451	.451	.000	1	1.000		
(Scale)	.024								
(Intercept)	*.364*	*.1143*	*.140*	*.588*	*10.136*	*1*	*.001**	*4.311*	*4.325*
NGL_D_Lag1	*-.077*	*.0645*	*-.204*	*.049*	*1.442*	*1*	*.230*		
(Scale)	*.022*								
(Intercept)	*.238*	*.1312*	*-.019*	*.495*	*3.284*	*1*	*.070*	*6.136*	*4.336*
NGL_D_Lag2	*.002*	*.0937*	*-.182*	*.185*	*.000*	*1*	*.985*		
(Scale)	*.024*								
Ngulia no Expn									
(Intercept)	.290	.1006	.092	.487	8.287	1	.004	4.884	4.346
NGL_D_Lag1	-.076	.1529	-.376	.223	.249	1	.618		
(Scale)	.023								
(Intercept)	.289	.0923	.108	.470	9.819	1	.002	4.866	4.332
NGL_D_Lag2	-.067	.1624	-.385	.251	.172	1	.679		
(Scale)	.024							.024	

			95% Wald Confidence Interval		Hypothesis Test				
Parameter	B	Std. Error	Lower	Upper	Wald χ^2	d.f.	Sig.	QIC	QICC
(Intercept)	*.290*	*.1007*	*.093*	*.488*	*8.304*	*1*	_.004*_	*4.893*	*4.346*
NGL_D_Lag1	*-.031*	*.0619*	*-.153*	*.090*	*.254*	*1*	*.614*		
(Scale)	*.023*								
(Intercept)	*.287*	*.0930*	*.105*	*.469*	*9.554*	*1*	_.002*_	*4.888*	*4.332*
NGL_D_Lag2	*-.026*	*.0660*	*-.156*	*.103*	*.157*	*1*	*.692*		
(Scale)	*.024*								
(Intercept)	.379	.1979	-.009	.767	3.668	1	.055	3.785	4.182
NNP_D_Lag1	-.116	.3707	-.843	.610	.099	1	.753		
(Scale)	.012								
(Intercept)	.455	.2593	-.053	.963	3.084	1	.079	5.688	4.138
NNP_D_Lag2	-.234	.4962	-1.207	.738	.223	1	.637		
(Scale)	.010								
(Intercept)	*.373*	*.1905*	*.000*	*.746*	*3.838*	*1*	_.050*_	*3.682*	*4.182*
NNP_D_Lag1	*-.036*	*.1210*	*-.273*	*.201*	*.088*	*1*	*.767*		
(Scale)	*.012*								
(Intercept)	*.456*	*.2610*	*-.055*	*.968*	*3.059*	*1*	*.080*	*5.776*	*4.137*
NNP_D_Lag2	*-.080*	*.1693*	*-.412*	*.252*	*.224*	*1*	*.636*		
(Scale)	*.010*								
(Intercept)	.199	.1327	-.061	.459	2.256	1	.133	3.832	4.415
OJS_D_Lag1	.431	.3057	-.168	1.030	1.991	1	.158		
(Scale)	.028								
(Intercept)	.181	.1151	-.045	.407	2.467	1	.116	3.499	4.393
OJS_D_Lag2	.503	.2709	-.028	1.034	3.448	1	.063		
(Scale)	.028								
(Intercept)	*.201*	*.1329*	*-.060*	*.461*	*2.277*	*1*	*.131*	*3.844*	*4.416*
OJS_D_Lag1	*.128*	*.0917*	*-.052*	*.308*	*1.943*	*1*	*.163*		
(Scale)	*.028*								
(Intercept)	*.180*	*.1145*	*-.044*	*.405*	*2.482*	*1*	*.115*	*3.494*	*4.393*
OJS_D_Lag2	*.151*	*.0807*	*-.007*	*.309*	*3.501*	*1*	*.061*		
(Scale)	*.028*								
(Intercept)	.359	.0747	.212	.505	23.070	1	.000*	3.384	4.184
OPC_D_Lag1	-.190	.1996	-.581	.202	.902	1	.342		
(Scale)	.012								
(Intercept)	.431	.0647	.304	.558	44.329	1	.000*	3.099	4.137
OPC_D_Lag2	-.469	.1595	-.781	-.156	8.631	1	.003*		
(Scale)	.010								
(Intercept)	*.361*	*.0758*	*.212*	*.509*	*22.638*	*1*	_.000*_	*3.402*	*4.183*
OPC_D_Lag1	*-.061*	*.0623*	*-.183*	*.062*	*.946*	*1*	*.331*		
(Scale)	*.012*								
(Intercept)	*.435*	*.0637*	*.310*	*.559*	*46.558*	*1*	_.000*_	*3.045*	*4.136*
OPC_D_Lag2	*-.148*	*.0479*	*-.242*	*-.054*	*9.584*	*1*	_.002*_		
(Scale)	*.010*								

*Significance at 95% Wald C.I.
Black text for absolute density
Red italic text for relative density

Appendix 3.2:

In appendices 3.2a, 3.2b and 3.2c, we assumed equal variances for slopes between populations and compared the slopes (a_1 and a_2; b_1 and b_2; *Abs* and *Rel*) by computing t-statistic as the difference between the two slopes divided by the standard error (SE) of the difference between the two slopes as follows:

$t = \frac{a_1 - a_2}{s_{a_1 - a_2}}$, where $s_{a_1 - a_2} = \sqrt{s_{a_1}^2 + s_{a_2}^2}$ at $df = n_1 + n_2 - 2$ (Wuensch 2007);

Appendix 3.2a: Comparison between slopes of absolute density and absolute density relative to maximum stocking density

Slope Tests	***Slope-Abs***	***Slope-Rel***	***SE_Abs***	***SE_Rel***	***Abs-Rel***	***SE_Abs-SE_Rel ($S_{abs-rel}$)***	***t-stat***	***P at d.f.=30***
Aberdare	-0.293	-0.293	0.1149	0.1149	0	0.1625	0.00	*ns*
Nakuru	-0.286	-0.147	0.3399	0.1747	-0.139	0.3822	-0.36	*ns*
Mara	-15.255	-1.753	6.7496	0.4423	-13.502	6.7641	-2.00	*ns*
Ngulia	-8.37E-06	0.002	0.2303	0.0937	-0.00201	0.2486	-0.01	*ns*
Ngulia noExpn♣	-0.067	-0.026	0.1624	0.066	-0.041	0.1753	-0.23	*ns*
Nairobi	-0.234	-0.08	0.4962	0.1693	-0.154	0.5243	-0.29	*ns*
Ol Jogi	0.503	0.151	0.2709	0.0807	0.352	0.2827	1.25	*ns*
Ol Pejeta	-0.469	-0.148	0.1595	0.0479	-0.321	0.1665	-1.93	*ns*

♣ Ngulia Rhino Sanctuary assuming it was not expanded from 63km^2 to 92km^2 in 2007; Abs=Absolute density; *Rel*=Absolute density relative to maximum stocking density.

Appendix 3.2b: t-statistic for pairwise comparison of slopes of yearly percentage of black rhino calving between seven populations of 2-year lagged absolute densities.

Paired populations	*a1*	*a2*	*SE_a1*	*SE_a2*	*a1-a2*	*SE_a1-SE_a2* (S_{a1-a2})	*t-stat*	*P at d.f.=30*
ANP:LNP	-0.293	-0.286	0.1149	0.3399	-0.007	0.3588	-0.02	ns
ANP:MMR	-0.293	-15.255	0.1149	6.7496	14.962	6.7506	2.22	<0.05
ANP:NGL	-0.293	-8.37E-06	0.1149	0.2303	-0.29299	0.2574	-1.14	ns
ANP:NGL NoExpn	-0.293	-0.067	0.1149	0.1624	-0.226	0.1989	-1.14	ns
ANP:NNP	-0.293	-0.234	0.1149	0.4962	-0.059	0.5093	-0.12	ns
ANP:OJS	-0.293	0.503	0.1149	0.2709	-0.796	0.2943	-2.71	<0.02
ANP:OPC	-0.293	-0.469	0.1149	0.1595	0.176	0.1966	0.90	ns
LNP:MMR	-0.286	-15.255	0.3399	6.7496	14.969	6.7582	2.21	<0.05
LNP:NGL	-0.286	-8.37E-06	0.3399	0.2303	-0.28599	0.4106	-0.70	ns
LNP:NGL NoExpn	-0.286	-0.067	0.3399	0.1624	-0.219	0.3767	-0.58	ns
LNP:NNP	-0.286	-0.234	0.3399	0.4962	-0.052	0.6015	-0.09	ns
LNP:OJS	-0.286	0.503	0.3399	0.2709	-0.789	0.4346	-1.82	ns
LNP:OPC	-0.286	-0.469	0.3399	0.1595	0.183	0.3755	0.49	ns
MMR:NGL	-15.255	-8.37E-06	6.7496	0.2303	-15.255	6.7535	-2.26	<0.05
MMR:NGLNoExpn	-15.255	-0.067	6.7496	0.1624	-15.188	6.7516	-2.25	<0.05
MMR:NNP	-15.255	-0.234	6.7496	0.4962	-15.021	6.7678	-2.22	<0.05
MMR:OJS	-15.255	0.503	6.7496	0.2709	-15.758	6.7550	-2.33	<0.05
MMR:OPC	-15.255	-0.469	6.7496	0.1595	-14.786	6.7515	-2.19	<0.05
NGL:NNP	-8.37E-06	-0.234	0.2303	0.4962	0.233992	0.5470	0.43	ns
NGL:OJS	-8.37E-06	0.503	0.2303	0.2709	-0.50301	0.3556	-1.41	ns
NGL:OPC	-8.37E-06	-0.469	0.2303	0.1595	0.468992	0.2801	1.67	ns
NGL:NGL NoExpn	-8.37E-06	-0.067	0.2303	0.1624	0.066992	0.2818	0.24	ns
NNP:OJS	-0.234	0.503	0.4962	0.2709	-0.737	0.5653	-1.30	ns
NNP:OPC	-0.234	-0.469	0.4962	0.1595	0.235	0.5212	0.45	ns
NNP:NGL NoExpn	-0.234	-0.067	0.4962	0.1624	-0.167	0.5221	-0.32	ns
OJS:OPC	0.503	-0.469	0.2709	0.1595	0.972	0.3144	3.09	<0.01
OJS:NGL NoExpn	0.503	-0.067	0.2709	0.1624	0.57	0.3158	1.80	ns
OPC:NGL NoExpn	-0.469	-0.067	0.1595	0.1624	-0.402	0.2276	-1.77	ns

ANP=Aberdare National Park; LNP=Lake Nakuru NP; MMR=Masai Mara NR; NGL=Ngulia Rhino Sanctuary; NNP=Nairobi NP; OJS=Ol Jogi Pyramid; OPC=Ol Pejeta Conservancy; NGL NoExpn=Ngulia Rhino Sanctuary assuming it was not expanded from 63km^2 to 92km^2 in 2007.

Appendix 3.2c: t-statistic for pairwise comparison of slopes of yearly percentage of black rhino calving between seven populations of 2-year lagged absolute densities relative to maximum stocking densities

Paired populations	*b1*	*b2*	*SE_b1*	*SE_b2*	*b1-b2*	*SE_b1-SE_b2 (S_{b1-b2})*	*t-stat*	*P at d.f.=30*
ANP:LNP	-0.293	-0.147	0.1149	0.1747	-0.146	0.2091	-0.70	ns
ANP:MMR	-0.293	-1.753	0.1149	0.4423	1.46	0.4570	3.19	<0.01
ANP:NGL	-0.293	0.002	0.1149	0.0937	-0.295	0.1483	-1.99	ns
ANP:NGL NoExpn	-0.293	-0.026	0.1149	0.066	-0.267	0.1325	-2.01	ns
ANP:NNP	-0.293	-0.08	0.1149	0.1693	-0.213	0.2046	-1.04	ns
ANP:OJS	-0.293	0.151	0.1149	0.0807	-0.444	0.1404	-3.16	<0.01
ANP:OPC	-0.293	-0.148	0.1149	0.0479	-0.145	0.1245	-1.16	ns
LNP:MMR	-0.147	-1.753	0.1747	0.4423	1.606	0.4756	3.38	<0.01
LNP:NGL	-0.147	0.002	0.1747	0.0937	-0.149	0.1982	-0.75	ns
LNP:NGL NoExpn	-0.147	-0.026	0.1747	0.066	-0.121	0.1868	-0.65	ns
LNP:NNP	-0.147	-0.08	0.1747	0.1693	-0.067	0.2433	-0.28	ns
LNP:OJS	-0.147	0.151	0.1747	0.0807	-0.298	0.1924	-1.55	ns
LNP:OPC	-0.147	-0.148	0.1747	0.0479	0.001	0.1811	0.01	ns
MMR:NGL	-1.753	0.002	0.4423	0.0937	-1.755	0.4521	-3.88	<0.001
MMR:NGLNoExpn	-1.753	-0.026	0.4423	0.066	-1.727	0.4472	-3.86	<0.001
MMR:NNP	-1.753	-0.08	0.4423	0.1693	-1.673	0.4736	-3.53	<0.002
MMR:OJS	-1.753	0.151	0.4423	0.0807	-1.904	0.4496	-4.23	<0.001
MMR:OPC	-1.753	-0.148	0.4423	0.0479	-1.605	0.4449	-3.61	<0.002
NGL:NNP	0.002	-0.08	0.0937	0.1693	0.082	0.1935	0.42	ns
NGL:OJS	0.002	0.151	0.0937	0.0807	-0.149	0.1237	-1.20	ns
NGL:OPC	0.002	-0.148	0.0937	0.0479	0.15	0.1052	1.43	ns
NGL:NGL NoExpn	0.002	-0.026	0.0937	0.066	0.028	0.1146	0.24	ns
NNP:OJS	-0.08	0.151	0.1693	0.0807	-0.231	0.1875	-1.23	ns
NNP:OPC	-0.08	-0.148	0.1693	0.0479	0.068	0.1759	0.39	ns
NNP:NGL NoExpn	-0.08	-0.026	0.1693	0.066	-0.054	0.1817	-0.30	ns
OJS:OPC	0.151	-0.148	0.0807	0.0479	0.299	0.0938	3.19	<0.01
OJS:NGL NoExpn	0.151	-0.026	0.0807	0.066	0.177	0.1043	1.70	ns
OPC:NGL NoExpn	-0.148	-0.026	0.0479	0.066	-0.122	0.0816	-1.50	ns

ANP=Aberdare National Park; LNP=Lake Nakuru NP; MMR=Masai Mara NR; NGL=Ngulia Rhino Sanctuary; NNP=Nairobi NP; OJS=Ol Jogi Pyramid; OPC=Ol Pejeta Conservancy; NGL NoExpn=Ngulia Rhino Sanctuary assuming it was not expanded from $63km^2$ to $92km^2$ in 2007.

Photo credit: B. Okita-Ouma

Chapter 4

The relationships between dietary and faecal mineral concentrations in black rhinoceros (*D. b. michaeli*) and the influence of population density on diet quality

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Abstract

Under a given Plant Available Moisture (PAM) and Nutrient (PAN) regime, a high population density of meso-herbivores has been shown to lead to intense foraging and depletion of high quality plants, thus lowering the quality of subsequent diets, possibly leading to population declines. However, mega-herbivores are more tolerant of low diet quality than meso-herbivores. Consequently, we expect that mega-browsers will show few (if any) signs of “nutritional stress” due to their own high density. Under high PAM, we expect a high quantity of browse leaves, and perhaps a higher leaf quality. Under high PAN, we expect both a higher quantity and a higher quality of browse leaves. In this paper we test the effects of density of eastern black rhinoceros (*Diceros bicornis michaeli*), a mega-browser on the quality of its diet while controlling for the effects of PAM and PAN. Our methods focused on faecal mineral concentration as an index of dietary quality of black rhinoceroses in the field. To test the faeces-diet mineral relationships, we used feeding trials with black rhinoceroses in zoos and found that the concentrations of minerals in faeces were significantly, positively correlated to concentrations of the same minerals in the diet. Next, we measured faecal Calcium (Ca), Phosphorous (P), Copper (Cu) and Zinc (Zn) from 473 fresh faeces obtained over one year from 37 male and 40 female adult black rhinoceroses in nine populations and tested for a correlation between these faecal minerals and PAM, PAN, and density by Generalized Linear Mixed Models. We found lagged PAM (lag PAM - lagged by 1 month to reflect rainfall over the 4 weeks preceding sampling) to be a significant explanatory variable for the mineral concentrations found in the faeces, whilst PAN did not explain faecal mineral content. We did not find significant relationship between density and dietary quality as measured via faecal mineral nutrients even after controlling for effects of PAM and PAN. We conclude that population density of black rhinoceros does not appear to affect the mineral concentration of the species’ natural diet.

Key words: *black rhinoceros, browser, density, diet, faeces, mega-herbivore, mineral nutrient, moisture*, *plants*

Introduction

Many herbivore species have a heterogeneous distribution in a given landscape and in these landscapes there are areas that are drier or wetter or of lower or higher soil fertility. In other words, most landscapes are patchy with respect to these important determinants of plant quality and quantity. Soil scientists and plant physiologists have studied the causes of this landscape heterogeneity and the effects of these on herbage production and herbage quality, and operationalized a standardization of the description of “drier or wetter” as “PAM”, *i.e.*, Plant Available Moisture: the water of the soil that can potentially be absorbed by plants in a soil horizon (Walker and Langridge 1997); and “soil fertility” as “PAN”, *i.e*., Plant Available Nutrient: the sum of exchangeable cations in the soil (Walker and Langridge 1997, Olff *et al.* 2002, Ahrestani *et al.* 2011). Since aboveground plant material, which is the food for the herbivores, is differentially affected by PAM and by PAN with respect to its quantity and its quality (*e.g.*, Solbrig *et al.* 1992, Mistry 2011), soil heterogeneity is of direct relevance for understanding herbivore distribution and population dynamics (*e.g.*, Fritz *et al.* 2002, Prins and Van Langevelde 2008, Pretorius *et al.* 2011). Under high moisture conditions (*i.e*., high PAM) a high quantity of browse leaves is expected (Olff *et al.* 2002, Sankaran *et al.* 2008) but also a higher quality of these leaves as shown in experiments, at least in seedlings (Singh and Singh 2004, Barbosa *et al.* 2014 *in press*). This is in contrast to the effect of increased PAM on grasses, because nitrogen (Olff *et al.* (2002) and phosphorus (Breman and de-Wit 1983) in grass leaves decrease with increasing PAM. When the soil nutrient status is high (*i.e*., high PAN) both a higher quantity and a higher quality of grasses is expected (Olff *et al.* 2002) whilst higher quantity (*see* Tilman 1984, Polis 1999) and insignificant changes in quality of browse leaves (Le Houérou 1980) is expected. These lack of changes in browse leaf nutrient quality may be due to the fact that woody plants differ widely in their response to differences in soil nutrients (Le Houérou 1980), for instance plants species from high fertility sites show fast growth rates and higher tissue nutrient concentrations compared to plants species from low fertility sites (Stuart Chapin 1980). Moreover, for species that are adapted to infertile soils it has been suggested that they store more nutrients in storage organs (*e.g.*, roots system) than species from fertile soils (Bryant *et al.* 1983). These stored nutrients can be readily used in conditions of stress (*e.g.*, high herbivory pressure, fire, nutrient shortage) to support growth or other important physiological processes (Chiariello and Roughgarden 1984).

Herbivores thus occur in a landscape that can be described as a PAM-PAN space (Olff *et al.* 2002, Ahrestani *et al.* 2011), and the associated heterogeneity may have large consequences for population dynamics of herbviores if that heterogeneity occurs at a large enough spatial scale (*e.g.*, Ludwig *et al.* 2008, Prins and Van Langevelde 2008, Treydte *et al.* 2009) because then in areas with much food of high quality it may be expected that herbivores show a high population growth rate, and in areas that are yielding low quality herbage, or low amounts of herbage, it may be expected that a herbivore (sub)population may exhibit low growth rates. In other words, spatial heterogeneity in PAM and PAN may lead to spatial intra-population inequality in fitness of the individuals that comprise a (meta) population (Illius and O’Connor 2000).

However, there is a potentially contrasting way of reasoning too: if there is a high density of herbivores, then this first leads to a decline in the quantity of herbage and subsequently, because of the selectivity of the herbivores, also to a further decline of the quality of the plant food. A persistent high density of herbivores leads to a low quality diet for grazers (*e.g.*, Owen-Smith 2008, Van Wieren and Bakker 2008) and browsers (*e.g.*, Van der Waal *et al.* 2003, Simard *et al.* 2008) alike, which then may induce population decline (Klein 1968, Caughley 1976a, Scogland 1985, Sinclair *et al.* 1985, Ellis and Swift 1988). To understand meta-population dynamics, it is thus not only necessary to know the spatial heterogeneity of PAM and PAN, and their interacting effects (*see* Chapter 5 this thesis), but also the spatial heterogeneity in density of these herbivores. This may have consequences on where to translocate endangered herbivores. What are the most optimal conditions for population growth in a PAM-PAN space? Mega-herbivores herbivores like white rhinoceros (*Ceratotherium simum*) has been found to be generally more tolerant of low diet quality than meso-herbivores (Owen-Smith 1988). The quality of leaves of woody plants especially from thorny *Acacia* spp. tends to be more invariable than the quality of grasses in African Savannas (Owen-Smith 2008). Consequently, we may argue that mega-browsers like the black rhinoceros will show few (if any) signs of “nutritional stress” compared to (mega-) grazers in areas where they occur in high densities.

Population dynamics and fitness differences between individuals in a heterogeneous landscape are thus affected by the quality and quantity of herbage on offer, which in turn is determined by plant available nutrients and plant available moisture, but also by the animals themselves. Can these factors be disentangled? We tried to do so in case of the eastern black rhinoceros (*D. b. michaeli*) (Hitchins 1970, Prins 1990, Foose *et al.* 1992), a mega-browser, with adults weighing between 800 - 1350 kg (Freeman and King 1969, Owen-Smith 1984, Emslie and Adcock 2013) thus classifying as mega-browser. Black rhino have a digestive system with hindgut fermentation which can process plants of low nutritional quality such as *Euphorbia* spp with high concentrations of secondary compounds (*e.g.*, Goddard 1968, 1970, Mukinya 1977, Hitchins 1979, Hall-Martin *et al.* 1982, Luske *et al.* 2009). Its tolerance for low quality browse and its twig-removing feeding behaviour suggest that the black rhinoceros may have a potentially large impact on its habitat, especially if enclosed at high density (Lent and Fike 2003, Luske *et al.* 2009). By virtue of its large body size, it does not have effective predators to regulate its population top-down, thus increasing the probability of detecting bottom-up processes.

Central in our study is the issue of diet quality and population density. Herbivores produce large quantities of faeces, and faeces has been a source of information on diet quality (*e.g.*, Putman 1984, Kohn and Wayne 1997, Van der Waal *et al.* 2003). Indeed, (i) the nutritional status of wild herbivores can be assessed indirectly from faecal mineral levels (Prins and Beekman 1989, Grant *et al.* 1995, Hodgson *et al.* 1996, Wrench *et al.* 1997). (ii) Faecal nitrogen (N) and phosphorus (P), for example, have been widely used to evaluate the nutritional value of diets for herbivores and to discern dietary differences between individuals (Robbins *et al.* 1987, Prins 1989, Grant *et al.* 1995). Other available methods for investigating diet selection involve chemical analyses of food plants (Oloo *et al.* 1994, Muya and Oguge 2000) by back-tracking the browsing path of a mega-herbivore (Helary *et al.* 2009, Van Lieverloo *et al.* 2009, Helary *et al.* 2012). The backtracking is, however, labour-intensive and often it is not easy to collect sufficient quantities and representative parts of plants for chemical analyses during different seasons and from different locations. These other methods may also result in errors in ascribing browsing of a plant to the species under study while in reality a different species browsed the plant. Faecal analyses omit the time consuming sampling and analysis of varied and heterogeneous diets because the study animals do this work themselves, which by definition is error-free with regards to which plants were selected. (iii) Micronutrients in the diet have been shown to be important in animal fertility and reproduction, for example copper (Cu) deficiency lowers testosterone, delays testicular development and results in defects in reproductive systems in males while in females, reproductive success is lowered (*review in* Bedwal and Bahuguna 1994). Koen (1988) attributed a low reproductive success of Kynsna forest elephants in South Africa to a deficiency in dietary Cu. Zinc (Zn) deficiency leads to gonadal dysfunctioning, decreases testicular weight, causes shrinkage of seminiferous tubules, impairs the action of the Mullerian inhibitory factor which is essential for testicular differentiation and is associated with malignant growth in the testis (*e.g.*, Gunn and Gould 1958, Bedwal and Bahuguna 1994, Yousofvand *et al.* 2013). In females, Zn deficiency leads to abnormal ovarian development, disruption of the oestrus cycle, frequent abortion, prolonged gestation period, teratogenicity, still-births, difficulty in parturition, and low birth weight of infants (*review in* Bedwal and Bahuguna 1994).

As a first step in our analysis of understanding differential population performance of browsing mega-herbivores in a heterogeneous savanna landscape, we tested the premise that diet and faecal mineral concentration are correlated in black rhinoceros by assessing the correlation between the concentrations of Calcium (Ca), Phosphorus (P), Copper (Cu) and Zinc (Zn) in the diet and faeces from data on feeding trials with adult black rhinoceros in zoos reported by Clauss *et al.* (2007b). We then analysed concentrations of these elements from faecal samples obtained in the wild from male and female black rhinos in nine populations of contrasting densities and different PAM and PAN environments. We hypothesized, firstly, that mineral concentrations in faeces would increase with increase in PAM and increase in PAN, and, secondly, that by controlling for the effects of PAM and PAN, mineral concentrations in faeces would decrease with increasing density of black rhinoceros. Using Generalized Linear Mixed Models (GLMMs), we explored the relationships between faecal mineral concentrations, PAM and PAN, and verified whether this approach is sensitive enough to detect black rhinoceros density effects on the nutritional status of the animals or *vice versa*.

Methods

Zoo study

We tested the premise that in Black rhinoceros mineral concentrations in faeces are correlated with mineral concentration in diet by using data from feeding trials with seven male and eight female adult black rhinos in various zoos (Clauss *et al.* 2007b). Not all minerals were analysed in all cases, resulting in different sample sizes for different minerals. The relationships between ingested and faecal mineral concentration cannot be considered directly causal, as they inherently ignore the effect of digestibility, which depends on the amount of diet ingested, faeces excreted and digestive physiology (with variable endogenous/metabolic losses, and different proportions of faecal and urinary excretion for different minerals). Nevertheless, they served to underline that assuming a link between mineral concentrations of diets and faeces is reasonable.

Field study

We selected nine different locations with Black rhinoceros populations in Kenya (Table 4.1). Selection criteria were based on the distinctive densities of rhinoceros population and the differences in Plant Available Moisture (PAM) and Plant Available Nutrients (PAN).

Table 4.1: Study areas and their geographic locations within Kenya with density estimates of the eastern black rhinoceros (*D. b. michaeli*) in 2010. The shortened names for the study areas as used in the main text are in brackets next to the full name.

Study area	**Area (km^2)**	**Density (Animals/km^2)**	**Geographic location**
Ngulia Rhino Sanctuary (Ngulia)	92	0.7	38^{0} 06’E - 38^{0} 10’E & 3^{0} 01’S - 3^{0} 06’S
Nairobi National Park (Nairobi)	117	0.6	36^{0} 23’E - 36^{0} 28’E & 2^{0} 18’S - 2^{0} 20’S
Lake Nakuru National Park (Nakuru)	144	0.5	36^{0} 20’E - 36^{0} 25’E & 0^{0} 50’S - 1^{0} 00’S
Ol Jogi Pyramid (Ol Jogi)	50	0.4	37^{0} 00’E - 37^{0} 05’E & 0^{0} 15’N - 0^{0}20’N
Ol Pejeta Conservancy (Ol Pejeta)	300	0.3	36^{0} 40’E - 37^{0} 00’E & 0^{0} 02’S - 0^{0} 07’N
Aberdare National Park (Aberdare)	70	0.1	36^{0} 25’E - 37^{0} 00’E & 0^{0} 05’S - 0^{0} 45’S
Masai Mara National Reserve (Mara)	1510	0.02	34^{0} 45’E - 35^{0} 25’E & 1^{0} 13’S - 1^{0} 45’S
Tsavo East National Park (Tsavo East)	1800♣	0.02	38^{0} 10’E - 39^{0} 25’E & 1^{0} 50’S - 3^{0} 30’S
Tsavo West National Park Intensive Protection Zone (Tsavo West IPZ)	1200♣	0.01	37^{0} 45’E - 38^{0} 45’E & 2^{0} 40’S - 4^{0} 02’S

♣This is the area that is used by black rhino (on basis of field reports by rangers) within the entire Tsavo East NP of *ca.* 11,000km^2 and Tsavo West NP *ca*. 9,000km^2.

PAM was calculated as the monthly average of the Log_{10} of the ratio of actual rainfall to potential evapo-transpiration (PET), *i.e.*, Log_{10} (Rainfall/PET) (Walker and Langridge 1997, Olff *et al.* 2002). Due to the limited available information, the Blaney-Criddle formula (Blaney and Criddle 1962) for deriving PET was preferred over other more detailed methods such as the Penman-Monteith equation (Penman 1948). PET was estimated as follows:

$PET_o = p \times (0.46 \times T_{mean} + 8)$

Where;

PET_o is the reference potential evapo-transpiration [mm day^{-1}] (monthly)

T_{mean} is the mean daily temperature [°C] given as $T_{mean} = (T_{max} + T_{min})/ 2$

p is the mean daily percentage of daytime hours (assumed 50% in equatorial regions)

Rainfall for 2010 and 2011 were estimated from decadal (10-day interval) measurements derived from raster images of 8 km^2 resolution (http://www.cpc.ncep.noaa.gov/products/fews/AFR_CLIM/afr_clim.shtml) because not for all study areas data from rainfall gauges were available Hawth's Analysis Tools for ESRI's ArcGIS™ 9.3 software was used to extract rainfall estimates from the raster images by overlaying GIS shape files of boundary for each study area on the raster images. The same source of data, analytical tools and procedures for rainfall were used to extract minimum and maximum temperatures from raster files of 4 km^2 resolution used in calculating potential evapo-transpiration.

PAN was measured as the mean of the sums of soil exchangeable cations (Ca^{2+}, Mg^{2+}, Na^+ and K^+) in milliequivalents of hydrogen per 100 g (mEq/100g) (Olff *et al.* 2002). Exchangeable cations were extracted from Kenya Soil Survey reports and datasets of scales 1:50,000 to 1:250,000 (Glover and Williams 1966, Glover and Trump 1970, Van der Weg *et al.* 1976, Siderius and Muriuki 1977, Wamicha and Gachene 1979, Wamicha *et al.* 1981, Sombroek *et al.* 1982, Van Wijngaarden and Van Engelen 1985, Waruru and Ita 1986, Ahn and Geiger 1987, GTZ *et al.* 1987, Kinyanjui 1996) and the World Soils Information (ISRIC) database (Batjes and Gicheru 2004, Batjes 2006, ISRIC 2011). To standardize comparisons, only exchangeable cations within 20 cm soil depth were used. Tsavo East, Ngulia and Tsavo West IPZ were classified under the same soil exchangeable cations for Tsavo (Sombroek *et al.* 1982).

We sampled fresh (< 18h old) faecal material from 77 individual adult black rhinoceroses comprising 37 males and 40 females that was not contaminated by urine or rainfall. Sampling was done between 0600 hr and 1200 hr from July 2010 to November 2011 in nine different study populations with varying population densities (Table 4.1) and variable levels of PAM and PAN (Fig. 4.1). On average we collected five faecal samples per animal (median value, with a range of one to 18; total N = 473). Only faecal samples that could be confidently allocated to known individual animals were used in these analyses. Individual animals were identified by rhino monitoring staff using ear-notches, body marks, home ranges and remote measures such as camera-traps and radio tracking (Mulama *et al.* 2005).

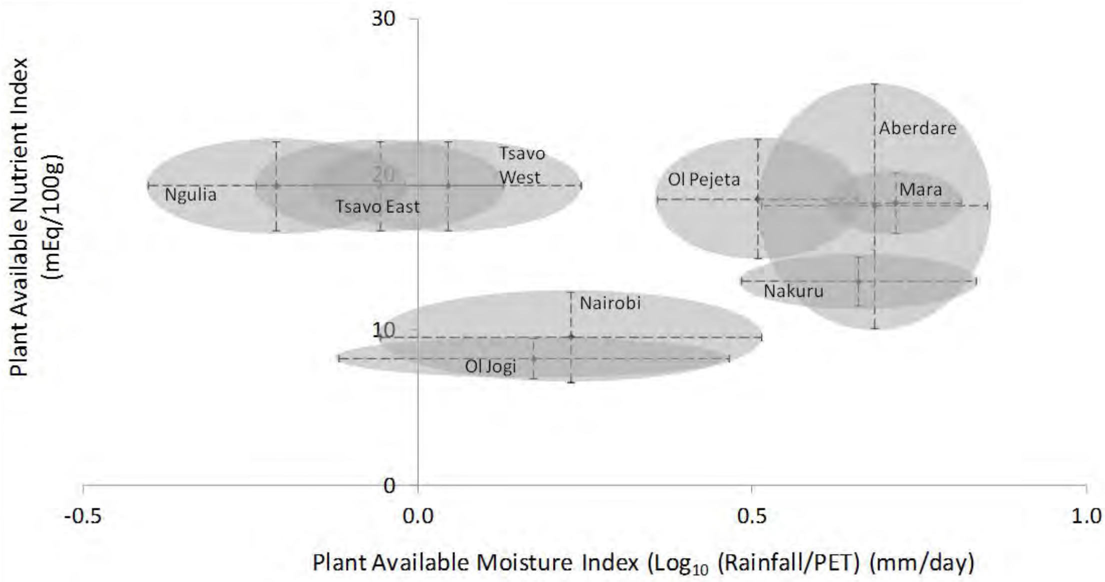

Figure 4.1: Nine black rhino conservation areas in Kenya plotted in the PAM-PAN plane. The dotted lines give the 95% Confidence Intervals. For all PAM values *n* = 10. For PAN, *n* = 179 in Ngulia, Tsavo East & Tsavo West-IPZ, *n* = 17 in Ol Jogi, *n* =28 in Nairobi, *n* = 19 in Ol Pejeta, *n* = 47 in Nakuru, *n* = 23 in Aberdare, and *n* = 43 in Mara. PAN-values are calculated from the literature (*see* Methods section).

Visible contaminants (*i.e.*, soil particles, insects) were removed using gloves. In order to ensure the sample collected in the field was representative of the faecal matter obtained at a single point of time from a known individual, each of these samples were thoroughly mixed by hands in gloves on a disposable polythene sheet surface of 50cm by 50cm for about 15min. To minimize cross-contamination, handling equipment and disposables such as the hand gloves, polythene sheets, and sampling bottles were not shared between samples. About 30 g of the hand-mixed faeces were put into airtight sampling bottles, labelled appropriately and immediately placed in an iced cooler box before being transferred into a -20 ^{0}C freezer for storage in the field. Samples were transported on ice in a mobile freezer to another freezer of -20 ^{0}C at Kenya Wildlife Service (KWS) Veterinary laboratory in Nairobi for storage before they were processed and chemically analysed at the industrial laboratories of the Ministry of Mines and Geology in Nairobi. The cooling protocol was followed because samples were also used for hormone essays (not reported here but *see* Chapter 6).

Standard procedures for preparation of samples for measuring mineral nutrients in plants (Plank 1992) were used for measuring nutrients in the rhino faeces. 20 - 30 g of fresh faecal samples was sun dried for 5 days then oven-air dried at 80 ^{0}C for 24 hours. A subsample of 2.5 g of oven-dried sample was ground and weighed into a 50 ml beaker and mixed with 3 ml distilled water to make slurry which was then digested with 15 ml concentrated hydrochloric acid and 5 ml concentrated nitric acid. After the reaction ceased, the beaker was heated on a hot plate while being agitated from time to time until 5ml of solution remained. 10 ml of distilled water was added and then allowed to settle. The cooled and settled digested solution was filtered using hardened ash-free filter paper grade no. 541, and then washed thoroughly with distilled water to the mark of 50 ml volumetric flask. The solutions were subjected to flame photometer for determination of Ca (Sherwood 2005) and Atomic Absorption Spectrometer (AAS) for determination of Cu and Zn (Levinson 2002).

For chemical analysis of P, 1.0 g of oven-air dried faecal sample was added to 1 ml of concentrated sulphuric acid and 5 ml of concentrated nitric acid and left to react until the solution became colourless (Plank 1992). 20 ml distilled water and 0.05 ml of phenolphthalein indicator was added then neutralized with 1N NaOH to Ph = 7. The solution was transferred into a 100 ml volumetric flask and distilled water added to the mark. The standard solutions were treated likewise. 10 ml of digested samples, blank and standard solutions were pipetted into 100 ml beakers and 10 ml of molybdovanadate added to the solution. 25 ml of distilled water was then added into each beaker, mixed and allowed to stand for at least 5 minutes. The percentage transmittance for each solution was then determined at 430 nm in a UV visible spectrometer using reagent blank as the reference blank solution (Plank 1992).

The precision at 95% confidence level of measures as obtained from the standard parallelism curves were: ±0.05 ppm for Ca; ±0.02 ppm for K; ±0.05 ppm for P, ±0.002 ppm for Cu; and ± 0.005 ppm for Zn. All measured mineral concentrations from rhino faeces yielded results parallel to the standard curves for each mineral (F-test, all $P > 0.05$).

Statistical Analyses

Correlations between the plotted dietary and faecal Ca, P, Cu and Zn concentrations for the feeding trials with the zoo animals were assessed using Spearman’s correlation coefficient; additionally, linear regression analyses were performed (in all cases, residuals of regressions had a Normal distribution), reporting 95% confidence intervals (95%CI) for parameter estimates. Faecal Ca, P, Cu and Zn concentrations determined from the field study were analysed with Generalized Linear Mixed Modelling (GLMM; (Goldstein 2003, Rasbash *et al.* 2012)), since these data consisted of repeated measurements, and were hierarchically structured, namely observations within rhinos (level 1) and between rhinos (level 2). This allowed Random Effects to be fitted such that variance within response variables (*i.e.*, the different minerals) was partitioned within and between rhinos, prior to the addition of Fixed Effects (broadly equivalent to fitting explanatory variables in Generalised Linear Models). We used MLwiN (version 2.26) (Rasbash *et al.* 2005) to fit the GLMM models.

Our response variables were Ca, P, Cu and Zn mineral concentrations measured in the faeces. The measurements of these minerals were $\log_{10}$ transformed to normalize their error distribution. In the first step of model exploration, the nine study areas were entered as explanatory variables (categorical fixed effects), as was PAM (and its one- and two-month lag preceding faeces deposition), and PAN. Significance of individual explanatory variables was assessed using the Wald χ^2 statistic. Quadratic fits of PAM were also checked for significance if the linear terms were statistically significant or not significant. We fitted 'Area' as a categorical term within the Fixed Effects before fitting constituents of PAM (temperature and rainfall) separately as estimated from satellite data (LSASAF 2010, Tchuenté *et al.* 2011), lagging these variables by up to 3 months prior to the collection of faecal samples, and the squares of these variables to assess curvilinearity . From a plant eco-physiological perspective, it was considered likely that cumulative rainfall and temperature effects impact on the nutrient content of plants: hence these initial tests of lagged rainfall and temperature. After these preliminary model explorations, it was found that the most consistent and parsimonious modelling was when temperature and rainfall were dropped, and replaced by PAM and its square. Since PAM takes into account more variables in addition to rainfall and temperatures (Penman 1948), this measure included more factors affecting nutrient uptake by plants.

The second step in building the model was to determine the effects of PAM and its quadratic fits on the variability of concentration of faecal mineral nutrients. We found high co-linearity between PAM and its lagged values ($r > 0.6$). This high co-linearity made it difficult to evaluate the consequence of adding each variable to changes in model log-likelihood; hence we decided that PAM_Lag1 was ecologically the most reasonable variable to explore further. This was also borne out by comparing models with PAM, PAM_Lag1 and PAM_Lag2; the addition of PAM_Lag1 caused the greatest decline in model variance.

The third step of the analyses was to examine whether there were any effects of the density of rhinos on faecal mineral concentration after first controlling for PAM and PAN. Density was added as a continuous variable ranging from 0.01 to 0.75 rhinos/ km^2.

Results

Zoo study

Data on dietary and faecal mineral concentration from feeding trials with black rhino in zoos indicated that an increase in dietary mineral concentrations is correlated with an increase in faecal mineral concentrations (Fig. 4.2). Correlations between dietary and faecal mineral concentrations (Fig. 4.2) were significant for the four elements (Spearman Ca: $\rho=0.718$, $p<0.001$, $n=50$; P: $\rho=0.715$, $p<0.001$, $n=36$; Cu: $\rho=0.734$, $p<0.001$, $n=50$; and Zn: $\rho=0.769$, $p<0.001$, $n=36$). The 95% C.I. for the slopes of the regression lines included 1 for Zn, were higher than 1 in the case of P and Cu, and lower than 1 in the case of Ca (Fig. 4.2).

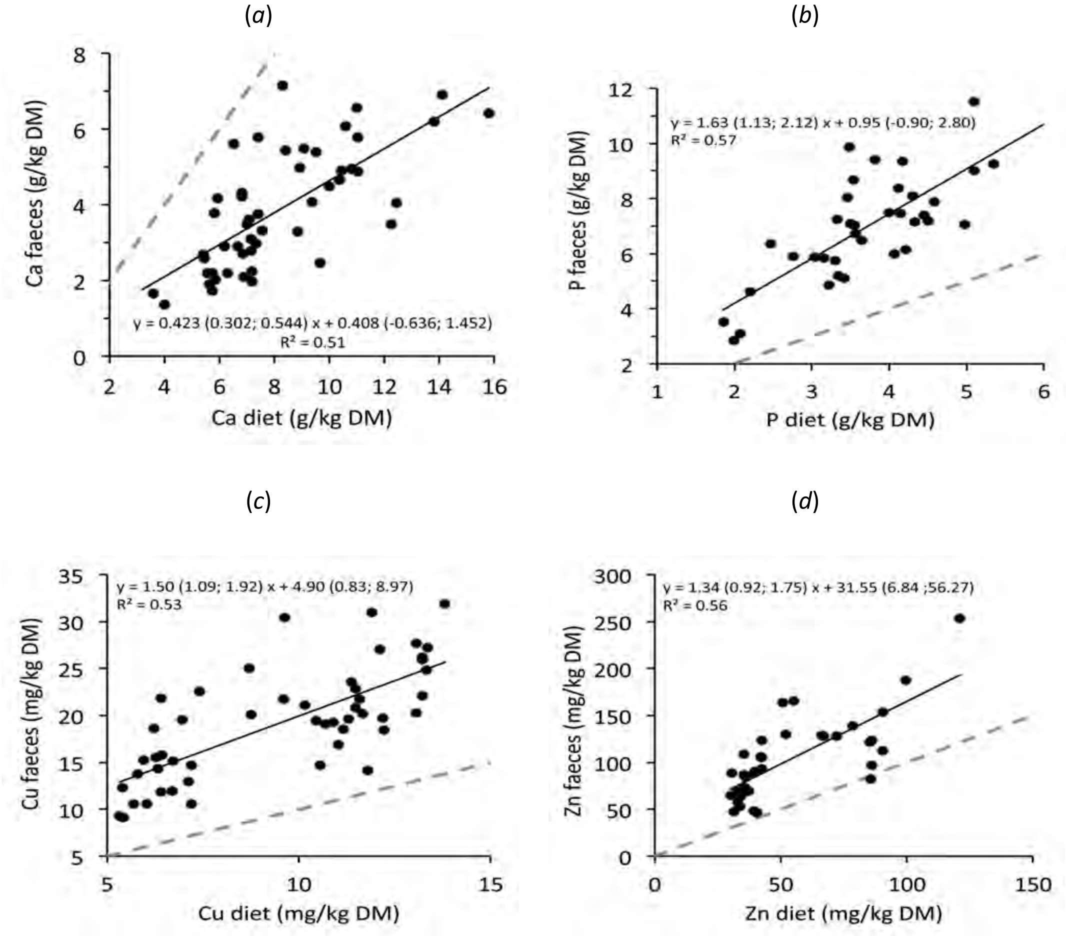

Figure 4.2: Relationship between dietary and faecal mineral concentrations for (*a*) calcium (Ca), (*b*) phosphorus (P), (*c*) copper (Cu), and (*d*) zinc (Zn) from feeding trials with 15 black rhinoceroses (*Diceros bicornis*) in zoos. The dotted line indicates $y = x$ to visualize if concentrations are higher in diet or in faeces. The bold line represents regression equations (in brackets for each parameter estimate the 95% C.I.).

Field study

Faecal mineral nutrient concentration differed significantly between study areas (fitting the model *Ln Nutrient Concentration ~ Area;* chi-square χ^2 values for Area each with 8 d.f. (Ca: $\chi^2 = 36$, $p<0.0001$, P: $\chi^2 = 59$, $p<0.0001$, Cu: $\chi^2 = 170$, $p<0.0001$, Zn: $\chi^2 = 383$, $p<0.0001$). Concentrations of all measured minerals in samples from the Aberdare NP had consistently higher values than those of any other area. Compared to the remaining areas Cu was highest in Nairobi while Zn was highest Nakuru and Nairobi. Cu and Zn were lowest in Tsavo East and Ol Pejeta (Fig. 4.3). When comparing the mineral concentration in faeces of black rhinoceroses from the field study (free-ranging animals) (Fig. 4.3) to those measured in the zoo study (Fig. 4.2), it was evident that both Ca and P occur at much higher concentrations in the free-ranging animals (mean ± SD Ca: 10.1 ± 3.9 *vs.* 3.9 ± 1.6 g/kg DM; P 13.2 ± 8.3 *vs.*

6.9 ± 1.9 g/kg DM). The micro-minerals Cu and Zn, in contrast, had lower concentrations in free-ranging animals than in zoo animals (Cu 12.2 ± 6.3 *vs.* 19.2 ± 5.8 mg/kg DM; Zn 51.6 ± 37.6 *vs.* 104.1 ± 44.0 mg/kg DM).

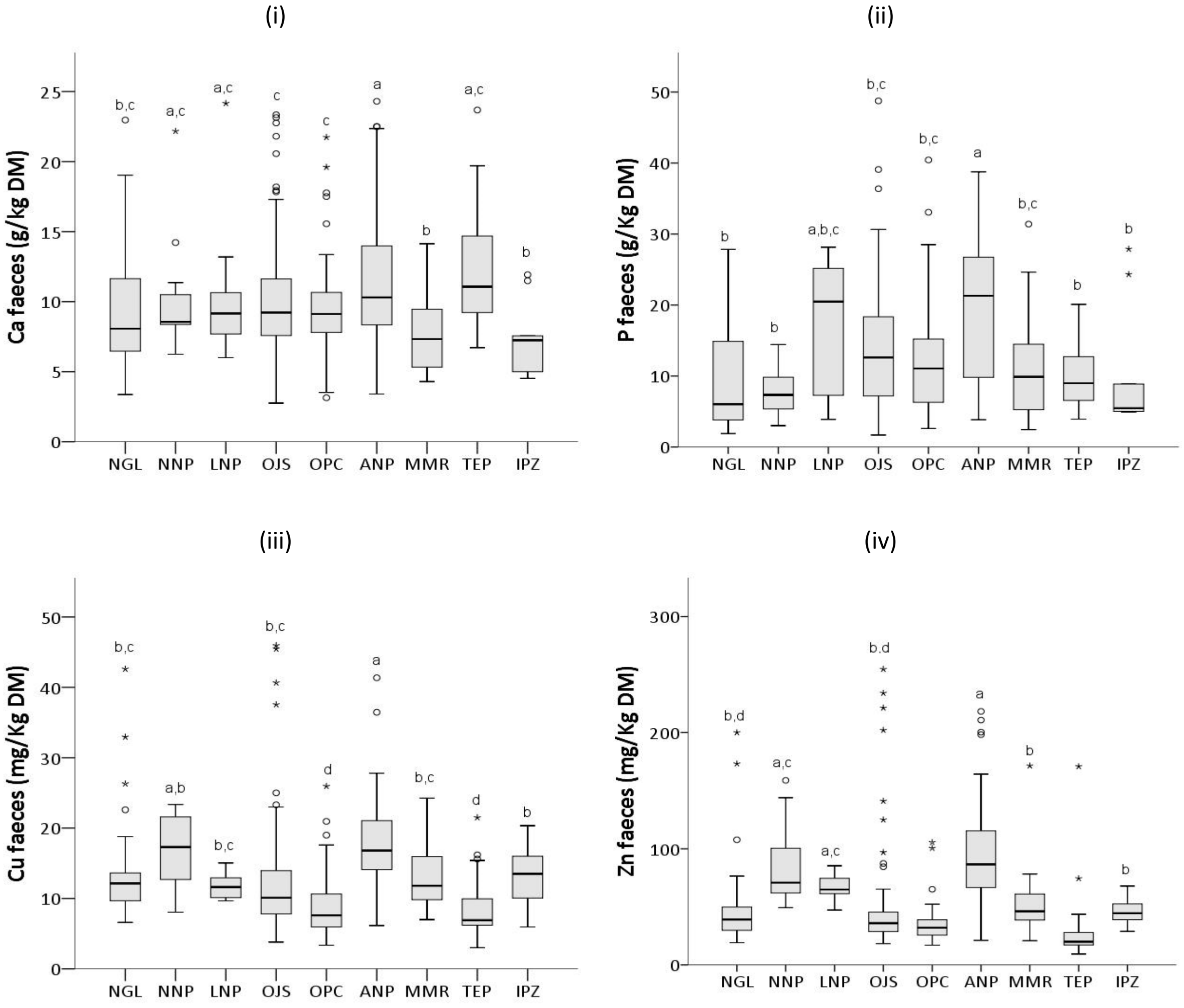

Figure 4.3: Box plots illustrating differences between study areas[†] in concentration of (i) calcium (Ca), (ii) phosphorus (P), (iii) copper (Cu), and (iv) zinc (Zn) as measured from faeces of black rhinoceroses in the field. The study areas are arranged in descending order (*L to R)* of population density as at 2010. The same superscript letters above the 95% C.I. bars indicate not significantly mineral concentration between study areas. The study areas that do not share the same letter above the 95% C.I. error bars had significantly different faecal mineral concentration as assessed by Wald χ^2 statistic at $P \leq 0.05$ (Appendix 4.1). For example, Zn in ANP, LNP and NNP are not significantly different, However Zn in ANP and IPZ are significantly different.

[†] NGL = Ngulia Sanctuary; NNP = Nairobi N. Park; LNP = Lake Nakuru N. Park; OJS = Ol Jogi Pyramid; OPC = Ol Pejeta Conservancy; ANP = Aberdare N. Park; MMR = Masai Mara N. Reserve; TEP = Tsavo East N. Park; IPZ = Tsavo West N. Park Intensive Protection Zone.

The addition of the one-month lag period of Plant Available Moisture (PAM_Lag1) to the models of nutrient concentration significantly reduced the variance observed in Ca, P, Cu and Zn. The parameter estimates indicate that each of these nutrients increased in the faeces with increasing values in PAM_Lag1, strongly suggesting higher nutrient availability in the vegetation as moisture increased (P <0.0001; Table 4.2). PAN did not explain faecal nutrient content: It never was a significant covariable (Table 4.2). Similarly, we did not find a significant relationship (P > 0.05) between density and any mineral nutrient measured in the faeces when controlling for effects of PAM and PAN.

Table 4.2: Results of Generalized Linear Mixed Models exploring the variability in faecal mineral concentration in faeces of black rhinoceros in relation to Plant Available Nutrient (PAN), Plant Available Moisture (lag PAM - lagged by 1 month to reflect rainfall over the 4 weeks preceding sampling) and black rhinoceros density in each of the nine study areas in Kenya 2010/2011

Effects	**Parameter Estimate**	**St error**	**Δ deviance**	**Δ d.f.**	**P**
Calcium					
Intercept	9.218	0.132	4852.74	1	< 0.0001
Area	-	-	36.70	8	< 0.0001
Density	-0.024	0.044	0.29	1	NS
PAN	-0.005	0.013	0.161	1	NS
Lag PAM	0.085	0.027	9.86	1	0.002
Phosphorus					
Intercept	9.787	0.223	1764.61	1	< 0.0001
Area	-	-	59.80	8	< 0.0001
Density	-0.009	0.028	0.11	1	NS
PAN	0.010	0.011	0.86	1	NS
Lag PAM	0.184	0.047	15.28	1	<0.0001
Copper					
Intercept	2.646	0.193	118.39	1	< 0.0001
Area	-	-	170.51	8	< 0.0001
Density	-0.116	0.061	3.61	1	NS
PAN	-0.009	0.018	1.49	1	NS
Lag PAM	0.141	0.029	23.16	1	<0.0001
Zinc					
Intercept	4.195	0.256	265.04	1	< 0.0001
Area	-	-	383.60	8	< 0.0001
Density	-0.118	0.080	2.16	1	NS
PAN	-0.026	0.024	1.16	1	NS
Lag PAM	0.171	0.031	30.18	1	<0.0001

When comparing the mineral concentration in faeces of black rhinoceroses from the field study (free-ranging animals) (Fig. 4.3) to those measured in the zoo study (Fig. 4.2), it was evident that both Ca and P occur at much higher concentrations in the free-ranging animals (mean ± SD Ca: 10.1 ± 3.9 *vs.* 3.9 ± 1.6 g/kg DM; P 13.2 ± 8.3 *vs.* 6.9 ± 1.9 g/kg DM). The micro-minerals Cu and Zn, in contrast, had lower concentrations in free-ranging animals than in zoo animals (Cu 12.2 ± 6.3 *vs.* 19.2 ± 5.8 mg/kg DM; Zn 51.6 ± 37.6 *vs.* 104.1 ± 44.0 mg/kg DM).

Discussion

Both Ca and P occurred at much higher concentrations in the field filed than in the zoo study. For Ca, this is expected, because browse usually contains higher levels of Ca than the diet items usually fed to zoo animals (Clauss and Hatt 2006). For P, this cannot be explained in the same manner, as P levels in browse are not higher than diet items fed to zoo animals (Clauss and Hatt 2006). Therefore, it can only be speculated that this might be an effect of increased microbial activity in free-ranging animals with higher microbial P excretion. In contrast, micro-minerals Cu and Zn had lower concentrations in faecal samples of free-ranging animals than in faecal samples of zoo animals. It is evidently not possible to extrapolate from faecal concentrations on the amount of mineral ingested. Yet, when using the regression line for Cu from Fig. 4.2, this average faecal Cu corresponds to a dietary concentration of approximately 5 mg/kg DM, which is distinctively lower than the maintenance recommendation for horses of 10 mg/kg DM (NRC 2007). Thus, these findings may tentatively support previous suspicions in the literature that free-ranging black rhinoceros might experience marginal dietary Cu concentrations (Maskall and Thornton 1989). Similarly, for Zn, such an extrapolation exercise yields hypothetical dietary concentrations of approximately 15 mg/kg DM, which again is distinctively lower than the maintenance recommendation for horses of 40 mg/kg DM (NRC 2007). While a critical deficiency appears unlikely in the wild, given that animals have been living in these habitats for more than decades, these indications for a marginal provision may support concepts of variations in micronutrient levels affecting reproductive success and hence population growth across habitats.

We hypothesized, firstly, that mineral concentrations in black rhinoceros faeces would increase with an increase in Plant Available Moisture (PAM) and an increase in Plant Available Nutrients (PAN), and secondly that, after controlling for the effects of PAM and PAN, mineral concentrations in faeces would decrease with increasing density of black rhinoceros, reflecting a decrease in diet quality.

Our reasoning that we could use faecal mineral concentrations as a reliable proxy for dietary quality, hinged on the assumption that there was a good correlation between faecal and dietary mineral concentrations, which we tested with data sets for feeding trials of rhinoceroses in zoos. These zoo feeding trials showed that an increase in faecal mineral concentrations is correlated with an increase in dietary mineral concentrations, and explained variances were quite good for minerals we investigated (P: 57%, Zn: 56%, Cu: 53% and Ca: 51%; *see* Fig. 4.2). At first sight, this correlation is straight forward to be explained because it can be expected that the animals are in a form of dietary steady state in zoos. In all cases except for Zn the faecal concentrations were significantly different from the dietary concentrations. Faecal Ca falls below the $y = x$ line which can be accounted for by high proportions of Ca being excreted in urine (Clauss *et al.* 2007b), thus explaining lower faecal than dietary concentrations (Fig. 4.2a). For Cu, Zn or P; the loss of the organic fraction of the diet due to digestion explains the higher concentration of these minerals in the faeces than in the diet (Fig. 4.2b, c & d). Nevertheless, the linear correlations and the reasonably high explained variances between diet and faecal mineral concentrations in the zoo study gave sufficient confidence to use faecal mineral nutrient for black rhinoceros as a measure of diet quality. Additionally, concentration of the measured minerals in faecal samples from the field study increased with increase in PAM_Lag1 (*i.e.*, rainfall over the 4 weeks preceding sampling). This correlation between PAM and mineral concentration strongly suggested that increase in moisture resulted in higher nutrient availability and higher concentration of nutrients for the browse (Singh and Singh 2004, Barbosa *et al.* 2014 *in press*). Diet quality have been shown to relate to plant mineral concentrations (*e.g.*, Hall-Martin *et al.* 1982, Howery and Pfister 1990, Hodgson *et al.* 1996, Wrench *et al.* 1997). On these bases, we interchangeably refer to faecal mineral nutrients as diet minerals or diet quality in this discussion.

One-month lag of plant available moisture (PAM_Lag1) consistently explained the variability of mineral concentration in faecal samples from the field study. This was consistent with the dependency of production of browse and growth of leaves of woody plants on precipitation that fell some time ago rather than on current precipitation (reflecting percolation to roots) (Rutherford 1984, Gaye and Edmunds 1996, Taylor and Howard 1996, Ludwig *et al*. 2004, Koduganti P. *pers. comm*.). In line with our hypothesis, the results were consistent with the reported increase in quality (Bell 1982, Huntley 1982) of woody plants with increase in rainfall in savanna ecosystems receiving annual rainfall between 200mm and 1000mm. Singh and Singh (2004) and Barbosa *et al.* (2014 *in press*) showed that generally the concentration of micro- and macro-minerals in leaves and twigs of woody plants increased with increase in water availability. This may explain why the Aberdare which had the highest PAM also had the highest concentration of dietary macro- and micro-minerals.

Apart from the broad categorization of soils in a PAM-PAN space, other soil characteristics such as parent rock type and soil texture have been shown to influence quality and quantity of woody plants (*e.g.*, Frost *et al.* 1985, Scholes and Walker 1993, Scholes and Archer 1997, Walker and Langridge 1997, Sankaran *et al.* 2008). In Nairobi, black rhinoceros showed higher concentrations of Cu in their faeces; and in Nairobi and Nakuru the black rhinoceroses exhibited higher Zn excretion. The rhinoceroses in Aberdare had highest Cu and Zn levels in their faecal samples compared to those in other study areas. Because the soils of these three different areas are of volcanic origin (McCall 1967, Sombroek *et al.* 1982, Onyancha *et al.* 2011), one could perhaps expect high concentrations of these metals in rhinoceros diets. However, low concentration of Cu in soils and a subsequent low concentration of Cu in two browse plants (*i.e*., yellow-backed acacia *Acacia xanthophloea* and Sodom apple *Solanum incanum*) have been reported for Nakuru (Maskall and Thornton 1989). Low concentration of Cu in soils of Nakuru and surrounding has been correlated to characteristics of volcanic ash and pumice that have been associated with copper deficiency in wheat (Chamberlain 1959, Pinkerton 1967) and pasture (Howard and Burdin 1962). Maskall and Thornton (1989) tried to find an explanation for the low Cu in browse plants they had studied in the high alkalinity of the soil (which decreases Cu uptake) and reported low Cu levels in the serum of impala (*Aepyceros melampus*) to be aggravated by the high molybdenum levels in the vegetation of Nakuru. Even so, we did not find a critical Cu deficiency in the browse diet of black rhinoceros in Nakuru as measured through faecal analysis. Our findings underscore the use of faecal analyses over chemical analyses of individual plants in determining quality of diet for browsers. This brings to fore higher likelihood that dietary quality for black rhinoceros in Nakuru may after all not be critically deficient of trace elements as earlier reported (Jonyo *et al.* 1988, Maskall and Thornton 1989). Based on these earlier studies, mineral supplementation for animals in Nakuru was recommended (*e.g.*, KWS 1993, Okita-Ouma *et al.* 2007b). Our results may also imply that mineral concentration in different browse plants are not much determined by soil fertility as assayed in chemical laboratories. The latter possible interpretation of our results was demonstrated by Le Houérou (1980) when he showed no significant differences in phosphorus concentration in legume browse between East and West African soils; West African soils are overwhelmingly deficient in phosphorus compared to East African soils. This lack of a clear relationship between soil mineral concentration and mineral concentration in woody plants explains, we think, why we did not a find significant effect of PAN on faecal mineral concentrations.

So where PAN appears to be of little use for understanding black rhinoceros diet quality, PAM appears to have a much a higher explanatory power. Tsavo East as compared to Aberdare, Nakuru and Nairobi was located in an area of low PAM (Fig. 4.1) and consequently had the lowest Cu and Zn in dietary browse of black rhinoceros as measured through faecal mineral analyses (Fig. 4.3). Given the availability and quality of key resources determine ideal-free habitat selection and animal distribution (McLoughlin *et al.* 2010), the significantly different density clusters of black rhinoceroses in Tsavo East between 1967 and 1968 (Goddard 1969) could be explained by PAM and PAN. PAM and PAN vary significantly over space and would therefore be expected to significantly vary within the vast Tsavo East. Goddard (1969) estimated some six to nine thousand eastern black rhinoceros in the Tsavo ecosystem area which then approximated 23,300 km^2. However, of this area *ca*. 15,000 km^2 comprised Tsavo East with 59% of it with black rhinoceros densities as low as 0.1 - 0.4 animals /km^2 at the time. The rest of the 41% area of Tsavo East then had densities of 1.2 - 4.0 rhinoceros/km^2. The highest density of 4 rhinoceroses/km^2 was recorded within one location measuring 544 km^2 in the North West of Yatta Plateau (Goddard 1969). In other words, the largest part of Tsavo East, mostly north east of Galana River, was not or not very much suitable for eastern black rhinoceros. Goddard (1970) attributed these density differences to food preferences of the black rhinoceros suggesting that the right food resources for this species did not occur in the northern part of Tsavo

East. Van Beest *et al.* (2014) did not find an ideal-free distribution of the Feral horse (*Equus ferus caballus* Linnaeus) in Sable Island National Park in Canada but they demonstrated, using isodars *i.e*., simple regression lines depicting the response of density in one habitat *vs.* another (*e.g.*, Morris 1988, Haugen *et al.* 2006) and resource selection functions which models relationships between use and availability of a resource unit (*e.g.*, Boyce and McDonald 1999, Manly *et al.* 2002, Fortin *et al.* 2008), that the densities and distribution of the horses were determined by key resources. Indeed the horses first favoured high quality vegetation but as their density increased habitat use spread out to lower quality vegetation. The Tsavo East example of 1969 could as well have been an example of an ideal-free habitat selection and animal distribution. On the basis of our results and the hypotheses underlying the ideal-free distribution, it is reasonable to suggest that the low density areas in Tsavo East (Goddard 1969) were not associated with low browse quality *per se* (Fig. 4.1) but possibly with low rainfall independent of browse quality.

It was not clear why Ol Pejeta Conservancy had a low Cu like Tsavo East despite being in volcanic soils (Sombroek *et al.* 1982) and having a high PAM. We speculate that this can be attributed to the lack of a definite pattern of mineral nutrients in browse plants *vs*. PAN. Our speculation hinges on, for example, the contrasting Cu levels in browse plants in Nakuru in this study *vis a vis* the study by Maskall and Thornton (1989) and the similar phosphorus levels in legume browse in the significantly different East and West Africa soils (Le Houérou 1980). Additionally heterogeneity of soil characteristics even when the soils are of similar origin can result in significant differences in mineral patches (Nyandat and Ochieng' 1976). The Ol Pejeta case further explains why we did not find a significant effect of PAN on dietary minerals.

Trace elements such as Zn and Cu have been reported to play important roles in reproduction and fertility in rats (*e.g.*, Gunn and Gould 1958, Bedwal and Bahuguna 1994, Yousofvand *et al.* 2013) and elephants (Koen 1988). The role of these elements as important modifiers of fertility or reproduction in domestic horses, the closest relative of rhinoceroses, has not been demonstrated (NRC 2007). We can therefore only infer from the studies in rats and elephant that the higher dietary Cu and Zn in Nairobi and Nakuru than in other areas, in addition to overall resource availability, could partly explain why black rhinoceros in these two populations reproduced optimally (Okita-Ouma 2004, Okita-Ouma *et al.* 2009, *also see* Chapter 3 this thesis). This could also mean that the low densities recorded by Goddard (1969) in a very large section of Tsavo East were probably associated with the lower concentration of trace elements as observed by us. The Aberdare population is not discussed in this context of fertility and reproduction because its population was adversely affected by poaching (Okita-Ouma and Wandera 2006).

Different herbivore guilds at high densities impact on their habitats differently but generally depress plant availability and quality (*e.g.*, Klein 1968, Caughley 1976a, Frost *et al.* 1985, Scogland 1985, Sinclair *et al.* 1985, Ellis and Swift 1988, Van Langevelde *et al.* 2003, Augustine and McNaughton 2004, Forsyth and Caley 2006, Simard *et al.* 2008). Density effects on the quality of dietary plants may be revealed only after controlling for influence of the variable precipitation (Owen-Smith 1990) and available nutrients. In our case, however, even after controlling for PAM and PAN, the variations observed in concentration of dietary minerals were not associated with the density of black rhinoceros ($P > 0.05$ in all instances). This could have meant four things; firstly, that there was no density dependence in any of the populations we studied; consequently implying (secondly) that density of black rhinoceros did not have a negative impact on quality of browse plants. Therefore, although browsers at high density reduce biomass of shrubs or woody plants (Van Langevelde *et al.* 2003, Sankaran *et al.* 2008) and quality of parts of plants browsed by (meso-) browsers (Van der Waal 2005), the quality of shrubs or woody plants ingested by mega-browsers is not necessarily reduced by these animals. Thirdly, the physiological ability of hindgut fermentation of black rhinoceros to process plants of low nutritional quality such as *Euphorbia* spp. with high concentrations of secondary compounds (*e.g.*, Goddard 1968, 1970, Mukinya 1977, Hitchins 1979, Hall-Martin *et al.* 1982, Luske *et al.* 2009) could override some density feedbacks. Fourthly, it could also have meant that faecal mineral concentrations used in this study were poor proxy for diet quality. Faecal nitrogen (N) has been widely used to evaluate the nutritional value of diets for herbivores and discern large dietary differences (Prins and Beekman 1989, Howery and Pfister 1990, Grant *et al.* 1995). It was therefore unfortunate that faecal N and in particular metabolic faecal N (Mason 1969, Van Soest 1994, Schwarm *et al.* 2009) could not be assessed in this study.

In conclusion, we did not find evidence for density dependent effect on diet quality in black rhinoceros, *i.e.*, the quality of browse plants of black rhinoceros were not lowered by browsing due to high densities of black rhinoceros. We found evidence that areas of high PAM have higher levels of faecal minerals and higher densities of black rhinoceros, implying this mega-browser is bottom-up controlled. We recommend further research on the role of PAN and the confounding soil characteristics on diet quality of browse plants, because based on our study and available old literature, PAN does not seem to influence mineral concentration in leaves of browse plants.

Acknowledgements

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Appendix 4.1: Differences between study areas[†] in concentration of Ca, P, Cu and Zn mineral nutrients as measured from analyses of faeces of black rhinoceros collected from nine study areas†. For each nutrient in the table, the signs given in each cell reading down the column compares whether the area in the row cell is significantly greater or less than the area given in the column header. For example, Cu concentrations in the faeces from IPZ were significantly less than mean concentration of Cu in faeces collected from ANP. Also mean Cu concentrations from TEP (or OPC) were greater than those found in IPZ. Statistical significance (assessed using the Wald Chi-square) is indicated by P ≤ 0.05 *; P ≤ 0.01 **; P ≤ 0.001 ***; P ≤ 0.0001 ****; and ns = not significant.

[†] ANP = Aberdare National Park; IPZ = Tsavo West National Park Intensive Protection Zone; LNP = Lake Nakuru National Park; MMR = Masai Mara National Reserve; NGL = Ngulia Rhinoceros Sanctuary; NNP = Nairobi National Park; OJS = Ol Jogi Ranch; OPC = Ol Pejeta Conservancy; TEP = Tsavo East National Park.

	ANP	IPZ	LNP	MMR	NGL	NNP	OJS	OPC
Ca								
ANP								
IPZ	-**							
LNP	ns	-*						
MMR	-****	ns	-*					
NGL	-*	ns	ns	-*				
NNP	ns	-*	ns	-*	ns			
OJS	-*	-*	ns	-**	ns	ns		
OPC	-*	-*	ns	-**	ns	ns	ns	
TEP	ns	-***	ns	-****	-**	ns	-**	-**
P								
ANP								
IPZ	-**							
LNP	ns	ns						
MMR	-****	ns	ns					
NGL	-****	ns	+**	ns				
NNP	-****	ns	+**	ns	ns			
OJS	-****	ns	ns	ns	-***	-**		
OPC	-****	ns	ns	ns	-**	-*	ns	
TEP	-****	ns	-*	ns	ns	ns	ns	ns
Cu								
ANP								
IPZ	-*							
LNP	-**	ns						
MMR	-***	ns	ns					
NGL	-****	ns	ns	ns				
NNP	ns	ns	-*	-*	-*			
OJS	-****	ns	ns	+*	+*	+****		
OPC	-****	+**	+**	+****	+****	+****	+****	
TEP	-****	+**	+**	+****	+****	+****	+***	ns
Zn								
ANP								
IPZ	-****							
LNP	ns	-*						
MMR	-****	ns	+*					
NGL	-****	ns	+**	ns				
NNP	ns	-***	ns	-****	-****			
OJS	-****	ns	+****	+**	ns	+****		
OPC	-****	+*	+****	+****	+***	+****	+**	
TEP	-****	+****	+****	+****	+****	+****	+****	+***

Chapter 5

Reproductive performance in black rhinoceros (*D. b. michaeli*) as a function of Plant Available Moisture and Plant Available Nutrients

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Abstract

Reproductive performance in mega-herbivores is hypothesised to be influenced by the quantity and quality of forage that are functions of Plant Available Moisture (PAM) and Plant Available Nutrients (PAN). PAM and PAN determine the quantity and quality of different plant guilds differently and therefore would be expected to influence reproductive performance of browsers and grazers differently. This influence is not well understood yet, but this understanding is important for selecting suitable areas for (re-)introducing rare mammalian herbivores. Here we focused on a mammalian mega-browser, the Critically Endangered eastern black rhinoceros (*Diceros bicornis michaeli*), in seven populations in Kenyan savanna habitat. Woody cover in savannas provides browse for black rhinoceros and is found to increase with PAM and decrease with PAN. The relation between reproductive performance and woody cover was such that age at first calving was earlier, inter-calving Interval was longer and Yearly Percentage of females calving was higher as woody cover increased. We found early age at first calving in areas with high PAM and low PAN, whereas inter-calving Interval was not affected by PAM but was longer in areas of high PAN. Yearly percentage of females calving was high in areas with high PAM and low PAN, but it was not affected in areas with low PAM irrespective of the levels of PAN. Our results suggest that age at first calving in black rhinoceros was influenced more by quantity of woody plants as estimated from measures of woody cover than were inter-calving intervals or yearly percentage of females calving. Besides the requirement of sufficient woody plants, these findings contribute a new criterion to use PAM and PAN to select conservation areas for maximising reproductive performance of mega-mammalian browsers particularly the black rhinoceros.

Key words: *black rhinoceros, moisture, nutrients, savanna, reproductive performance, woody cover*

Introduction

Mammalian herbivores exhibit better reproductive performance in habitats that provide sufficient quality and quantity of plants (Sinclair *et al*. 1985, Berryman 2004, White 2004, Jones *et al*. 2010), and this applies to mega-herbivores too (Owen-Smith 1988, Atkinson 1995, Birkett 2002, Danell *et al.* 2006, Clauss *et al.* 2007a). Quality and quantity of plants are functions of Plant Available Moisture (PAM), *i.e.*, the water potential of the soil that can be absorbed by plants in every soil horizon (Walker and Langridge 1997) and soil characteristics, particularly Plant Available Nutrients (PAN). Soil fertility is determined by a variety of factors (Walker and Langridge 1997); however, PAN is the simplest index to represent soil fertility, because it is derived from the sum of exchangeable calcium-, magnesium-, sodium- and potassium cations. The PAM/PAN concept has been used in comparing structure and functions of savanna habitats from a subcontinent scale to a global scale, where the highest diversity of grazing mammalian herbivores is found to occur in areas with intermediate PAM and high PAN (Frost *et al*. 1986, Walker and Menaut 1988, Solbrig 1990, Olff *et al*. 2002, Ahrestani *et al*. 2011).

However, PAM and PAN determine the quantity and quality of different plant guilds differently and therefore would be expected to influence reproductive performance of browsers and grazers differently. Moreover, this concept has not been used to explain reproductive performance in mega-mammalian herbivores or to use its potential for selecting suitable areas for (re-)introducing rare mammalian herbivores. Appropriate selection of suitable areas for reproduction is important for maintaining rare or Critically Endangered species in the wild, especially when a species is facing significant poaching threat.

Generally, high PAM decreases quality of herbaceous plants as forage for grazers but increases quantity of herbaceous plants, whereas high PAN increases both quality and quantity of herbaceous plants (Olff *et al*. 2002). However, for woody plants that provide nutrition and energy for reproductive performance in browsers, the quality of browsing forage (twigs and leaves) increases with increase in PAM (Singh and Singh 2004, Barbosa *et al*. 2014 *in press*). Furthermore, the strong negative correlation between woody cover and soil nitrogen (Sankaran *et al*. 2008) suggest higher quantity of woody cover in areas of low PAN. The influences of PAM on the quality of herbaceous plants (Fig. 5.1a) and influences of PAN on quantity of herbaceous plants (Fig. 5.1b) are expected to be opposite to the influences of PAM on quality and PAN on quantity of woody plants (Figs. 5.1c & 5.1d).

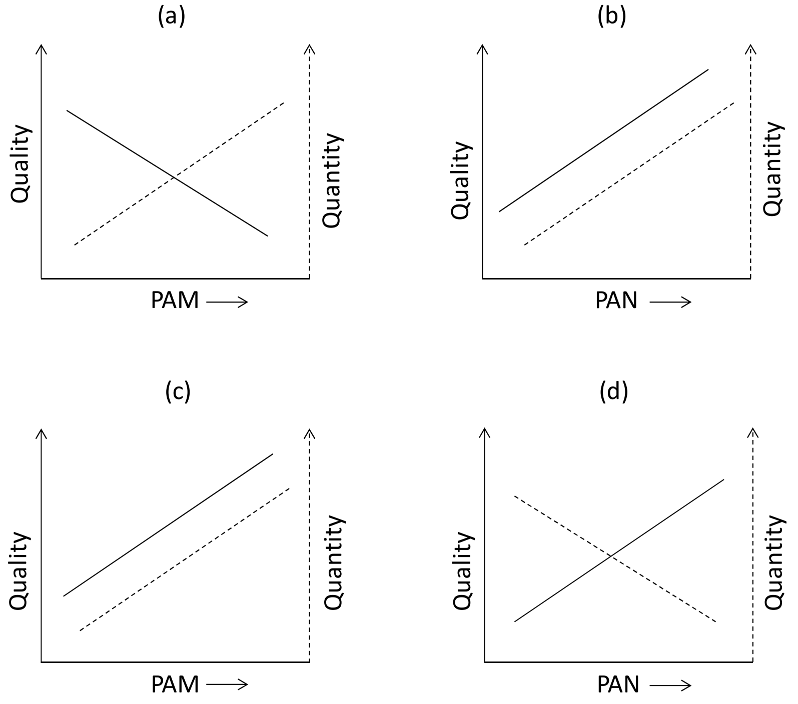


Figure 5.1: The relationships between quality (solid line) and quantity (dotted line) of herbaceous plants with Plant Available Moisture = PAM (Fig. 5.1a) and with Plant Available Nutrient = PAN (Fig. 5.1b); and the relationships between the quality and quantity of woody plants (leaves and twigs) with PAM (Fig. 5.1c) and with PAN (Fig. 5.1d). Fig. 5.1a shows decrease in quality of herbaceous plants with increase in PAM whereas Fig. 5.1c shows the quality of woody plants increase with increase in PAM. Fig. 5.1b shows increase in quantity of herbaceous plants with increase in PAN, whereas Fig. 5.1d shows decrease in quantity of woody plants with increase in PAN.

It is therefore expected that nutrient-dependent reproductive performance as observed in browsers such as white-tailed deer (*Odocoileus virginianus*) (Bryant *et al*. 1980), black-tailed deer (*Odocoileus hemionus*) (Parker *et al*. 1999), greater kudu (*Tragelaphus strepsiceros*) (Owen-Smith and Cooper 1989) and black rhinoceros (*Diceros bicornis*) (Atkinson 1995) would be affected differently by PAM and PAN compared to nutrient-dependent reproductive performance in grazers.

Here we select black rhinoceros, a mega-browser, to better understand its reproductive performance in relation to PAM and PAN. This species is Critically Endangered (IUCN 2013b) hence requires amongst many well informed biological management including effective ways of selecting suitable areas to maximize its reproduction (Atkinson 1995). Three measures of reproductive performance namely; age at first calving (AFC), average inter-calving interval (ICI) and yearly percentage of females calving (%FC) are used in this study, because they are directly influenced by browse quality and quantity (du Toit 2001). Sufficient food quality and quantity promotes attainment of approximately 80% of full adult body weight that a female requires for successful conception and parturition (Owen-Smith 1988) with females' ages at first calving being about 7 years (du Toit 2001). The shortest possible black rhinoceros' average ICI, *i.e.*, the time from the birth of one calf to the next, can be between 1.4 to 1.5 years considering its gestation period of 1.25-1.30 years and an oestrus cycle of about 30 days (Hildebrandt *et al*. 2007). A short ICI is very rare; on average an ICI of 2.5 years has been recorded for best performing black rhinoceros populations *in situ* (Adcock 1999). The period between one calf and the next requires quality nutrition for the lactation phase (Hildebrandt *et al*. 2007) and survival of the new born calf. Low quality browse in this phase may lead to delays in conception or abortions. Neonate mortality may also increase when quality of browse is low (Emslie 1999). The yearly percentage of females calving (%FC) is an equally important measure of population performance. This measure assumes that all females ≥ 7 years old are calving. This measure fluctuates a lot on a year to year basis because of the >1 year gestation period, and because most black rhinoceros populations are small (<100 animals) at present, thus giving rise to randomness in this parameter hence the need to average %FC over ≥3 year periods. Overall, %FC is considered excellent if >40%, good if ranging from 33 - 40%, and below average if <33% (du Toit 2001).

Given the opposite effects of PAM-PAN on forage for browsers and grazers, we hypothesize (a) early age at first calving (< 7 years), (b) short average inter-calving Interval (<2.5 years) and high yearly percentage of females calving (>40%) for black rhinoceros in areas with high PAM and low PAN. We first related these three measures of reproductive performance to woody cover that provides browse for black rhinoceros. Second, we tested whether the relation with woody cover could be explained by PAM and PAN. Therefore we used data of seven populations in Kenyan savanna habitats recorded between 1993 and 2010.

Study Areas

Aberdare National Park (Aberdare)

Aberdare lies between 36^0 25'E to 37^0 00'E and 0^0 05'S to 0^0 45'S (Fig. 5.2). It was gazetted as a National Park in 1950 and in 1988 listed as a rhinoceros conservation area in Kenya. The Park is ring-fenced and covers an area of 766km^2 but rhinoceroses are concentrated in a section the Park called Salient that covers about 70km^2. The mean annual rainfall (± SD) in the period of study was 607 ± 149 mm (www.cpc.ncep.noaa.gov). The vegetation is characterized by montane forest habitat with and undergrowth of mixed species of bush land thickets (Adcock *et al*., 2007). Higher altitudes are dominated by bamboo forest and grass tussocks on the moorlands. Soil typology is described by Sombroek *et al*. (1982).

Lake Nakuru National Park (Nakuru)

Nakuru is located between 36^0 20'E to 36^0 25'E and 0^0 50'S to 1^0 00'S (Fig. 5.2) approximately 4 km from Nakuru town. The Park was gazetted in 1961 and established as a rhinoceros sanctuary in 1986. It is completely fenced, covering an area of 188 km^2, of which 44 km^2 lies in the shallow highly alkaline soda lake in the central rift valley.

The mean annual rainfall over the period of study was 755 ± 169 mm (www.cpc.ncep.noaa.gov). The vegetation is a mixture of open grassland, *Acacia*, *Tarchonanthus* bush land, deciduous and *Euphorbia* forests and riverine bush land (Maskall and Thornton 1989, Adcock *et al.* 2007). Soils are dominated by lacustrine and riverine sediments and volcanic ash (Sombroek *et al.* 1982).

Masai Mara National Reserve (Mara)

Mara is located between 34^0 45’ to 35^0 25’E and 1^0 13’ to 1^0 45’S (Fig. 5.2). The reserve has been a black rhinoceros conservation area since it was gazetted as a protected area in 1958. Mara is not fenced and covers an area of 1510 km^2 in the south-west of Kenya and is part of Tanzania’s Serengeti National Park ecosystem. The mean annual rainfall over the period of study was 911 ± 147 mm (www.cpc.ncep.noaa.gov). The vegetation comprises mainly grassland with isolated scrublands and woodlands especially along drainage lines (Walpole 2002, Adcock *et al.* 2007, Kanga *et al.* 2011). Soil typology is described by Sombroek *et al*. (1982).

Ngulia Rhinoceros Sanctuary (Ngulia)

Ngulia is located between 38^0 06’E to 38^0 10’E and 3^0 01’S to 3^0 06’S (Fig. 5.2) within Tsavo West National Park. It was formed in 1985 as a way of re-establishing a large free-ranging black rhinoceros population within the Park. The completely fenced sanctuary has been progressively expanded from 3 km^2 in 1985 to 20 km^2 in 1987 to 63 km^2 in 1990 and to the current 92 km^2 in 2007. The sanctuary suffered habitat deterioration due to high density of mega-herbivores mainly elephant, buffalo and black rhinoceros in the mid-2000s (Brett and Adcock 2002). The situation was managed by removing 255 elephant (Okita-Ouma *et al.* 2008a, Okita-Ouma *et al.* 2009), 200 buffalo and 12 black rhinoceroses. The mean annual rainfall over the period of study was 385 ± 146 mm (www.cpc.ncep.noaa.gov). The vegetation is predominantly *Commiphora* and *Acacia* woodland with scattered baobab trees (Leader-Williams *et al.* 1993, Brett and Adcock 2002, Adcock *et al.* 2007). Soil typology is described by Sombroek *et al*. (1982).

Nairobi National Park (Nairobi)

Nairobi is located between 36^0 23’E to 36^0 28’E and 2^0 18’S to 2^0 20’S covering an area of 117 km^2 (Fig. 5.2). It was gazetted as a protected area in 1946 and made a rhinoceros sanctuary in 1963. The Park is ring-fenced but approximately 20 km perimeter on its southern boundary is unfenced to link with a wildlife migratory corridor (Gichohi 2000). However, current extensive infrastructural and housing development is gradually blocking the migratory corridor. The mean annual rainfall over the period of study was 540 ± 151 mm (www.cpc.ncep.noaa.gov). Major vegetation communities comprise deciduous forest, riverine thorn forests, shrubs and grasslands (Muya and Oguge 2000, Adcock *et al.* 2007). Soil typology is described by Sombroek *et al*. (1982).

Ol Jogi Pyramid (Ol Jogi)

Ol Jogi is located between 37^0 00’E to 37^0 05’E and 0^0 15’N to 0^0 20’N (Fig. 5.2). It is completely fenced; covers 50 km^2 and was established as a rhinoceros sanctuary in 1979. Nine black rhinoceroses died from clostridial enterotoxaemia in 2010 (Ndeereh *et al*. 2011). The mean annual rainfall over the period of study was 459 ± 113 mm (www.cpc.ncep.noaa.gov). The vegetation comprise of a mosaic of grassland, *Acacia* woodland and shrubs (Mizutani 1999, Adcock *et al.* 2007). Soil typology is described by Sombroek *et al* (1982).

Ol Pejeta Conservancy (Ol Pejeta)

Ol Pejeta is located between 36^0 40’E to 37^0 00’E and 00^0 02’S to 00^0 07’N (Fig. 5.2). It was designated as a black rhinoceros sanctuary in 1988. Its fence-line is designed to allow migration of other wildlife species except for rhinoceroses. The conservancy covers an area of 300 km^2 after an expansion from 93 km^2 in 2007. The mean annual rainfall with ±SD over the period of study was 557 ± 115 mm (www.cpc.ncep.noaa.gov). The vegetation is a mosaic of grassland, *Acacia* woodland, *Euclea* shrub and riverine woodland grassland (Birkett 2002, Adcock *et al.* 2007). Soil typology is described by Sombroek *et al*. (1982).

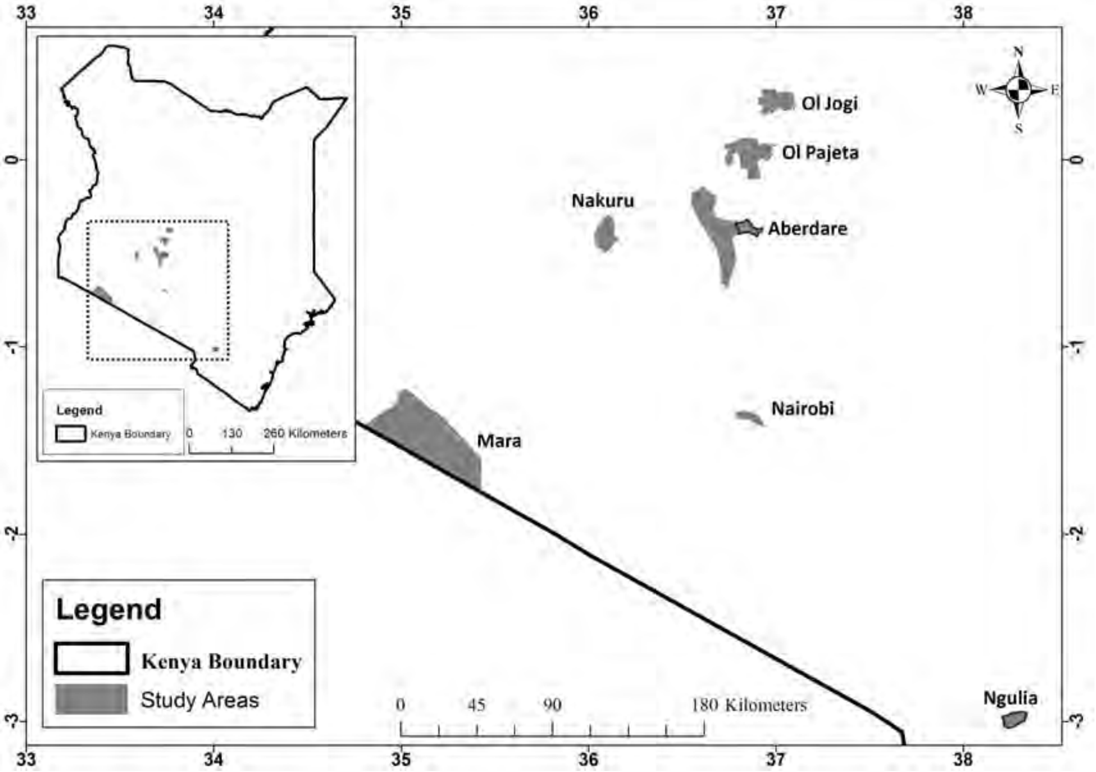


Figure 5.2: Locations of the seven study areas on the map of Kenya. The section of Aberdare where the study was carried is shown by bold border line.

Data collection

Reproductive performance

Reproductive performance measures were derived from demographic data stored in Kenya's rhinoceros information system (Okita-Ouma *et al*. 2011). All the field data underwent a process of quality control and entered into the information system by the field staff of the Kenyan rhinoceros monitoring programme.

For each study area, age at first calving (AFC) was calculated as the difference in years between the date of birth of a female and the date of birth of her first calf. Only females of ±1 year of birth-date accuracies, all totalling 64 animals, were considered. The frequency distribution of AFC for the 64 females is shown in Figure 5.3.

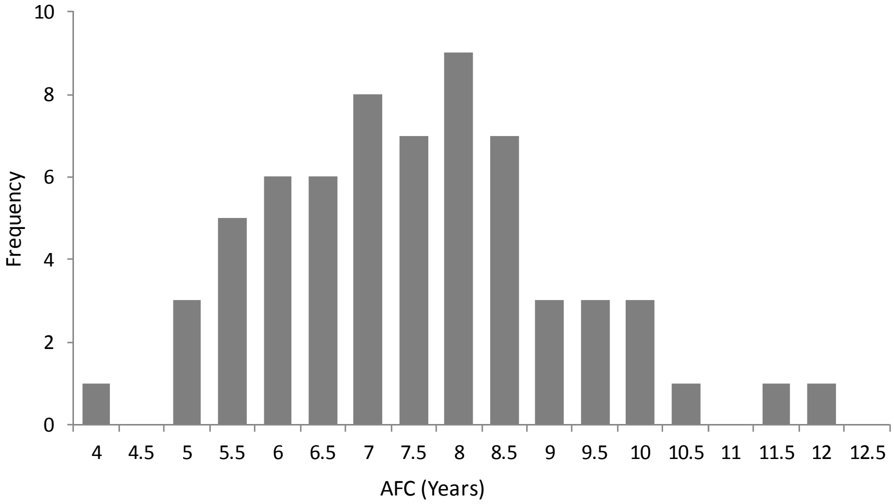


Figure 5.3: Frequency histogram of ages at first calving (AFC) for female black rhinoceroses (*n*=64) of ±1year birth-date accuracies in six populations in Kenya namely Nakuru, Mara, Ngulia, Nairobi, Ol Jogi and Ol Pejeta, between 1993 and 2010. Aberdare is excluded due to lack of data for females with ±1year birth-date accuracies.

The average inter-calving interval (ICI) in years for a known female was derived by dividing the difference between the date of birth of first calf and date of birth of the last calf as at the end of 2010, by the female's total number of calves minus one. The frequency distribution of average ICI for 100 females is shown in Figure 5.4.

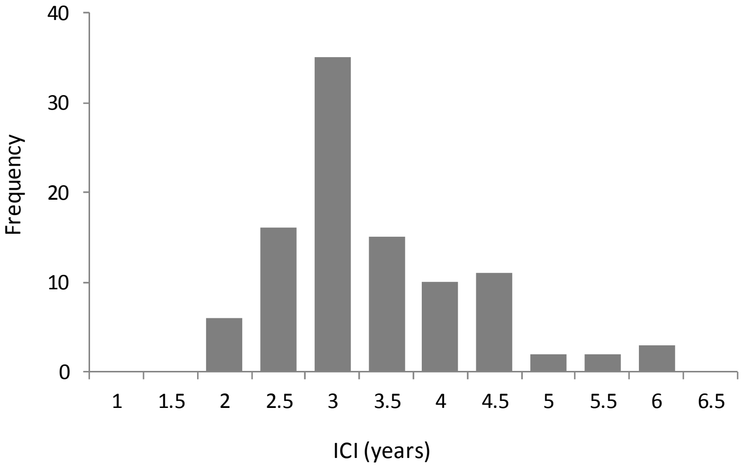
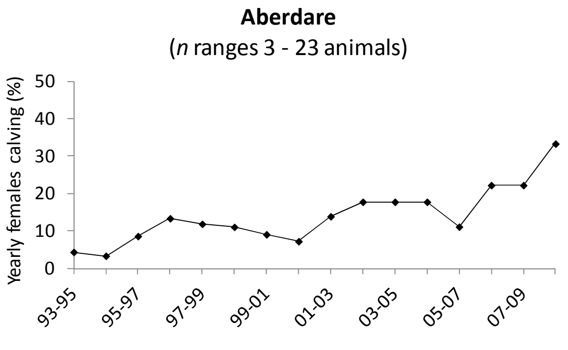
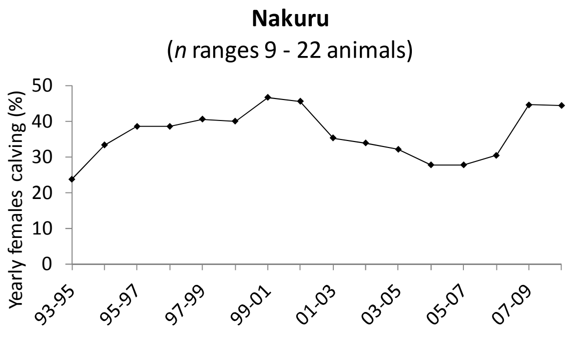
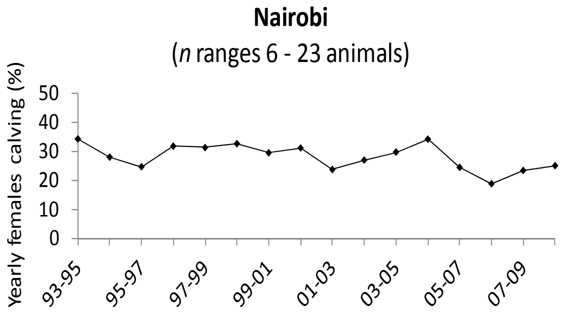


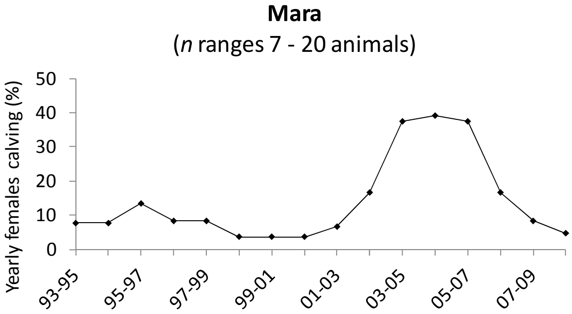
Figure 5.4: Frequency histogram of average Inter-calving Interval (ICI) for female black rhinoceroses (*n* = 100) with ≥ 2 calves in seven populations in Kenya namely Aberdare, Nakuru, Mara, Ngulia, Nairobi, Ol Jogi and Ol Pejeta between 1993 and 2010.

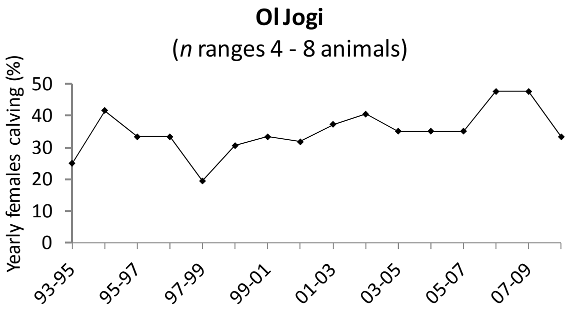
Yearly percentage of females calving (%FC) was derived as the proportion of number of calves born in year "*t*" to the number of live adult females (≥ 7years) in year "*t*". The year to year variations in %FC due to the long gestation period of about 1.3 years for black rhinoceros and the possibility of synchronized calving in small populations was solved by averaging %FC over 3-year moving windows. %FC in the seven study areas between 1993 and 2010 are shown in Figure 5.5.

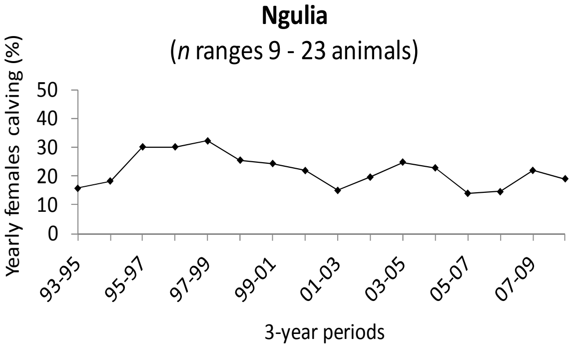












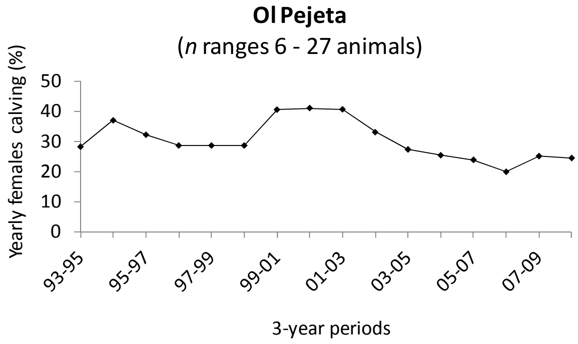


Figure 5.5: A 3-year moving average of yearly percentage of female black rhinoceros calving (%FC) between 1993 and 2000 in seven study areas in Kenya. %FC >40% is excellent, 33 - 40% is good, while <33% is below average performance (du Toit 2001).

Plant Available Moisture (PAM) index

PAM index for each study area was calculated by averaging the area's yearly PAM. Yearly PAM was derived as the monthly average of the Log_{10} of the ratio of actual Rainfall (RAIN) and Potential Evapo-Transpiration (PET) *i.e.*,Log_{10} RAIN/PET (Walker and Langridge 1997, Olff *et al*. 2002). PAM ranged between -1.0 and +1.0 representing the lowest and highest Log_{10} RAIN/PET values in the study area. The Blaney-Criddle formula (Blaney and Criddle 1962) was preferred over the detailed Penman-Monteith equation (Penman 1948) due availability of data. PET was thus derived as follows:

$$PET_o = p \times (0.46 \times T_{mean} + 8)$$

Where;

PET_o is the reference Potential Evapotranspiration [mm day^{-1}] (monthly)

T_{mean} is the mean daily temperature [°C] given as $T_{mean} = (T_{max} + T_{min})/2$

p is the mean daily percentage of daytime hours (assumed 50% for equatorial regions over the year)

Rainfall data included field measurements and decadal (10-day interval) estimates derived from rainfall raster images of 8 km resolution (http://www.cpc.ncep.noaa.gov/products/fews/AFR_CLIM/afr_clim.shtml). Hawth's Tools for ArcMap9.3.1© and ArcGIS™ software was used to extract specific rainfall estimates from raster images by overlaying GIS shape files of boundary for each study area on the raster images. The same data source, analytical tools and procedures for extracting rainfall data were used in the derivation of minimum and maximum temperatures from raster files of 4 km resolution.

Plant Available Nutrient (PAN) index

PAN was measured as the mean of the sums of soil exchangeable cations (Ca^{2+}, Mg^{2+}, Na^+ and K^+) in milliequivalent of hydrogen per 100 grams (mEq/100g) (Olff *et al*. 2002, Ahrestani *et al*. 2011). Exchangeable cations were extracted from Kenya Soil Survey reports and datasets of scales 1:50,000 to 1:250,000 (Glover and Williams 1966, Glover and Trump 1970, Van der Weg *et al.* 1976, Siderius and Muriuki 1977, Wamicha and Gachene 1979, Wamicha *et al.* 1981, Sombroek *et al.* 1982, Van Wijngaarden and Van Engelen 1985, Waruru and Ita 1986, Ahn and Geiger 1987, GTZ *et al.* 1987, Kinyanjui 1996) and World Soils Information (ISRIC) database (Batjes and Gicheru 2004, Batjes 2006, ISRIC 2011). To standardize comparisons only exchangeable cations within 20 cm soil depth were used. Study areas that lacked estimates of exchangeable cations were represented by exchangeable cations estimates of neighbouring sites on the basis of Sombroek (Sombroek *et al.* 1982).

Woody cover

Woody cover was used as broad-scale indicative measure of forage and assumed to be correlated with reproductive performance in black rhinoceros. This was derived from Moderate Resolution Imaging Spectroradiometer (MODIS) land cover maps comprising the vegetation continuous field layers. The layers included percent bare ground, herbaceous and woody cover and, for woody cover, percent evergreen, deciduous, needle-leaf and broad-leaf (Hansen *et al*. 2002). We estimated woody cover of the seven study areas from these maps that were basically of 500 m sub-pixel. MODIS uses annual phenological metrics as the independent variables to predict woody cover. This methodology was developed based on a very large dataset and has the potential to fairly estimate available forage of ≤ 2 m (Adcock *et al.* 2007) for black rhinoceros in savanna ecosystem (Bucini and Hanan 2007) because it combines vegetation classes including bush land and shrub land of ≤5 m.

Statistical analyses

Averages of PAM and PAN for each study area were used to distinguish the study areas in a PAM-PAN plane. IBM® SPSS® Statistics19 was used for all statistical analyses. To test the relationship between reproductive performance and PAM, PAN, and woody cover, we used curve estimation procedures to investigate the shape of relationships between the means of AFC and ICI for each study area and PAM, PAN and woody cover. We also related the standard deviations of the means of AFC and of ICI as dependent variables to PAM, PAN and woody cover as independent variables to investigate whether the variation between the individuals in each area could be explained by these independent variables. We tested for linear, logarithmic, inverse, power, S-shape and exponential relationships and used the highest R^2_{adj} values to measure goodness of fit. We used the Generalized Estimating Equation approach (GEE) (Zeger *et al.* 1988) to investigate the relationship between %FC as dependent variable and PAM, PAN (and the interaction between PAM and PAN), and woody cover as independent variables. GEEs can be used as extensions of generalized linear models for the analysis of longitudinal data such as %FC. We applied 'Year' as within-subject variable and 'Area' as subject variable. The 3-year moving average of the %FC was used as dependent variable, and we checked that the residuals were normally distributed.

Results

The seven black rhinoceros study areas were plotted on a PAM-PAN space as shown in (Fig. 5.6). Ngulia had the lowest average PAM while Aberdare, Nakuru and Mara had the highest PAM. Ngulia had the highest PAN while Ol Jogi had the lowest PAN.

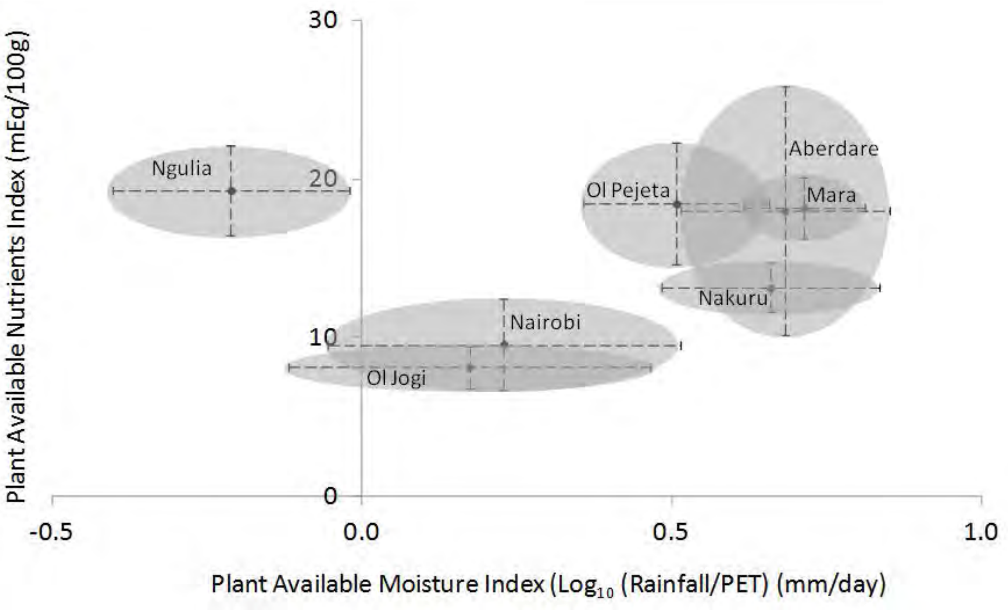


Figure 5.6: Seven black rhinoceros conservation areas plotted on a plane of PAM-PAN plane. The x and y error bars are 95% Confidence Interval. The shading indicates the 95% CI of PAM and PAN in each of the rhinoceros conservation area. *n* = 10, for PAM in all areas. For PAN, *n* = 179 in Ngulia, *n* = 17 in Ol Jogi, *n* =28 in Nairobi, *n* = 19 in Ol Pejeta, *n* = 47 in Nakuru, *n* = 23 in Aberdare and *n* = 43 in Mara. PAN-values are from the literature (*see* Methods section).

Reproductive performance measures as functions of PAM, PAN and woody cover

Mean AFC was negatively related with woody cover (Fig. 5.7a), with the linear model as the best model (R^2_{adj} = 0.84, *n* = 6, coefficient b_1 ± SE for woody cover = -16.02 ± 3.10, t = -5.170, P = 0.007). The curve estimation procedure showed no significant relationship between mean AFC or the standard deviation of the mean AFC as dependent variables and PAM, PAN, or woody cover as independent variables (all P>0.05). AFC ranged between 4 and 12 years (Fig. 5.3).

The average ICI as related to PAN could be best described by an S-shaped curve ($\ln(Y) = b_0 + (b_1/X)$; PAN: R^2_{adj} = 0.51, *n* = 7, coefficient b_1 ± SE for 1/PAN = -2.96 ± 1.11, t = -2.692, P = 0.043), and a power function best described the relationship between the average ICI and woody cover ($\ln(Y) = \ln(b_0) + (b_1 * \ln(t))$; woody cover: R^2_{adj} = 0.77, *n* = 6, coefficient b_1 ± SE for ln(woody cover) = 0.12 ± 0.03, t = 4.663, P = 0.006). We found an increase of the mean ICI with increasing PAN (Fig. 5.7b) and increasing woody cover (Fig. 5.7c). There was no significant relationship between the mean ICI or the standard deviation of the mean ICI as dependent variables; and PAM, PAN or woody cover as independent variables (all P > 0.05). Average ICI ranged between 1.5 and 6.0 years (Fig. 5.4).

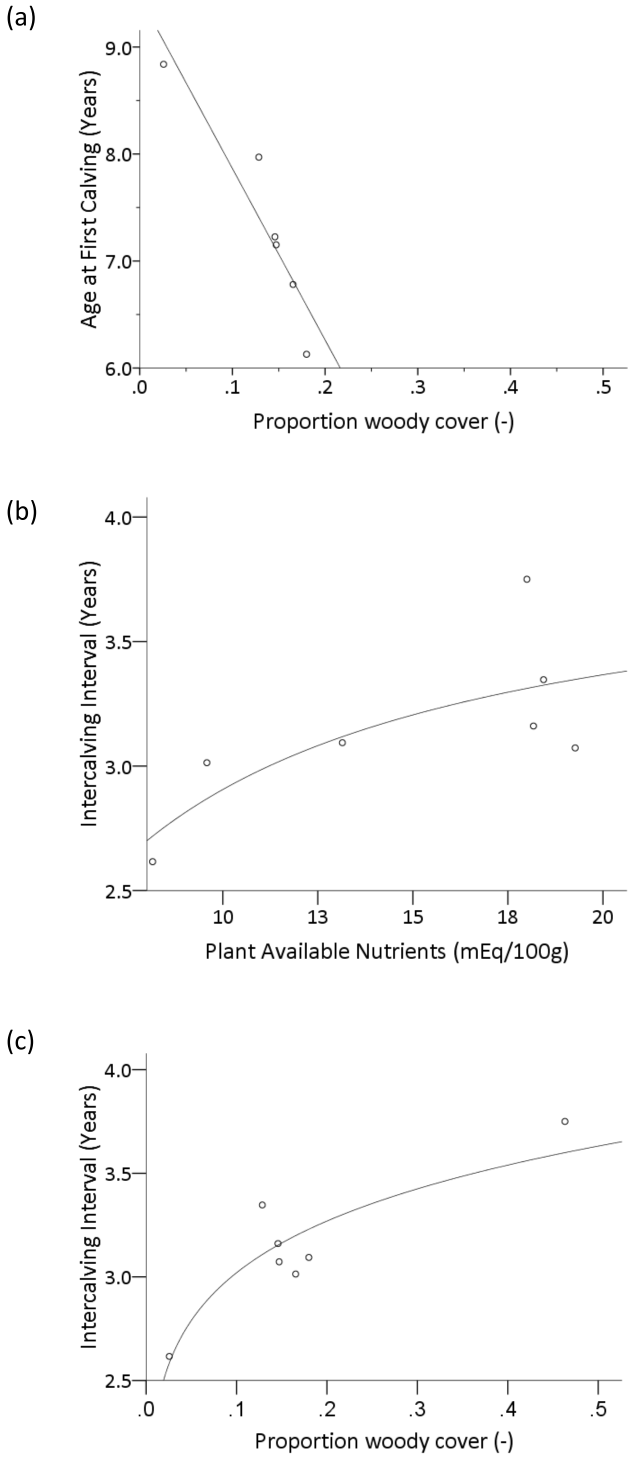


Figure 5.7: The relationships between (a) the age of first calving and woody cover (*linear function*); (b) the inter-calving interval and plant available nutrients (*S-shape function*) and; (c) the inter-calving and woody cover (*power function*). The lines in the graph represent the best fitting regression models. (*see* text for statistics. *Note*: the Y-axes do not start at 0; Aberdare is excluded in (a) for lack of data).

There was a significant relationship between yearly percentage of females calving (%FC) and PAM, and the interaction between PAM and PAN (Table 5.1). This model relationship is illustrated in Figure 5.8, which shows that %FC decreased at high levels of PAM with increasing PAN, but was not significantly affected at low levels of PAM irrespective of levels of PAN. The %FC averaged between 10% and 45% between study areas (Fig. 5.5). There was no significant correlation between %FC and woody cover (P > 0.05).

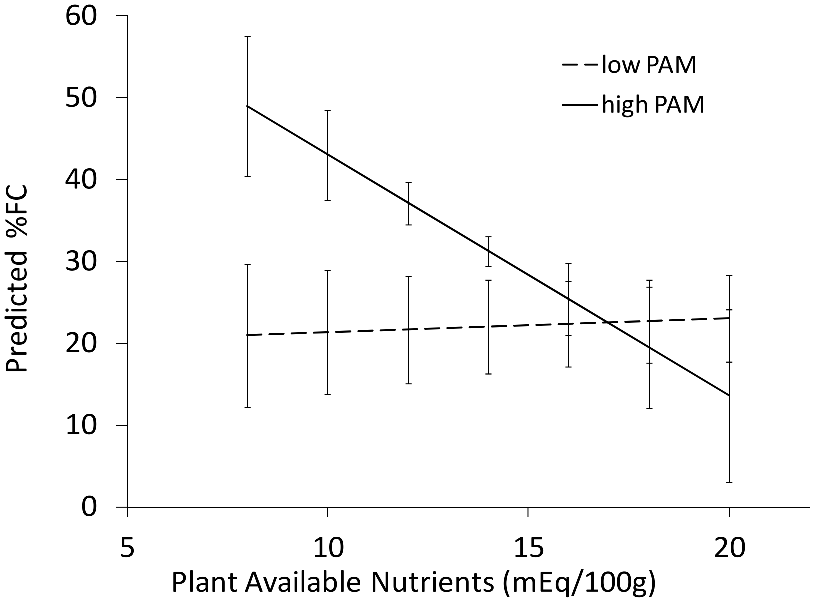


Figure 5.8: The predicted relationship between the yearly percentage of female black rhinoceros calving and the interaction between Plant Available Moisture (PAM) and Plant Available Nutrients (PAN) in the seven study areas namely Aberdare, Nakuru, Mara, Ngulia, Nairobi, Ol Jogi and Ol Pejeta (*see* Table 5.1 for statistics). The error bars indicate the 95% confidence intervals of the predicted values. PAM index range from -0.1 to 0.6.

Table 5.1: Model relationship between the yearly percentage of female black rhinoceros calving (%FC) and the interaction between Plant Available Moisture (PAM) and Plant Available Nutrients (PAN) in the seven study areas namely Aberdare, Nakuru, Mara, Ngulia, Nairobi, Ol Jogi and Ol Pejeta between 1993 and 2010 using Generalized Estimating Equations (Zeger *et al.* 1988). The model shows a significant relationship between %FC (*n* =111) and PAM and interaction between PAM and PAN.

Independent variable	**Parameter estimate of GEE model (± SE)**	**Wald Chi-square (χ^2)**	**P**
Constant	27.13 (5.43)	24.99	<0.001
PAN (mEq/100g)	-0.27 (0.30)	0.80	0.371
PAM (mm/day)	75.56 (22.29)	11.49	0.001
PAN x PAM	-4.46 (1.39)	10.26	0.001

Discussion

The observed early age at first calving (AFC) in areas with high woody cover (Fig. 5.7a) was consistent with the generally shorter inter-calving intervals (ICI) in low PAN-areas (Fig. 5.7b). Short ICI of ≤2.5 years was found in areas with PAN of less than7.5 mEq/100 g, which supports the improved reproductive performance as PAN decreased. Our study supports the hypothesis that reproductive performance in mega-herbivores is influenced by the quantity and quality of forage that are functions of Plant Available Moisture (PAM) and Plant Available Nutrient (PAN). However, in contrast to findings for grazers that are found to occur in areas with intermediate PAM and high PAN (*e.g.*, Frost *et al*. 1986, Walker and Menaut 1988, Solbrig 1990, Olff *et al*. 2002, Ahrestani *et al*. 2011), our study suggests that reproductive performance of browsers is best in areas with high PAM and low PAN. These findings contribute to our understanding that PAM and PAN determine the quantity and quality of different plant guilds differently and influence reproductive performance of browsers and grazers differently. More so, historical evidence (Mitchell 1953, Sidney 1965) show black rhinoceros occurred in abundance in areas such as coastal *nyika* (hinterlands) in Kenya, *Brachystegia* woodland of Malawi, and Rufiji delta in Selous Game Reserve in Tanzania where precipitation is high but soil fertility is low.

We suggest that PAN of ≤7.5 mEq/100 g allows for sufficient quantity woody cover, leaves and twigs at, such that above this threshold increases in PAN lead to decreases in quantity of woody cover (Sankaran *et al*. 2008). However, ICI was longer (> 2.5 years) in high woody cover (Fig. 5.7c). These seemingly contradictory results are of interest and we attribute them to the following two reasons:

Firstly, an early AFC of <7 years but longer ICI of >2.5 years in woody cover of >15% meant that early AFC is associated with vegetation characteristics of high woody cover, *i.e.*, abundant leaves, twigs, forbs and shrubs, while short ICI is associated with vegetation characteristics of low woody cover of ≤15%, that is, less abundant leaves, twigs, forbs and shrubs; a characteristic of savanna ecosystems of high PAN (Walker and Langridge 1997, Sankaran *et al*. 2008). Areas of low woody cover generally provide plants of high palatability (Prins and Van der Jeugd 1992) and protein (Wright *et al*. 2001) with low lignin and low chemical compounds (Dierenfeld *et al*. 1995, Wright *et al*. 2001). In our case, it could therefore be justifiably assumed that even though areas of low woody cover yielded less abundant leaves, twigs, forbs and shrubs, they were of high protein and of low chemical compounds.

Secondly, the gestation and lactation are energy and nutritionally demanding phases for mega-herbivores (Geist 1974, Prins 1996, Ahrestani *et al*. 2012), hence the higher the nutritional and energy requirements an area can provide, particularly areas with high woody cover or areas with high PAM and low PAN, the earlier the AFC is expected. Lactation phase is more nutritional than energy demanding for both female's body conditioning and survival of the calf as exhibited in herbivores that synchronize calving with seasons when forage is of higher protein (Estes and Estes 1979). Areas of low woody cover are characterized by high quality forage (Prins and Van der Juegd 1993, Dierenfeld *et al*. 1995, Wright *et al*. 2001) and therefore likely to favour shorter ICI and subsequently higher yearly percentage of females calving (%FC). However, given lactation naturally down-regulates the ovarian activity in black rhinoceros (Hildebrandt 2011), the resultant ICI can be long in areas of high PAN.

The observed decrease in %FC at high levels of PAM with increasing PAN and the lack of significant relationship between %FC and PAN at low levels of PAM (Fig. 5.8) can be explained by the influences of PAM and PAN on quantity and quality of woody cover (leaves, twigs, forbs and shrubs). Quantity of leaves, twigs, forbs and shrubs increase with increase in PAM (Owen-Smith 1994, Singh and Singh 2004, Owen-Smith 2008, Barbosa *et al.* 2014 *in press*) whereas quantity increase with decrease in PAN (Sankaran *et al*. 2008). Reproductive performance in the browsing black rhinoceros as influenced by quantity of forage would therefore be expected to be better in areas of high PAM with low PAN. This explains the good %FC (33 - 40%)(du Toit 2001) generally recorded in areas of high PAM particularly Nakuru. Similar better reproductive and better stocking potentials for black rhinoceroses in Nakuru has been reported using a different approach of browse availability and suitability assessment (Adcock *et al.* 2007). The low %FC of <29% and moderate ICI of slightly above 3 in Ngulia despite being located in a favourable low PAM and high PAN for diet browse can be attributed to habitat degradation by intense inter specific competition between 2000 and 2006 (Brett and Adcock 2002, Okita-Ouma *et al.* 2008a).

Yearly percentage of females calving (%FC) was the reproductive performance measure that was best explained as a function of PAM and PAN. This is mainly because inherently %FC has a large sample size that takes into account all females of breeding age; the average ICI excludes females that have calved once, whereas AFC excludes females whose birth date accuracy is greater than ±1 year.

Conclusion

Areas of high PAM, low PAN and sparse woody cover yielded the best reproductive performance measures. The relations between reproductive performance and woody cover showed that AFC was earlier, ICI was longer and %FC was higher as woody cover increased. We found early AFC in areas with high PAM and low PAN, whereas ICI was not affected by PAM but was longer in areas of high PAN. The %FC was high in areas with high PAM and low PAN, but it was not affected in areas with low PAM irrespective of the levels of PAN. We suggest that AFC in black rhinoceros is determined more by quantity of forage than are ICI and %FC. Besides the requirement of sufficient woody cover, these findings contribute a new criterion on use of PAM and PAN to selecting conservation areas for maximising the reproductive performance of mega-mammalian browsers particularly the black rhinoceros.

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Photo credit: William Mutua

Chapter 6

Physiological stress levels in the eastern black rhinoceros (*D. b. michaeli*) in relation to population density and extrinsic factors

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Abstract

Chronic levels of physiological stress have been linked to lowered reproductive performance and other maladies in animals. It is important to understand intrinsic and extrinsic factors that elevate physiological stress levels in animals to alleviate negative consequences. To achieve this, we seek to understand changes in corticosterone metabolites (stress hormones) in black rhinoceros as influenced by intrinsic (population density and sex) and extrinsic (plant minerals, rainfall, temperature) factors. We hypothesised i) that physiological stress levels are higher in populations of high density than in populations of low density; ii) that corticosterone metabolite levels decrease with increase in rainfall when food resources are abundant but increase with increase in temperature; iii) that corticosterone metabolite levels would decrease with increase in concentration of dietary minerals as measured via faecal minerals; iv) that female black rhinoceros would be more physiologically stressed than male black rhinoceros. We applied non-invasive faecal sampling techniques on animals of known sex, age and dominance in seven populations of contrasting densities over one year covering dry and rainy seasons. We measured variability in faecal corticosterone metabolites in male and female black rhinoceros in relation to population density, sex, faecal minerals as indicator dietary minerals, rainfall, and temperature. Faecal samples were analysed for corticosterone metabolites by radio immunoassay techniques. Calcium concentration was determined using flame photometer, Phosphorous concentration was determined using a UV visible spectrometer, whereas Copper and Zinc concentration were determined using Atomic Absorption Spectrometer. We used linear mixed models (LMM) to analyse the data. There was no significant relationship between density and physiological stress levels. Physiological stress levels increased as rainfall and maximum temperature increased; stress levels were higher in females than in males, but were inversely related to concentration of phosphorous in dietary browse as measured via faecal mineral concentration. These results suggest that secretion of stress hormones are influenced more by climate and dietary minerals than by population density. As reported in many species, females have generally elevated corticosterone metabolites than males.

Key words: *black rhinoceros, faeces, corticosterone, minerals, rainfall, physiological stress, temperature*.

Introduction

Understanding the effect of population density on animal physiology is important in understanding reproductive performance (Creel 2001, Wingfield and Sapolsky 2003). For mega-herbivores, density feedback is theorised to occur at 70% – 80% of the maximum stocking density (*e.g.*, Owen-Smith 1988, McCullough 1999). Constant reviews of the concept of density feedbacks are necessary especially when the concept is used to manage Critically Endangered mega-herbivores such as black rhinoceros *(Diceros bicornis)* to maximise reproduction. Density dependence may manifest itself through physiological changes in the animal whereby populations of high densities exhibit higher physiological stress than populations at low densities. The physiological stress, may be described as the increase in concentration of glucocorticoids secretion due to activation of the hypothalamic–pituitary–adrenal (HPA) system by external stimulus related to demography, environment or behavior (Creel 2001). Symptoms of adrenal exhaustion in dense populations (Selye 1946) was first advanced by Christian (1950) when he hypothesised activation of physiological systems of stress in meadow voles (*Microtus* spp.) at high population density. Li *et al.* (2007) also found high adrenocortical secretion in Pére David's deer (*Elaphurus davidianus*) living in small enclosures at high densities in captivity. Chronic physiological stress levels have been found to lead to low survival probability, immunosuppression, diseases (Christian 1950), loss of body condition and poor reproductive capability (Wasser and Starling 1988, Singer and Zumoff 1992, Kirby *et al.* 2009) or disruption of metabolism and gastrointestinal functions (*see review in* Li *et al.* 2007, Smith *et al.* 2012) if high densities lower quantity and quality of key resources.

Dietary fibre (Dantzer *et al.* 2011) and plant minerals for example nitrogen (N), Phosphorous (P), Calcium (Ca), Copper (Cu) and Zinc (Zn) (Breman and de-Wit 1983, Lester *et al.* 2010), as influenced by plant phenology (Millspaugh *et al.* 2001a; Huber *et al.* 2003; Millspaugh & Washburn 2004) and population density (Coomes *et al.* 2003) would affect food quality and subsequently influence gut microbial metabolism of stress hormones (Wasser *et al.* 1993). Corticosterone metabolites would increase with increase in intake of dietary fibre (Dantzer *et al.* 2011). Females generally exhibit higher physiological stress than males (Millspaugh *et al.* 2001, Kudielka and Kirschbaum 2005, Munshi-South *et al.* 2008, Bangasser *et al.* 2010). The sexual differences in stress levels are attributed to male and female brain dimorphisms (Luque *et al.* 1992, Pinos *et al.* 2001) and to ovarian cycles (Ter Horst *et al.* 2009) in humans. Social species are more vulnerable to physiological stress than territorial species (Kuznetsov *et al.* 2004, Weingrill *et al.* 2004, Engh *et al.* 2006b, Creel *et al.* 2012) whereas less dominant animals are more physiologically stressed than the dominant animals (Creel 2001). Seasonal changes, rainfall and temperature can modulate basal and elevated glucocorticoid secretion in birds, mammals, amphibians and reptiles in a predictable pattern during the annual cycle (Harper & Austad 2001; Millspaugh *et al.* 2001a; Millspaugh *et al.* 2002; Millspaugh & Washburn 2004). Faecal glucocorticoid concentrations among free-ranging North American elk (*Cervus elaphus*) for example, are low in winter and peak in summer (Millspaugh *et al.* 2001).

Here we report physiological stress responses to population density and its interaction with rainfall, temperature, plant mineral concentration measured via faecal minerals in black rhinoceros, and sex differences. This investigation provides insight into likely effects of activation of the HPA system by these stressors for a Critically Endangered species. We hypothesised i) that physiological stress levels are higher in populations of high density than in populations of low density; ii) that corticosterone metabolite levels decrease with increase in rainfall when food resources are abundant but increase with increase in temperature; iii) that corticosterone metabolite levels would decrease with increase in concentration of dietary minerals as measured via faecal minerals; iv) that female black rhinoceros would be more physiologically stressed than male black rhinoceros.

We deployed a non-invasive faecal sampling technique to collect faecal samples for twelve months covering dry and rainy seasons. Black rhinoceros from which faecal samples were collected were of known identity, sex, age, dominance, and reproductive status from seven populations of contrasting densities in Kenyan savanna ecosystems. The faecal sampling technique facilitates data on metabolic products consisting of digestive tract cells, secretions, and microbes to be gleaned from the faeces without the sampling procedures themselves stressing the animal (Möstl and Palme 2002, Millspaugh and Washburn 2004). Excreted hormones via faeces represent average values pooled over time, rather than a single point-in time measure (Schwarzenberger *et al.* 1996, Palme 2005, Li *et al.* 2007).

Apart from its Critically Endangered status and the need for well-informed scientific management, we chose black rhinoceros for several reasons. Firstly, it is a selective browser known to significantly impact on its habitat especially if enclosed in high density; however, there is still no evidence of density effects on its stress hormones either directly or via quality of food resources. Secondly, by virtue of its large body size, the black rhinoceros does not have effective predators to regulate its population top-down therefore making it possible to detect density effects via food resources. Thirdly, no synchronised reproduction is expected for black rhinoceros given that its gestation period stretches over more than a year, hence no biased seasonal stress hormone secretion is expected. Males of black rhinoceros are territorial whiles females are not (*see review in* Emslie and Adcock 2013) and hence sexual differences in secretion of stress hormones are expected. Dietary and faecal mineral concentration of black rhinoceros indicate that, in general, an increase in faecal mineral concentration is linked to an increase in dietary mineral concentration (Clauss *et al.* 2007b; also *see* Chapter 4 this thesis). This offers an opportunity to understand the influence of dietary quality on corticosterone metabolite levels as measured via faecal minerals.

Methods

We aimed to collect one faecal sample per animal per month between July 2010 and July 2011 inclusive from 29 individually known male and 31 individually known female black rhinoceros from seven varying population densities (Fig. 6.1, Table 6.1). We managed to collect 198 faecal samples from 29 males and 189 faecal samples from the 31 females for mineral analysis. Of these, 151 faecal samples from 25 males and 137 faecal samples from 20 females were assayed for corticosterone metabolites, resulting in a mean of 6.45 faecal samples per animal for mineral nutrients and a mean of 6.40 faecal samples per animal for corticosterone metabolites, both randomly distributed over the thirteen months and seasons of data collection. Individual animals were identified by use of ear-notches, body marks, home ranges and remote measures such as camera-traps and radio tracking.

Three populations (Ngulia, Ol Jogi and Ol Pejeta) exceeded their estimated maximum stocking densities, whereas four populations (Mara, Aberdare, Tsavo East and Tsavo West IPZ) had densities below the estimated maximum stocking density (Adcock *et al.* 2007).

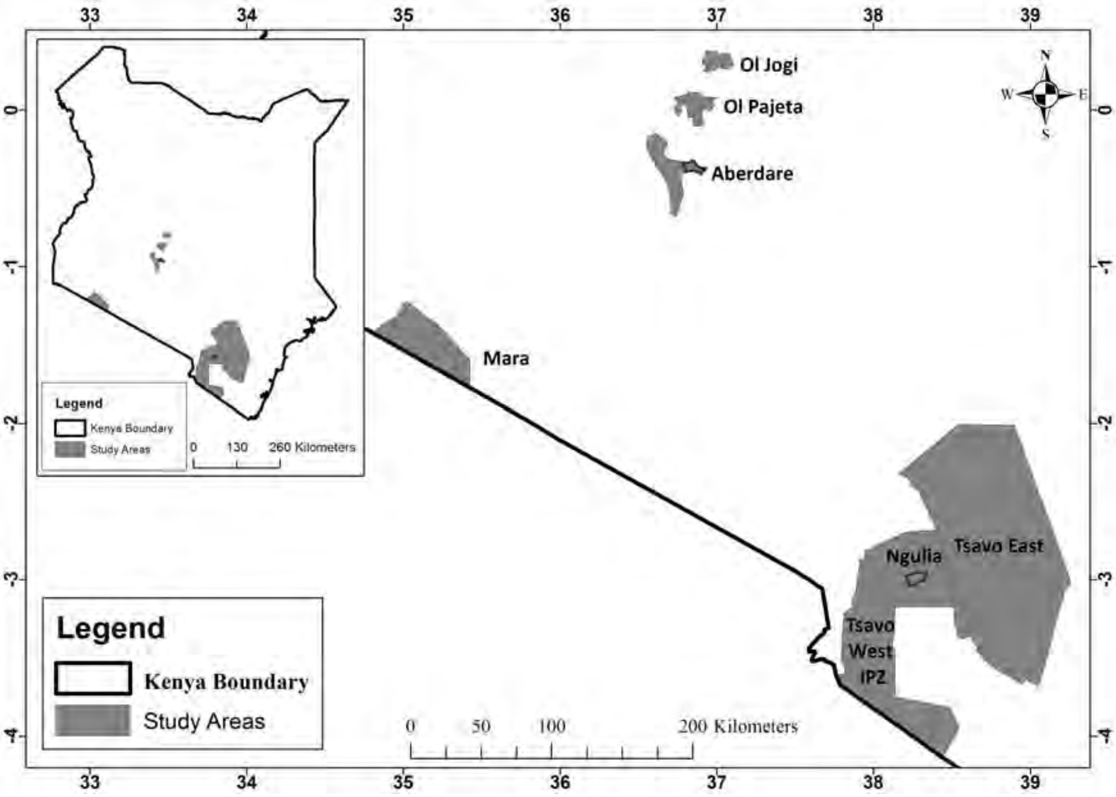


Figure 6.1: Locations of the seven study areas within the map of Kenya. The section of Aberdare where the study was carried is shown by bold border line; similarly for Ngulia.

Table 6.1: A summary of attributes measured within study areas. Sizes of study areas, black rhinoceros population (*N*), population densities, estimated maximum stocking density and number of animals that were repeatedly sampled for corticosterone metabolites and faecal mineral concentration are shown.

Study area	Area (km^2)	*N*	Estimated maximum stocking density (Adcock *et al.* 2007)	Density (Animals/km^2)	No. animals for corticosterone metabolite		No. animals for faecal mineral concentration	
					males	females	males	females
Ngulia	92	69	0.405	0.75	5	0	5	6
Ol Jogi	50	21	0.229	0.42	5	5	5	5
Ol Pejeta	300	87	0.307	0.29	5	6	5	6
Mara	1510	37	0.069	0.02	2	3	5	4
Aberdare	70	8	1.002	0.11	2	3	2	3
Tsavo East	1800♣	31	0.405	0.02	4	3	4	5
Tsavo West (IPZ)	1200♣	10	0.405	0.01	2	0	3	2

♣This is the area that is used by black rhinoceros (on basis of field reports by rangers) within the entire Tsavo East NP of *ca.* 11,000km^2 and Tsavo West NP *ca.* 9,000km^2.

Field sampling and collection procedures

Fresh (<18 hours old) faecal samples that were not contaminated by urine or rainfall were collected between 0600hr and 1200hr from identifiable adult (>7 years old) rhinoceroses. Visible contaminants like surface soil particles or flies were removed. Each of the faecal samples was thoroughly mixed by hand in gloves to achieve an even distribution of metabolites within the faeces sample (Millspaugh and Washburn 2003). To minimize contamination, handling equipment and disposables such as the hand gloves, polythene sheets, and sampling bottles were not shared between samples. About 30 g of the hand-mixed faeces was put into airtight sampling bottles. Samples were then immediately placed in an iced cooler box before being transferred into a -20 ^{0}C freezer for storage in the field. From the field, samples were transported over ice in a mobile freezer to another freezer of -20 ^{0}C at Kenya Wildlife Service (KWS) Veterinary laboratory in Nairobi.

Sample preparations and analyses

Samples were processed and sent in two lots for analyses between May 2011 and April 2012 for hormones at the University of Missouri USA and for minerals at the industrial laboratories of Ministry of Mines and Geology in Nairobi. 472 faecal samples from 25 adult male and 20 adult female black rhinoceroses were analysed for corticosterone metabolites whereas 485 faecal samples from 29 adult male and 31 adult female black rhinoceroses were analysed for mineral concentration as representative of diet mineral concentration.

Health and safety requirement required that subsamples are tested for Foot-and-Mouth Disease (FMD) at the disease' laboratory in Nairobi and soaking approximately 5 g of faecal samples in 3 ml of 2% acetic acid before they were shipped to USA. This kind of treatment is known to have the least effects on hormones compared to other treatments (Millspaugh *et al.* 2003) and is acceptable the USA Food and Agriculture Department. Prior to shipment to USA over dry ice, the subsamples were freeze-dried in a BOC EDWARDS, Minifast 04 freeze drier for at least 48 hours to zero moisture at the Kenya Medical Research (KEMRI) Laboratories. The freeze-drying bottles were rubber-cocked and sealed with aluminium caps to prevent absorption of moisture by the sample. A separate set of frozen subsamples were sent to the Ministry of Geology and Mines Laboratory in Nairobi, Kenya for faecal mineral analyses.

Stress hormonal assay

Approximately 0.2 g of dried faeces was placed in a test tube with 2.0 ml of 90% methanol and vortexed at high speed in a multi-tube vortex mixer for 30 min. Samples were then centrifuged at ~1900*g* for 20 min, and the supernatant was saved and stored at –20 °C until analysed using radioimmunoassay procedures. Corticosterone metabolites in rhinoceros faeces were measured using a commercially available corticosterone I^{125} double-antibody radioimmunoassay kit (Cat. #07120103, MP Biomedicals, Solon, Ohio). The manufacturer's method for the corticosterone I^{125} radioimmunoassay was followed, except that the volumes of all reagents were halved (Wasser *et al.* 2000).

A standard assay validation including assessment of parallelism, recovery of exogenous analyte, intra- and inter-assay precision, and assay sensitivity (Jeffcoate 1981, Grotjan and Keel 1996, O'Fegan 2000) were conducted to confirm the assay accurately and precisely measured corticosterone metabolites in rhinoceros faeces. Parallelism and recovery of exogenous corticosterone validation assays was conducted on two pooled faecal extract samples (each pool consisted of faeces from three individuals). Parallelism ensures the assay maintains linearity under dilution, and recovery of exogenous corticosterone verifies accurate measurement throughout the working range of the assay (Jeffcoate 1981). Exogenous corticosterone was added to the low and high pooled faecal extracts to obtain corticosterone values under higher dilution levels. Tests for equal slopes (parallelism) were used to determine if log-transformed curves of serially diluted pooled faecal extracts were parallel to log-transformed corticosterone standard curves. Intra-assay variation was calculated by averaging the coefficient of variation (CVs) of replicate tubes from 20 randomly chosen samples (Jones and Payne 1997).

The assay accurately and precisely measured faecal glucocorticoid metabolites in rhinoceros samples. Serial dilutions (1:4 up to 1:128) of rhinoceros faecal extracts yielded displacement curves that were parallel (all $\underline{P} > 0.5$) to the corticosterone standard curve (Fig. 6.2). Mean recovery of added exogenous corticosterone (range = 0.25-1.25 ng/mL) was 110.9% at 99% confidence limit for $\underline{n} = 10$). Acceptable recovery of exogenous corticosterone (within 90-110%) and demonstration of parallelism suggested no sample matrix effects (Jeffcoate 1981, Grotjan and Keel 1996, O'Fegan 2000). Assay sensitivity was 1.25 ng/g. The manufacturer's reported cross-reactivity of the antisera was 100% with corticosterone and < 1% for other steroids. Inter-assay variation for the assays was 6.2% and average intra-assay variation was 1.3%.

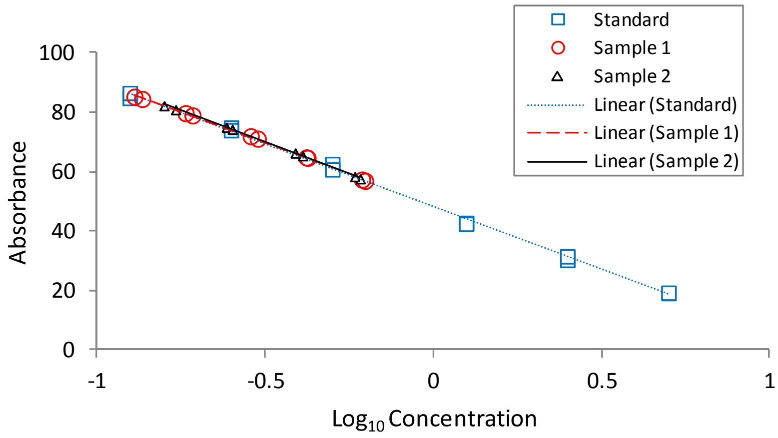


Figure 6.2: Parallelism curve used to assess accuracy and precision of corticosterone metabolites measured in black rhinoceros faeces.

Mineral analyses

Standard procedures (Plank 1992) for preparation of samples for determination of mineral concentration in plants were used. We sun dried a 20-30 g of fresh faecal sample for 5 days then oven-air dried samples at 80 ^{o}C for 24 hours. A subsample of 2.5 g of oven-dried sample was ground and weighed into a 50 ml beaker and mixed with 3 ml distilled water to make slurry which was then digested with 15 ml concentrated hydrochloric acid and 5 ml concentrated nitric acid. After the reaction ceased, the beaker was heated while agitating on a hot plate from time to time until 5 ml of solution remained. Next, 10 ml of distilled water was added and then allowed to settle. The cool and settled digested solution was filtered using hardened ashless filter paper grade no. 541, and then washed thoroughly with distilled water to the mark of 50 ml volumetric flask. The solutions were subjected to Atomic Absorption Spectrometer (AAS) for determination of Cu and Zn (Levinson 2002) and to flame photometer for determination of Ca (Sherwood 2005).

To analyse P, 1.0 g of oven-air dried faecal sample was added to 1 ml of concentrated sulphuric acid and 5 ml of concentrated nitric acid. Digestion was done to a volume of 1 ml until the solution became colourless. Next, 20 ml distilled water and 0.05 ml (one drop) of phenolphthalein indicator was added then neutralized with 1N NaOH to Ph = 7. The solution was transferred into a 100 ml volumetric flask and distilled water added to the mark. The standard solutions were treated likewise. Next, 10 ml of digested samples, blank and standard solutions were added into 100 ml beakers and 10 ml of molybdovanadate added to the solution. Next, 25 ml of distilled water was then added into each beaker, mixed and allowed to stand for at least 5 minutes. The percentage transmittance for each solution was then determined at 430 nm in a UV visible spectrometer using reagent blank as the reference blank solution.

The precision at 95% confidence level of measures as obtained from the standard parallelism curves were; ±0.002ppm for Cu; ± 0.005 ppm for Zn; ±0.02 ppm for K; ±0.05 ppm for P and ±0.05 ppm for Ca. All measured minerals from rhinoceros faeces yielded results parallel to the standard curves ($P<0.05$).

Statistical procedures

Rainfall and temperature were used to characterize seasonality (LSASAF 2010, Tchuenté *et al.* 2011). The estimates were derived from raster images of 8 km resolution for rainfall and 4 km resolution for temperatures for the period 2010 to 2011 inclusive (http://www.cpc.ncep.noaa.gov/products/fews/AFR_CLIM/afr_clim.shtml). Hawth's Tools for ArcMap9.3.1© and ArcGIS™ software was used to extract specific rainfall and temperature estimates from satellite raster images by overlaying the boundary of each study area on the raster images. Even though the satellite derived measurements of rainfall and Land Surface Temperatures (LST) follow quite well the field measurements (LSASAF 2009), we reconfirmed this by correlating satellite-derived rainfall and the reliable rain gauge rainfall since it was critical for our analysis (Appendix 6.1). Rainfall and temperature were not expected to have an immediate influence on food resources and consequently physiological stress levels. We lagged their effects by counting 29 days back for a 1-month lag and 59 days preceding faeces deposition for a 2-month lag.

For eleven faecal samples mineral values were missing. We imputed these missing values by fitting two-way ANOVA models per mineral, using area, month and area*month as factors, and replacing the missing values by predictions from the two-way ANOVA models.

Because of the complex data collection process, resulting in repeated measurements on areas, rhinoceroses (per area), faecal samples (per rhinoceros), and measurements of corticosterone metabolites in two lots at different times, whereby each sample in the first lot was assayed with one repeat per sample but samples in the second lot were assayed once, we used linear mixed models (LMM) to analyse the data by applying the procedure MIXED of the SAS® software Version 9.2 (Littell *et al.* 2006). Corticosterone metabolites values were log-transformed for a more symmetrical distribution of residuals. In the mixed model for log (corticosterone) the fixed effects included area (rhinoceros density), area-time combination (rainfall and temperature), rhinoceros (sex), and rhinoceros-time combination (minerals measured in the faeces). The random effects included different sources of variation: area, rhinoceros, dung sample, and residual error. The residual error variance quantifies the variability of the duplicates of dung samples analysed in the first lot of samples.

The following steps were taken to build a parsimonious model to explain variability in observed corticosterone metabolites:

Step 1: As a starting model a mixed model was used with fixed variables: density, sex, the set of four mineral nutrients (Ca, P, Cu, Zn, all log-transformed), and the three un-lagged climatological variables (maximum and minimum LST, rainfall), and random effects for area, rhinoceros, faecal sample, and random effect for error.
Step 2: Determination of best lag for climatological variables. As climatological effects from the past may play a role in the present, the best lag for temperature and rainfall was determined, by evaluating mixed models comparing -2 log likelihood (-2LL) containing combinations of unlagged, 1-month lagged, and 2-months lagged climatological variables. From a plant physio-ecological perspective, it was considered likely that cumulative rainfall and temperature effects may impact on the nutrient content of plants: hence we lagged both rainfall and temperature. The model with lowest -2LL was chosen. Climatological variables needed to be statistically significant ($P<0.01$) to remain in the model.
Step 3: Backward elimination was used to select the important minerals, starting with all minerals in the model, and removing the insignificant ones. Forward selection, starting with the model without minerals, and trying each of the mineral separately was used to check for model stability. The P-value to retain a variable in the model was set at 0.01.
Step 4: Variability of faecal corticosterone may be different for males and females. Models with constant and sex dependent variance components were compared with likelihood ratio tests.

All LMM's were fitted by maximizing the full likelihood. The resulting -2LL is used as criterion for model comparison, *e.g.*, for hypothesis testing. Degrees of freedom were obtained using the method by Kenward and Rogers (Littell *et al.* 2006).

Results

The final linear mixed model obtained for corticosterone (y) after the four steps was:
$\log(\underline{y}) = \beta_0 + \beta_1$ density + $\underline{\text{area}}$ + β_2 sex + $\underline{\text{rhinoceros}}$ + β_3 2month rainfall lag + β_4 2month max temperature lag + β_5 log(Phosphorous) + $\underline{\text{faecal sample}}$ + $\underline{\text{error.}}$

With (y) corticosterone, all underlined terms represented random effects with variance components: var(area) = σ^2_a, var(rhinoceros) = σ^2_r, var(faecal sample) = σ^2_d, and var(error) = σ^2_{eM} for males and σ^2_{eF} for females.
The results are summarised in Table 6.2 based on the final model.

Table 6.2: Results of the linear mixed model obtained for variability in corticosterone metabolites in faeces of black rhinoceros as explained by population density, climatological variables and faecal mineral nutrients characterizing plant nutrients in 2010/2011 in Kenya. Two-months lagged maximum temperature and rainfall, and phosphorous significantly explained the variability in faecal corticosterone metabolites ($P<0.000$). Population density did not significantly explain the observed variability in faecal corticosterone metabolites ($P=0.660$). Females had significantly higher corticosterone metabolites than males ($P=0.025$). -2 log likelihood (-2LL) = -542.7

Effect	**Sex**	**Standard Estimate**	**Error**	**d.f.**	**t Value**	**Pr > |t|**
Intercept		1.096	$2.17*10^{-1}$	163.00	5.05	<.000
Density (rhinoceroses/km^2)		$3.68*10^{-2}$	$7.98*10^{-2}$	6.47	0.46	0.660
Max. Temp ^{0}C (2-months lag)		$1.46*10^{-4}$	$2.2*10^{-5}$	253.00	6.55	<.000
Rainfall in mm (2-months lag)		$3.2*10^{-3}$	$7.2*10^{-4}$	290.00	4.42	<.000
	Female	$5.1*10^{-2}$	$2.18*10^{-2}$	36.50	2.34	0.025
	Male	0	.	.	.	.
Log Phosphorus (mg/kg)		$-1.16*10^{-1}$	$3.87*10^{-2}$	302.00	-3.00	0.003

Even though corticosterone metabolite levels increased with increases in density, this positive relationship was not significant [($b_1 \pm se(b_1)$: $3.68*10^{-2} \pm 7.98*10^{-2}$]. Rainfall effects on corticosterone metabolite levels were not apparent at the onset of rainfall but later. We found the 2-month lag of rainfall to have a strong significant positive relationship with corticosterone metabolite [($b_3 \pm se(b_3)$: $3.2*10^{-3} \pm 7.2*10^{-4}$]. Corticosterone metabolite levels peaked in May, which is two months after the highest rainfall was recorded in March (Fig. 6.3a).

As expected corticosterone metabolite levels increased with increase in temperature, however, it was the 2-month lag of maximum land surface temperature that had a significant positive relationship with corticosterone metabolite [($b_4 \pm se(b_4)$: $1.46*10^{-4} \pm 2.2*10^{-5}$] (Fig. 6.3b). All minerals except P were removed from the model after backward elimination and forward inclusion of important minerals into the model showed that Log P had strong enough explanatory power. Concentration of phosphorous in faeces was found to have a negative and significant relationship with corticosterone metabolite [($b_5 \pm se(b_5)$: $-1.16*10^{-1} \pm 3.87*10^{-2}$]. Sex influenced corticosterone metabolite levels in two ways. Firstly, females had significantly higher corticosterone metabolites than males [($b_2 \pm se(b_2)$: $5.1*10^{-2} \pm 2.18*10^{-2}$] and secondly, the variability in measurements within female faecal corticosterone metabolite was significantly higher than in male faecal corticosterone metabolite: (females $s^2_{eM} = 7.6*10^{-3}$, males $s^2_{eM} = 3.8*10^{-3}$, $P=0.001$).

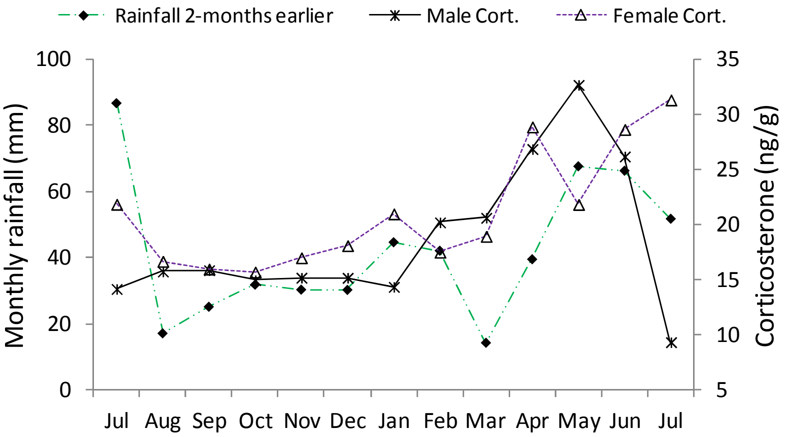


Figure 6.3a: The relationships between 2-months lag of rainfall and corticosterone metabolite (Cort.) levels in male and female black rhinoceros, 2010/2011, Kenya. A peak in Cort. was recorded two months after a peak in rainfall.

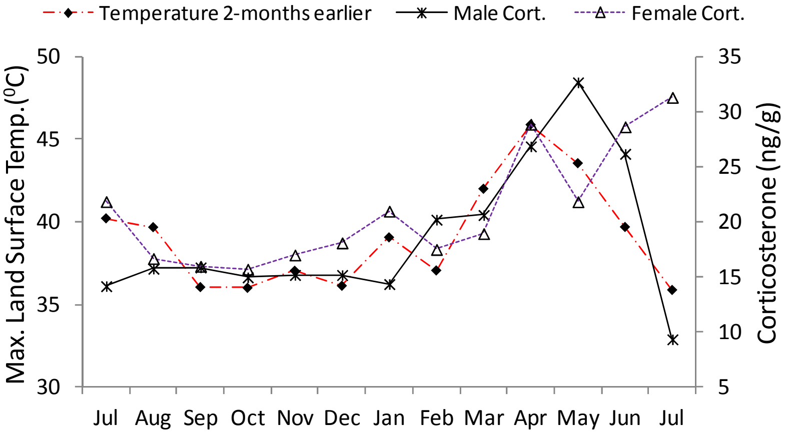


Figure 6.3b: The relationships between 2-months lag maximum land surface temperature and corticosterone metabolite (Cort) levels in male and female black rhinoceros, 2010/2011, Kenya. A peak in Cort. was recorded two months after a peak in rainfall.

Discussion

To some, mega mammalian herbivores are thought to be controlled by density feedback mechanisms particularly when population near or attain maximum stocking levels (*e.g.*, Owen-Smith 1988, McCullough 1999, Eberhardt 2002, Pastor *et al.* 2006, Owen-Smith 2011, Law *et al.* 2013). Other scholars argue that mega mammalian herbivore populations *in situ* subject to sufficient environmental variability are governed by fundamentally different processes other than density (*e.g.*, Fowler 1981, Gough and Kerley 2006, Morgan *et al.* 2009). Our results did not show significant effects of density on corticosterone metabolite levels despite densities of Ngulia, Ol Jogi and Ol Pejeta relative to estimated maximum stocking levels (Adcock *et al.* 2007) exceed the 70-80% of maximum stocking density theoretically assumed to result in negative density dependence feedback (Caughley 1976b, McCullough 1992, Kirkwood *et al.* 1994, Robinson 2000). Our results did not show density dependence feedback on physiological stress despite some populations having exceeded their estimated maximum stocking density. This was thus contrary to Christian's (1950) hypothesis that high population density leads to physiological mechanisms that trigger the HPA system to secrete elevated corticosterone metabolites. Kuznetsov *et al.* (2004) found similar results in midday gerbils (*Meriones meridianus*), whereas Evsikov *et al.* (1999) found an inverse relationship between population density and physiological stress levels in water vole (*Arvicola terrestris L.*). Territorial species are generally less prone to physiological stress compared to social species at high population densities (Kuznetsov *et al.* 2004, Creel *et al.* 2012). Lower physiological stress levels were found in the territorial gerbil (*Meriones unguiculatus*) when grouped in mixed-sexes compared to when grouped in same-sex (Hull *et al.* 1973). In these respects, the territorial nature of the black rhinoceros, the fact than males allow females into their territories, and the occasional social tolerance at shared resources for example at drinking-water points, may partly explain why there was no significant effect of population density on stress hormones.

The strong and direct significant relationships between physiological stress levels in black rhinoceros and two-month lag of rainfall and two-month lag of maximum temperatures are of clear interest. Recent researches in various animals indicate relationship between glucocorticoid levels and environmental conditions (Millspaugh *et al.* 2001, Kuznetsov *et al.* 2004, Millspaugh and Washburn 2004). Rainfall is an important factor that seasonally modifies a range of influential abiotic and biotic factors in an environment, such as forage availability, and mobilizes exchangeable plant available nutrients in the soil for uptake by plants which in turn affects physiological processes within an animal. An intake of high quantities of dietary fibre, for example, has been found to increase secretion of faecal corticosterone metabolite in red squirrels (*Tamiasciurus hudsonicus*)(Dantzer *et al.* 2011). We had expected a negative correlation between corticosterone metabolites and rainfall; however, increases in fibre content in rhinoceros forage two months after the rainy season could partly explain the observed positive correlation between rainfall and faecal corticosterone metabolite in black rhinoceros. The mechanism by which consumption of high quantities of fibre leads to increase in concentration of faecal corticosterone metabolite remain speculative Some studies have suggested that frequent defecation as a result of high dietary fibre leads to decreased re-absorption of hormone metabolites thus higher secretion of metabolites (Dantzer *et al.* 2011). Ingestion of forage from rainy season that succeeds a drought can alter the gastrointestinal tract environment of mammalian herbivores (Blood and Radostits 1989, Wasser *et al.* 1993) and can permit excessive multiplication of bacteria *Clostridium* species to lethal levels in black rhinoceros (Ndeereh *et al.* 2012). We speculate that such conditions can be physiologically stressful and over-task the HPA system leading to elevation of secreted corticosterone metabolites.

In this study concentration of minerals in faecal samples was assumed to reflect concentration of dietary mineral concentration (Clauss *et al.* 2007b; also *see* Chapter 4 this thesis). Only phosphorus from the four faecal minerals nutrients (Ca, P, Cu and Zn) as hypothesised showed a significant negative correlation with corticosterone metabolite levels. The threshold of this inverse correlation however require further research to determine the concentration levels that as discussed would alter gastrointestinal environment to increase levels of corticosterone metabolites.

Increases in temperature stimulates the pituitary gland to gonadotrophic activity (Wells and Zalesky 1940) to increase levels of secreted corticosterone metabolites as observed in North American elk (Millspaugh *et al.* 2001). Our results showed that levels of corticosterone metabolites in black rhinoceros increased with increases in maximum land surface temperatures (LST). For thermoregulation purposes, black rhinoceros and many large terrestrial mammals tend to be more active in the evening, throughout the night into early morning when temperatures are low (*see review in* Emslie and Adcock 2013). As was therefore expected, corticosterone metabolite was elevated in periods when maximum temperatures were high (Millspaugh *et al.* 2001), thus confirming the reasons for this species' inactivity during day-time when temperatures are high. Circadian rhythm in corticosterone metabolite secretion in this species is therefore expected and should always be considered in its future endocrinal researches. Nevertheless it was not clear why there was a 2-month lagged effect of maximum LST on levels of corticosterone metabolites. We speculate that maximum LST increased soil temperature which, in turn, increased availability of total nitrogen, ammonium and phosphates to plants (Kelly 1993) at the onset of rainfall. Quality (Singh and Singh 2004, Barbosa *et al.* 2014 i*n press*) and quantity (Owen-Smith 1994, Olff *et al.* 2002, Owen-Smith 2008, Sankaran *et al.* 2008) of browsing forage (twigs and leaves) increased with increase in water availability. Therefore the effects of temperature, as for rainfall, if linked to food resources, could not be instantaneous but evident afterwards in this case two months later when there was abundant forage. As discussed increases in consumption of fibre increases secretion of faecal corticosterone metabolites (Dantzer *et al.* 2011).

Our observed higher corticosterone levels in female than in male black rhinoceros were not surprising but important because it is the first time it is reported for black rhinoceros *in situ*. The generally higher physiological stress levels in females than males is well documented for many species (*e.g.*, Kudielka and Kirschbaum 2005). Female African forest elephants in the Gamba Complex of Gabon reportedly had higher glucocorticoid levels than their male counterparts when exposed to disturbance (Munshi-South *et al.* 2008). Female rats also showed higher levels of baseline corticosterone metabolites, larger corticosterone metabolite secretion after ACTH injections, and enhanced responses to stressors like handling, ether, restraint, shocks, or higher conflict situations (Kirschbaum *et al.* 1992). Sexual dimorphism in levels of corticosterone metabolites are partly explained by the sexual dimorphism of an animal's brain (Luque *et al.* 1992, Pinos *et al.* 2001) and by ovarian cycles in humans whereby the cyclic release of oestrogen and progesterone creates an unstable endogenous environment that can by itself have a significant effect on the physiology and behaviour of female animals (Ter Horst *et al.* 2009). As observed in rats by Bangasser *et al.* (2010), the receptor for corticotrophin-releasing factor (CRF), the neuropeptide that orchestrates the physiological stress response, signals and is trafficked differently in females than in males in a manner that can result in greater response and decreased adaptation to stressors. Similar brain dimorphism and physiological processes could be expected in black rhinoceros and probably explain our observed sex differences in levels of corticosterone metabolites. Additionally, less dominant animals (Creel 2001) and less territorial animals (Kuznetsov *et al.* 2004) exhibit higher physiological stress levels than the dominant animals or the territorial animals. In this case the female black rhinoceros is known to be less dominant and less territorial than the male black rhinoceros (*see review in* Emslie and Adcock 2013) hence exhibited higher levels of physiological stress.

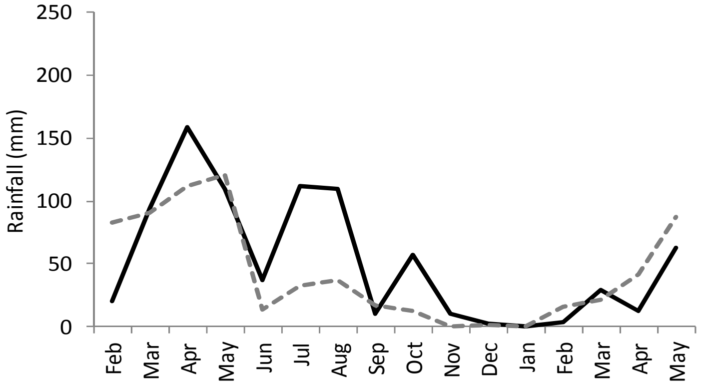
Conclusion

We demonstrate for the first time that increases in two-month lagged rainfall and Least Surface Temperature (climatic variables) lead to increases in levels of physiological stress and overrides any influence of population density as a stressor. The same climatic variables influence quantity and quality of food resources which in turn influence physiological stress as shown by the inverse correlation between concentrations of phosphorous and corticosterone metabolites. We also demonstrate for the first time that levels of faecal corticosterone metabolites are higher in female than in male black rhinoceros. Our results provide a range of both basal and elevated corticosterone metabolites that could be used as references in future black rhinoceros endocrinal studies. These findings have significant implications for conservation and management of the Critically Endangered black rhinoceros *in situ*.

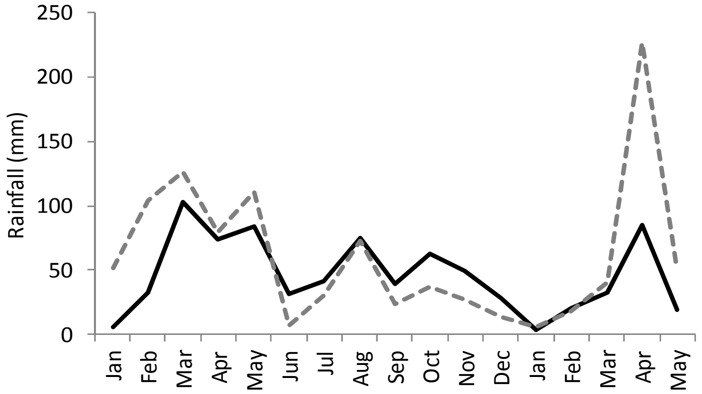
Acknowledgments

Rhinoceros monitoring staff helped in collecting faecal samples. WWF ESARPO Nairobi and The Mohamed bin Zayed Species Conservation Fund financed faecal collection and shipping costs. Ol Jogi Ranch and Ngulia Safari Lodge provided extra freezers for storing samples in the field. Hesbon Odongo of Chiromo Campus, University of Nairobi and Paul Okwach of Pathcare Kenya Laboratories helped with initial trials of hormonal assays. Frank Basiye and George Owiti-Otiang' helped with linkages to local laboratories. Hawi Jane helped with sorting samples. Dominic Mijele and Abraham Sangula facilitated testing of samples for Foot-and-Mouth Disease. Peter Maina helped with GIS work. Frank van Langevelde and Rajan Amin provided invaluable insights. Marcus Clauss, Shelley Waterland and Yussuf Wato provided helpful comments. We thank Rami Woods of University of Missouri for assistance with assays of faecal glucocorticoid metabolites.

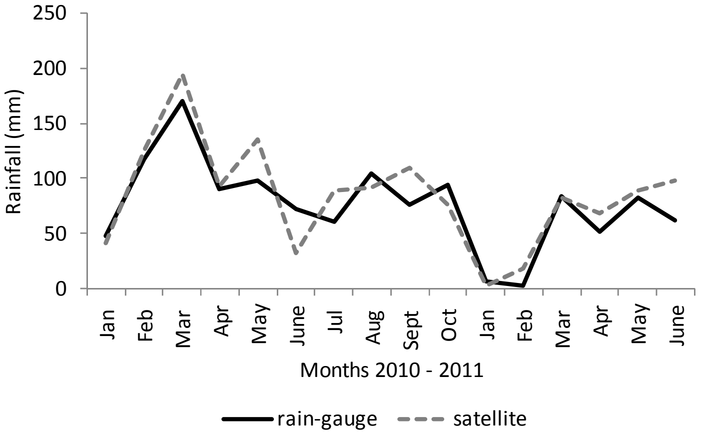
Appendix 6.1: The correlations between reliable rain gauge and satellite-derived rainfall. Only available and reliable rain-gauge rainfall were used hence the inconsistent sequence of months on x-axis.



Appendix 6.1a: The correlation between satellite (*dotted*) and rain gauge (*bold*) rainfall in Ol Jogi Ranch (Corr. Coef. R = 0.68).



Appendix 6.1b: The correlation between satellite (*dotted*) and rain gauge (*bold*) rainfall in Ol Pejeta Conservancy (Corr. Coef. R = 0.68).



Appendix 6.1c: The correlation between satellite (*dotted*) and rain gauge (*bold*) rainfall in Lake Nakuru National Park (Corr. Coef. R = 0.89).

Photo credit: Renaud Fulconis/Awely

Chapter 7

Density dependence and conservation of a mega-herbivore: a synthesis

Benson Okita Ouma

Introduction

The concept of density dependence and its link with reproductive performance and population regulation continues to be important in ecology, since it was first advanced by Nicholson (1933). The debate on this concept and how to empirically quantify density feedbacks in a natural setting has continued to date (*e.g.*, Turchin 1995, Cromsigt *et al.* 2002, Berryman 2004, White 2004, Okita-Ouma *et al.* 2009, Getz and Owen-Smith 2011, Owen-Smith 2011). Density dependence occurs when population performance (as measured or indicated by fecundity, growth rates and mortality, for example) is primarily regulated by density or population size (*N*) for a given area. The relationships between density and population performance are often different forms of linearity but the common form of linearity is described by the classic logistic growth model (*e.g.*, Verhulst 1838, Gabriel *et al*. 2005, Ross 2009). In applying the concept of density dependence and the mathematical models associated with it, animal numbers are kept around a putative maximum sustained yield (MSY) to avoid negative density feedbacks. In the classic logistic model MSY is at K/2 (half the maximum stocking density or “carrying capacity – ‘K’”) (**Chapter 1, Figure 1.1**). However, it has become clear that this model does not fit mega-herbivores (>1000 kg body mass) (Fowler 1987). Recently, Peacock and Garshelis (2006) concluded from many long term studies of large mammals, that these species exhibit strong density dependence near K, while MSY occurs between 50% K and K. For mega-herbivore MSY has been assumed at 70 - 80% of K (*e.g.*, Owen-Smith 1988, McCullough 1992, McCullough 1999). Many conservation managers responsible for managing and breeding mega-herbivores *in situ* have assumed that they are subject to density dependence and have managed them accordingly (Law *et al.* 2013). These include emblematic or key-stone species (Mills *et al.* 1993, Baudron and Giller 2014) hence attracting wider interests too. Most of the mega-herbivores also suffer from population declines due to anthropogenic factors (*e.g.*, Waithaka 2004, Milliken and Shaw 2012, Ayling 2013, Baudron and Giller 2014) and climate change (*e.g.* Quintero and Wiens 2013, Fordham 2013, Thomas *et al.* 2004, Warren *et al.* 2013). Because of the need to increase the numbers of especially endangered, slow-breeding and long-living vertebrates, some institutions have consequently embedded the concept of density dependence into their policy guidelines for the conservation of such endangered mega-herbivores (*e.g.*, Okita-Ouma *et al.* 2007b, Goodman *et al.* 2008, KWS 2012, Litoroh *et al.* 2012, MET 2012, KWS 2013).

Numbers of rhinoceroses in Africa and Asia are slowly increasing from the catastrophic declines in population of the 1960s and 1970s brought about mainly by poaching for their horn (*e.g.*, Leader-Williams 1992, Emslie and

Brooks 1999, Amin *et al.* 2006b). The need to rapidly grow rhinoceros populations is even more urgent given the upsurge in rhino poaching since 2007 (*e.g.*, Lucy *et al.* 2007, Graham-Rowe 2011, Ferreira and Okita-Ouma 2012, Milliken and Shaw 2012), coupled with the ever increasing negative anthropogenic impacts on conservation areas. Implementation of policy guidelines that consider the concept of density dependence to achieve rapid population growth as advocated by the IUCN's African and Asian Rhino Specialist Groups (Emslie and Brooks 1999, Emslie *et al*. 2009) has resulted in regular translocations and establishment of new populations. The source and recipient populations are increasingly located within intensively managed fenced areas. This fragmentation of small rhino populations (<100 animals) presents conservation challenges requiring an in-depth knowledge of their population dynamics including density dependence. Additionally, poaching pressure and anthropogenic pressures on conservation areas have made it very expensive for conservation agencies and private landowners to keep rhinos safely in larger conservation areas. One challenge resulting from adopting the density dependence concept is related to the need to translocate animals to reduce population densities, namely the availability of large areas of suitable habitat where animals can be safeguarded and properly managed.

Consequently, understanding how density dependence influences reproductive performance of rhinoceroses is of utmost importance to the future management and conservation of these species. Also, appropriate and enabling policy and legislative foundations for the management of rhino populations require significant technical expertise and knowledge. Without this knowledge and motivation within management authorities, efforts to conserve remnant rhino populations may not yield the desired outcomes. However, where adaptive management and sound, well advised conservation measures are implemented, they not only result in the effective conservation of rhinoceros populations, but are also likely to boost the general conservation of biodiversity in areas where rhinos exist and thus act as a potent catalyst for conservation land use as practised by some private land owners in many rhino range States in Africa (*e.g.*, Emslie and Brooks 1999, IUCN-SSC-AfRSG 2013).

Of the two species of rhinoceros in Africa, the black rhinoceros (*Diceros bicornis*) numbers only about 5,100 animals compared with white rhinoceros (*Ceratotherium simum*) estimated at 20,000 animals (IUCN-SSC-AfRSG 2013). Three subspecies of black rhinoceros currently exist after the fourth one, the western black rhinoceros (*D. b. longipes*) was declared extinct in 2006 (IUCN 2013b). As of 2012, the Southern-central black rhinoceros (*D. b. bicornis*) was estimated at 2,300 animals, the south-western black rhinoceros (*D. b. minor*) numbered 2,000 animals while the eastern black rhinoceros (*D. b. michaeli*), of which 75% are found in Kenya (Emslie and Adcock 2013, IUCN-SSC-AfRSG 2013), numbered 800 animals (IUCN-SSC-AfRSG 2013) with a majority of populations in fenced-in areas. Some of these populations based on predictions using the only available 'carrying capacity' model for black rhinoceros (Adcock 2001), were reported to have exceeded maximum stocking densities (Adcock *et al.* 2007, Okita-Ouma *et al.* 2007a).

Central theme of this study

The central theme of this thesis is to assess the evidence for density dependence in mega-herbivores, particularly a mega- browser; then use the findings to suggest solutions to the complex conservation issues facing mega-browsers such as the black rhinoceros populations as a whole. I chose to study the Critically Endangered (IUCN 2013b) eastern black rhinoceros (*D. b. michaeli*) in Kenya for this central theme for reasons outlined in **Chapter 1**, this thesis. I selected nine populations on the basis of different densities, fenced or open, and varying Plant Available Moisture ('PAM' *i.e.*, 'soil moisture') and Plant Available Nutrients ('PAN' *i.e.*, 'soil fertility') (*see* **Chapter 1**, this thesis). I investigated density dependence in the reproductive performance of black rhinoceros using two approaches: One directly through measures of vital rates of population growth, and another, indirectly through diet and physiological stress hormones as measured through woody cover and faecal analyses. I considered edaphic variables mainly PAM and PAN and climatic variables mainly rainfall and temperatures in interpreting results of the two approaches.

Structure of this synthesis

In this synthesis, I first expand on the challenges facing the conservation of black rhinoceros, and then discuss my findings and how, in relation to density dependence, they contribute to solving these conservation challenges. I approach this from ecological and socio-economic perspectives. Prior to the conclusion I provide a critique of my study alongside recommendations for future research.

Challenges facing conservation of rhinoceroses

Loss of biodiversity implies tremendous cascading negative effect on biodiversity itself (*e.g.*, Janzen and Martin 1982, Pimm *et al.* 1995, Cowlishaw 1999, Koh *et al.* 2004). These losses are predicted to accelerate with either by the direct influences of poaching as an economically-driven crime (*e.g.*, Milliken and Shaw 2012, Ayling 2013) and expansion of agriculture and settlement (*e.g.*, Waithaka 2004, Baudron and Giller 2014), or and by the indirect influences of climate changes (*e.g.* Quintero and Wiens 2013, Fordham 2013, Thomas *et al.* 2004, Warren *et al.* 2013). The rhinoceros like many other remaining terrestrial mega-herbivores face these challenges. This species can be considered an emblematic taxon for biodiversity conservation, but at the same time its extinction would constitute an important cultural and economic loss for the countries concerned. Rhinoceroses, like other charismatic mega-herbivores such as the African elephant (*Loxodonta africana*), require large areas and abundant forage to support viable populations. To ensure their longer-term survival, land sparing as opposed to land sharing conservation strategies (Baudron and Giller 2014) would best suit them; otherwise they could go extinct as the mega-herbivores in Australia, Europe, North America and South America did in the late Pleistocene epoch (Owen-Smith 1988).

Throughout the 20th and 21st centuries, all other African and Asian rhinoceroses have been brought to the verge of extinction by killing mainly for trade in their horn. Recent, 2014, wildlife statistics by conservation agencies in Kenya and South Africa, for instance, showed that in 2013, the numbers of rhinoceroses poached in these two countries amounted to about 5% of their total numbers that year. If one considers the annual mortality rate of up to 4% and the annual 8 - 9% maximum intrinsic growth rate for rhinoceroses (Owen-Smith 1988, Emslie 1999, Kingdon and Hoffmann 2013), then it is not surprising that these two countries registered quite stagnant or very limited growth, and in some individual populations, even negative population growth rates. The recent upsurges in rhino poaching is linked to deep and fairly complex socio-economic, criminal networks and political factors (*e.g.*, Lin-Easton 2001, Graham-Rowe 2011, Milliken and Shaw 2012, Ayling 2013). Several solutions ranging from proposals to legalise trade in rhinoceros horn (Biggs *et al.* (2013) to proposals for combination of different strategies (*e.g.*, Ferreira and Okita-Ouma 2012, Prins and Okita-Ouma 2013) have been proposed to address some of these challenges. An example of combination of different strategies is shown in Box 7.1.

Box 7.1: Rhino Poaching: Unique Challenges

IN THEIR POLICY FORUM "LEGAL TRADE OF Africa's rhino horns" (1 March, p. 1038), D. Biggs *et al*. advocated legalizing trade in rhino horn through harvesting horns of 5000 white rhinos in South Africa as the panacea to the current rhino poaching crisis. Their arguments were based on the law of supply and demand and supported by the example of crocodile farming. The law of supply and demand only applies to commodities for which supply is independent of demand. It does not apply specifically to white rhino horn because the market does not differentiate between horns of the five extant rhinoceros species, and therefore the supply exceeds the quantity of horns of the white rhino alone. Because the rhino horn has no medicinal properties (*1, 2*), drug producers can dilute it at will, making the price formation normally dictated by the law of supply and demand unpredictable. Furthermore, legal access to rhino horn risks reawakening demand in older markets, such as Taiwan, Japan, Singapore, and Yemen, where demand for rhino horn was prevalent in the 1970s and 1980s and has since decreased (*3*). If demand grows again in these markets, indiscriminate poaching may increase accordingly, putting look-alike black, Indian, Javan, northern white, and Sumatran rhinoceros species at even higher risk. The costs of securing horns for the proposed Central Selling Organization for legal trade might make poaching even more lucrative. The comparison with the crocodile skin market is unjustified. Farmed crocodiles occur in millions, whereas the five rhino species number about 30,000 animals and reproduce much more slowly. Crocodile skin trade involves processing and was established in well-regulated industries; rhino horn is used unprocessed in China and Vietnam (*3, 4*), known for weak control of their drugs industries. Unlike rhino horn, crocodile skin has substitutes, is not consumed, and is not believed to have medicinal or religious applications. The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) upheld the ban on trade in rhino horn by enacting tighter controls in March 2013 (*5*). We propose education, awareness, and diplomacy (*5, 6*) for squashing horn demands in China and Vietnam that continue to destabilize rhino conservation globally and undermine national security (*7*) in source countries.

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Internationally, many governments are collectively playing their part in different ways to curtail illegal trade in rhinoceros horns (CITES 2013a). Nationally, stiffer penalties to punish wildlife offenders are being enacted; for example, the Kenyan government passed a new wildlife conservation and management bill containing penalties of life imprisonment or a minimum fine of KSh 20 Million, equivalent to US$ 250,000 for poaching rhinoceros or African elephant (*Loxodonta africana*) or trafficking their parts or derivatives (GoK 2013).

Density dependence and solving conservation challenges for mega-herbivores

The immediate and most obvious challenge facing conservation of rhinoceroses is the lack of adequate secure habitats devoid of significant human interference and poaching threat. Some solutions to these will be found through better understanding of the concept of density dependence in the black rhinoceros. Subsequently, this study contributes the following six possible solutions towards solving these challenges:

1. Reproductive performance at high population densities

The results of **Chapters 2 & 3** showed a lack of density effects on reproductive performance of the black rhinoceros. Some populations such as Lake Nakuru National Park, Nairobi National Park and Ol Pejeta Conservancy performed optimally at densities that were higher than their estimated maximum stocking densities or "carrying capacity - K" estimated using a carrying capacity model for black rhinoceroses (Adcock 2001). Assuming K for these populations was correctly estimated in 2005 by Adcock *et al.* (2007) then my results contradicted the general reporting by Emslie and Adcock (2013) that black rhinoceros exhibit density dependence when their numbers exceed K. In **Chapter 4** I demonstrated the link between faecal mineral concentrations and dietary mineral concentrations using data from feeding trials of 15 black rhinoceroses in zoos. This was supported by a significant positive correlation between increases in faecal mineral concentrations and plan available nutrients. The population density of black rhinoceros did not show a significant relationship with the quality of dietary browse as measured through faecal minerals. These findings imply that black rhinoceroses can exhibit optimal reproductive performance without showing signs of nutritional stress at densities higher than the currently estimated carrying capacities (Adcock *et al.* 2007). The concept of density dependence may therefore not always be applicable for making management interventions such as translocation, if the aim of such an intervention is to minimise negative density feedbacks and enhance reproductive performance. Therefore, in the "short term", as a way of buying time during the current poaching upsurge, conservation managers should consider amending policies that require black rhinoceroses to be translocated to new areas once their numbers reach MSY, *i.e.*, 70 - 80% of K. Such amendments of policies would firstly reduce the vulnerability of rhinoceroses to poaching posed by translocating them to open areas. Secondly, the costs associated with protecting open areas would be significantly reduced.

However, given that animal numbers cannot increase infinitely within finite (fenced) areas and the fact that the term "short term" is difficult to define within the current uncertainties on trends of illegal demand for rhino horn (*e.g.*, Milliken and Shaw 2012, Prins and Okita-Ouma 2013, Rademeyer 2013), alternative translocation strategies are proposed: I advise conservation managers to consider set percentage translocation as opposed to translocating at an imaginary MSY. A set percentage translocation for rhinoceroses was proposed by Peter S. Goodman of Ezemvelo KwaZulu-Natal Wildlife and Professor John Hearne of Natal University. This involves translocating rhinoceroses at fixed percentages below their intrinsic growth rate of 8 - 9% p.a., monitoring population responses and adjusting the next translocation percentage accordingly (*see* Goodman 2001).

2. *Economies of scale: Halt illegal killings to benefit the tourism sector*

The black rhinoceros, like other mega-herbivores, is not regulated top-down through predation (Hunter and Price 1992) unless by man through hunting and habitat loss (*see review in* Gandiwa 2013). However, their calves and sub-adults are vulnerable to predation by the spotted hyena (*Crocuta crocuta*), lion (*Panthera leo*) and leopard (*Panthera pardus*) (*see review in* Emslie and Adcock 2013) but this is not significant. So, as in many other mega-herbivores, black rhinoceros populations are controlled bottom-up, *i.e.*, by available and suitable food resources, as influenced by environmental stochasticity (White 1978, Glenn-Lewin *et al*. 1992, Illius and O'Connor 2000) (**Chapters 3, 4, 5 &6**). Curtailing unregulated, anthropogenic top-down control such as poaching and allowing bottom-up processes to control population growth of mega-herbivores could lead to maximal intrinsic growth rates. Maximal reproductive performance was achieved when heavy poaching of rhinoceroses was halted in Africa in the mid-1980s, and protection and biological management enhanced (Emslie and Brooks 1999). Rhinoceroses then became a marketing tool for tourism as one of the 'big five' terrestrial mammals

Industrial whaling is another case where halting of unregulated top-down control occurred and consequently the number of whales increased. Illegal whaling significantly reduced numbers of the southern right whale (*Eubalaena australis*) and the humpback whale (*Megaptera novaeangliae*) (*e.g.*, Yablokov 1994, Clapham and Baker 2002, Jackson *et al.* 2008). When the International Whaling Commission imposed whaling restrictions in the 1960s (Tønnessen and Johnsen 1982, Clapham and Baker 2002), these were strongly enforced in some countries such as South Africa. These interventions led to whales reproducing maximally, with an 8 -10% p.a. intrinsic growth rate. The increase in number of whales led to whale watching. Whale watching has become a multibillion dollar tourism venture for coastal communities in Australia, the Canary Islands of Spain, Canada, Namibia, Taiwan, the Solomon Islands, South Africa, USA and in many other countries that have protected their oceanic waters (Herremans 2006). These whales have several similarities with rhinoceroses in measures of their reproductive performance. Both whales and rhinoceroses have gestation periods of between 12 and 16 months, and inter-calving intervals between 2 and 4 years; they become sexually mature between 5 and 10 years, while their intrinsic rate of growth also falls within 8% and 10% per annum. All is not lost, as black rhinoceroses can be kept at high densities, reproduce maximally and hopefully boost the tourism sector if the current international and national efforts to curtail illegal demand and trafficking of rhino horn is sustained (*e.g.*, CITES 2013b, a, GoK 2013).

3. *Survival on infertile soils with high moisture*

I deduced the optimal habitat conditions for black rhinoceros from reproductive performance measures, particularly the yearly percentage of females calving, ages at first calving and inter-calving intervals (**Chapter 5**). The results showed that best reproductive performances in the black rhinoceros were in areas of high soil moisture with sparse, woody cover, and that soil nutrients did not play a significant role. In other words, areas of low nutrients but with high moisture still supported optimal reproductive performances. Predictions made from Adcock (2001) showed unexpectedly low densities of black rhinoceros in habitats with low soil nutrients - PAN and sufficient soil moisture - PAM with abundant available browse plants with high levels of polyphenols and tannins (Emslie and Adcock 2013). The reasons why areas of low PAN still had optimal reproductive performance in black rhinoceros could have three possible explanations. Firstly, reproductive performance is controlled by browse quality, yet woody plants differ widely in their response to soil nutrients. For example, conspecific browse plants may have similar concentration of mineral nutrients in their leaves and twigs despite being located in areas that are significantly different in PAN (Le Houérou 1980); or they may significantly differ in mineral concentrations even when the PAN of their localities are the same (Wu *et al*. 2007) (**Chapter 4**). Additionally, it has been suggested that browse plants that are adapted to low PAN store more nutrients in storage organs (*e.g.*, the root system) than species from fertile soils (Bryant *et al*. 1983). These stored nutrients can be used quickly in stress conditions (*e.g.*, a high herbivory event, fire, or nutrient shortage) to support growth or other important physiological processes (Chiariello and Roughgarden 1984). Secondly, black rhinoceros is known to browse on over 200 plant species (Adcock *et al.* 2007, Emslie and Adcock 2013). The advantage of this feeding behaviour is that, in spite of the lack of definite response of woody plants to PAN, by eating a diversity of plants, the diet of black rhinoceros is naturally mixed to provide sufficient nutrient quality for optimal reproduction. Thirdly, even though woody plants species from low PAN have higher plant secondary chemicals (Cates and Rhoades 1977, Stuart Chapin 1980, Bryant *et al*. 1991) than their conspecific counterparts from high PAN, the hind gut digestive system of black rhinoceros can tolerate low quality diets and digest a diversity of plants with potentially harmful plant secondary chemicals (Emslie 1999, Muya and Oguge 2000, Lieverloo *et al.* 2009, Luske *et al.* 2009, Emslie and Adcock 2013). As most detoxification processes occur in the liver, the large liver of the black rhinoceros relative to its body mass (Hofmann 1989, Kock and Garnier 1993) could also play a potentially important role in detoxifying the poisonous chemicals in their browse plants from low PAN. This may explain why I did not find a significant relationship between PAN and mineral nutrients in the dietary browse as measured via faecal samples.

4. Land sparing and not land sharing is ideal for conservation of mega-herbivores

My results showed that black rhinoceros can potentially reproduce optimally in areas with low PAN and high PAM in savanna ecosystems receiving annual rainfall of 200 mm to less than 1,000 mm (Bell 1982, Huntley 1982, Sankaran *et al*. 2008) (**Chapter 5**). The ability of black rhinoceros to survive on infertile soils (low PAN) has already been discussed. However, sufficient PAM is required. High PAM leads to high plant biomass (Olff *et al*. 2002, Sankaran *et al*. 2008) and may lead to higher quality of leaves and twigs of woody plants that constitute the main forage for black rhinoceros. Increases in mineral nutrient concentration in leaves and twigs with increases in soil water (PAM) have been shown in experiments with seedlings of woody plants (Singh and Singh 2004, Barbosa *et al*. 2014 *in press*). This finding that black rhinoceros can thrive in area of low soil fertility and high precipitation is exciting as it has positive implications for what sort of habitats to use for the conservation of this mega-herbivore.

Lands that are of low PAN and high PAM are generally not favourable for human habitation or for agriculture. Selection of such areas for conservation of black rhinoceros would benefit the animal and at the same time lead to a reduction in human-wildlife conflicts. Relatively fewer anthropogenic pressures would be expected in such areas, allowing wildlife to thrive while communities derive benefits from enterprises related to wildlife tourism. Where such wooded areas are used for charcoal burning, providing that woody cover is only a little reduced and not depleted, then black rhinoceros would still not suffer because my results showed that sparse woody cover of 10 -15% correlated with best reproductive performance (**Chapter 5**). Charcoal burning itself usually leads to more vegetation of *Euphorbia* plants which is not suitable for charcoal burning but is easily eaten by black rhinoceros (*see* Lieverloo *et al.* 2009, Luske *et al.* 2009, Okita-Ouma B. *pers. observ*.).

Historical evidence and anecdotal reports (Mitchell 1953, Sidney 1965) indicate that black rhinoceros occurred in abundance in areas such as coastal *nyika*, *i.e.*, hinterlands in Kenya, *Brachystegia* woodland of Malawi, and the Rufiji delta in Selous Game Reserve in Tanzania, where soil fertility is low but precipitation is high. For the long term conservation of black rhinoceros in Africa, conservation managers and financial institutions should therefore focus attention on securing areas with sparse, woody cover where human densities and soil nutrients are low, and precipitation is high. This would turn the malaria-infested and degraded hinterlands to prime biodiversity conservation areas or as described by (Myers *et al.* 2000), biodiversity 'hotspots', for some threatened herbivores, without displacing people. However, potential diseases particularly trypanosomiasis from tsetse fly *Glossina* spp. that are potential in such environmental conditions (Wint and Rogers 2000) and affect humans (*see review in* Févre *et al.* 2008), livestock (*e.g.*, Kamuanga 2003) and wildlife (*e.g.*, Kock and Garnier 1993, Mihok *et al.* 1994, Kock *et al.* 1999, Kock *et al.* 2010a, Kock *et al.* 2010b, Obanda *et al.* 2011) would have to be controlled. Whereas on the one hand high parasitaemia might influence health and reproduction in black rhinoceros (Kock R. *pers. comm*.), on the other hand, this infection might be an acceptable trade off especially as it will discourage use of such lands by people and livestock. If such areas are secured and black rhinoceros are kept at high densities, then it could potentially spur wildlife tourism for the benefit of the national economy and presumably, local economies and livelihoods as well. Sparing of marginal lands would therefore be ideal for the longer term conservation of mega-herbivores and mega predators (Baudron and Giller 2014). The living example of such land sparing is the *ca*. 22,000km^2 Etosha National Park in the Namib Desert in Namibia where mega-herbivores and mega predators seem to thrive and sustain wildlife tourism.

5. Towards standardized method for selection of suitable habitats

When selecting appropriate areas for conservation of black rhinoceros, my choice to use PAM and PAN (Frost *et al*. 1985, Walker and Menaut 1988, Solbrig 1990) to characterise habitats simplified the environmental complexities but still allowed for sufficient data to be obtained to deduce expected environmental changes (**Chapters 4 & 5**). Combining PAM and PAN with woody cover estimates (Hansen *et al*. 2002) and satellite-derived rainfall and temperature estimates (LSASAF 2010, Tchuenté *et al.* 2011) gave fairly accurate predictions of suitable habitat for black rhinoceroses. Other methods for selecting suitable habitats for black rhinoceros in African Savanna are being developed to also include browsing height of black rhinoceros, number of other browsers and primary productivity (*e.g.*, Adcock *et al.* 2007, Okita-Ouma *et al.* 2007a). The approach used in this thesis and the others being developed (Adcock 2001) yield more or less similar results. However, where large-scale habitat suitability assessment is required, the latest approach described in this thesis may be more suitable since it is less laborious and easier to standardise, since it does not have subjective parameters. The use of the PAM-PAN approach and of woody cover is therefore novel in the sense that data collection can be better standardised and can potentially be used for rapid assessments and characterization of suitable habitats for black rhinoceros.

6. Lower physiological stress in females improves reproductive performance

This study demonstrated sexual differences in stress levels in black rhinoceros for the first time (**Chapter 6**). Higher stress levels in females than in males have been reported in humans (*e.g.*, Davis and Emory 1995, Kudielka and Kirschbaum 2005), elephants (Munshi-South *et al*. 2008), rats (*e.g.*, Ahima *et al.* 1992, Kirschbaum *et al.* 1992, Bangasser *et al.* 2010) but have never before been studied in black rhinoceros. The sexual differences in stress levels are attributed to male and female brain dimorphisms (Luque *et al*. 1992, Pinos *et al*. 2001) and to ovarian cycles (Ter Horst *et al*. 2009) in humans. The receptor for corticotrophin-releasing factor (CRF), the neuropeptide that stimulates the stress response, has a different signal in female Sprague-Dawley rats that results in a stronger response and decreased adaptation to stressors (Bangasser *et al*. 2010). The cyclic release of oestrogen and progesterone creates an unstable endogenous environment that can by itself have a significant effect on the physiology and behaviour of females (Ter Horst *et al*. 2009). Similar arguments could be advanced to explain my findings in black rhinoceros. Dominance also plays a role in determining stress levels amongst animals, where the subordinates show higher stress levels than the dominant individuals (Creel 2001), whereas territorial species are generally less prone to stress compared to social species at high densities (Kuznetsov *et al*. 2004, Creel *et al*. 2012). In the case of black rhinoceros, the male dominance over females and the male territorial behaviour compared to females (*see review in* Emslie and Adcock 2013) could also have led to their lower levels of physiological stress compared to the females.

Several other factors lead to physiological stress in animals (*see review in* Millspaugh and Washburn 2004). For a rhinoceros population such as that found in the Masai Mara National Park, several forms of disturbances ranging from the annual wildebeest migration, cattle incursion, agricultural activities in dispersal areas, fires, to tourism related activities have been reported (*e.g.*, Karanja 2002, Waithaka 2004, Walpole *et al.* 2004, Okita-Ouma *et al.* 2007a). If such disturbances are allowed to continue and lead to chronic stress, then reproductive performance could be significantly lowered from that reported in **Chapter 3**. Chronic physiological stress levels have been found to lead to low survival probability, immunosuppression, diseases loss of body condition and poor reproductive capability (*e.g.*, Christian 1950, Wasser and Starling 1988, Singer and Zumoff 1992, Li *et al.* 2007, Kirby *et al.* 2009). For maximal reproductive performance, any form of stressor should be removed from rhino conservation areas. Ngulia Rhino Sanctuary in Kenya is a good example of a controlled rhino viewing area with the aim of minimising physiological stress.

A critique of the study and recommendations

i. I did not have a study running in parallel, a control experiment, or a similar previous study on density dependence on black rhinoceros with which to compare my results. The number of populations, N = 9, might have been too low to run a between-populations analysis with a high statistical power. Similar research that samples more populations in contrasting PAM and PAN is encouraged. In the meantime, the models used for estimating ‘carrying capacities’ and the current estimated ‘carrying capacities’ for the populations I studied require immediate revision.

ii. There was no significant correlation between faecal mineral nutrients and plant available nutrients (PAN). An experiment in which conspecific woody plants are grown to maturity in plots that are significantly different in PAN but similar in plant available moisture over several years would provide more insight into the relationship between mineral nutrients and soil nutrients in browse plants. Such knowledge would lead to a better understanding of the influence of soil nutrients on the physiology and reproductive performance of browsing herbivores.

iii. I was unable to test the link between corticosterone and reproductive performance due to limitations of time. I could also not obtain anywhere in the literature the baseline corticosterone levels for black rhinoceros upon which to gauge whether or not the measured corticosterone levels were below or above normal. Such a comparison would have yielded a deeper understanding of controls of black rhino reproductive performance.

iv. I calculated estimates of plant available nutrients (PAN) from the only available but relatively old (20-30 years) literature and databases. This made it difficult to differentiate PAN, particularly for Tsavo East, Tsavo West and Ngulia that occur in one ecosystem. This led to less detailed individual discussions of these three areas with regards to effects of PAN on dietary browse. Future research should consider new soil profiling for each of the study areas.

v. Faecal nitrogen (N) has been widely used to evaluate the nutritional value of diets for herbivores and to discern large dietary differences. It was therefore unfortunate that for logistical reasons, faecal N and in particular metabolic faecal N could not be assessed in this study to compare with many other studies. The faecal mineral concentrations used in this study are proxies for dietary minerals that have not been extensively researched compared to faecal N.

In conclusion, this study demonstrated that:

i. Densities of black rhinoceros in the areas studied did not affect their reproductive performances, the quality of browse diet, or the stress hormonal levels. Reproductive performances tended to be better where PAM was high. PAM was found to be directly correlated with quality of dietary browse. PAN did not play a significant role in determining reproductive performance. These results as illustrated in Figure 7.1 confirmed that black rhinoceros reproductive performance is controlled bottom-up by food resources. The populations I studied can still reproduce optimally at densities higher than current estimates of ‘carrying capacities”.

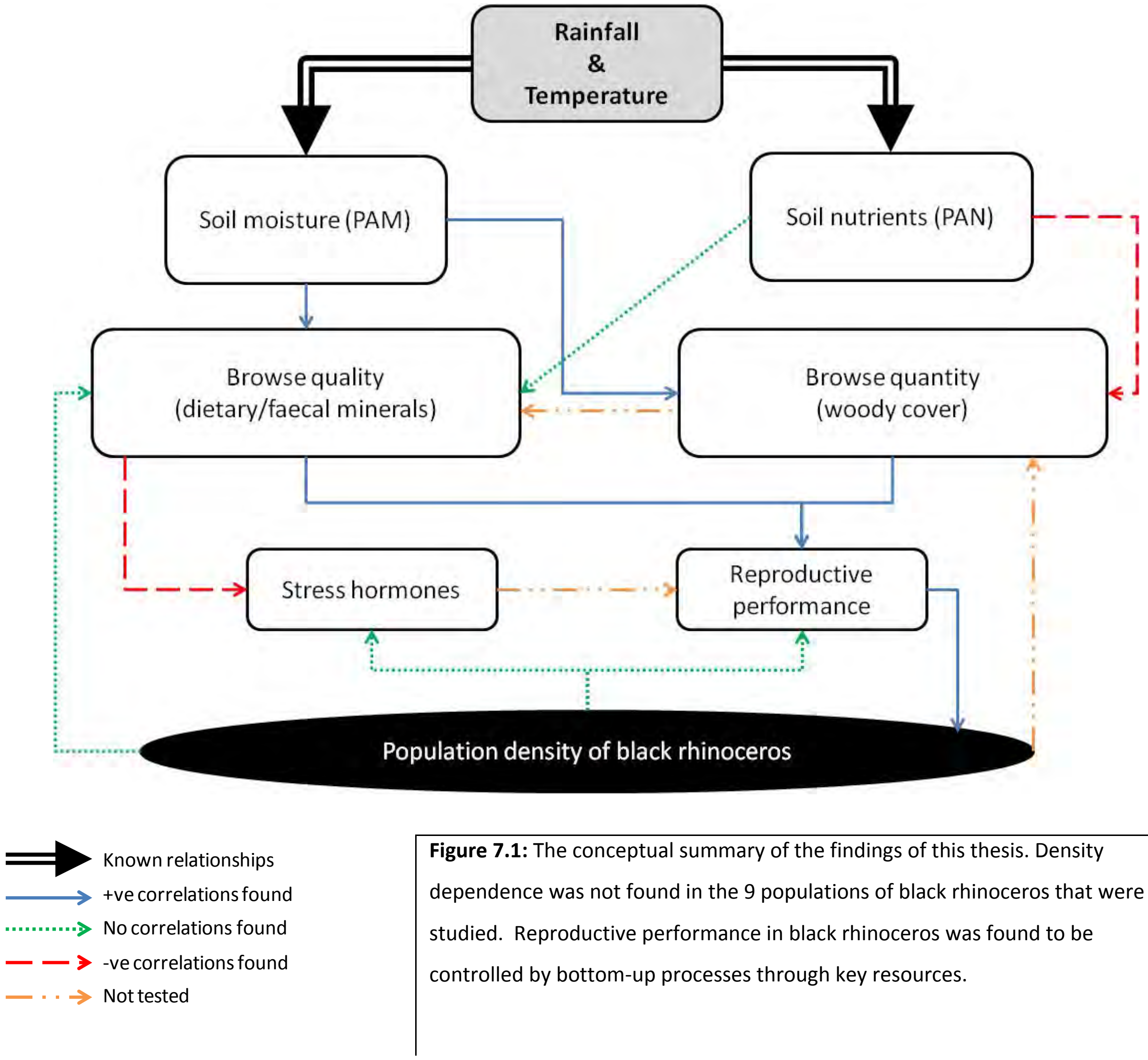

Figure 7.1: The conceptual summary of the findings of this thesis. Density dependence was not found in the 9 populations of black rhinoceros that were studied. Reproductive performance in black rhinoceros was found to be controlled by bottom-up processes through key resources.

ii. Black rhinoceros can potentially be conserved in marginal areas of low soil fertility but high rainfall in sub-tropical ecosystems. These are areas that do not compete with agriculture and human habitation and can thus be classical models of land sparing for long term conservation of mega-herbivores. Deliberate efforts should be made to secure such marginal lands for conservation and to encourage wildlife tourism.

iii. As far as my dataset was concerned, black rhinoceros populations are not density dependent, but are controlled by bottom-up processes through key resources. It is apparent that the density dependence concept and the concept of bottom-up control still require more investigation eventhough the densities I studied were perhaps too low to fully support my alternative view of bottom-up control**.** As was said by Charles Krebs (2014) in his Foreword in Prins and Gordon (2014); we ecologists should "*walk slowly, we have much to do*" because theories and concepts must be revised continuously, based on empirical evidence, before using them to make generalized ecological predictions.

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Summaries in English, Dutch, French and Swahili

Summary (*English*)

Population densities of eastern black rhinoceros: Unravelling the controls

Understanding the forces that cause variability in population sizes is a central theme in ecology. Limiting factors comprise to a large extent deterministic processes, shaped by stochastic events. Deterministic processes include feedbacks from population density (density dependence) through births and deaths to population growth rate. Density dependence results from competitive interactions within a population. These processes interact to generate complex patterns of animal abundance over time and space. The limiting factor in populations of large mammals which are not controlled top-down by predation is food; in other words, such populations are controlled by bottom-up processes. Food limitation as a result of high density results in decreased birth rates and increased mortality rates. However, there is little evidence of density dependence in large- to mega-herbivores. Many mega-herbivores are Critically Endangered, from which stems the urgent need to let them increase in numbers as fast as possible to counteract for instance the devastating effects of poaching. Many conservation institutions and managers responsible for managing and breeding mega-herbivores *in situ* assume, without much evidence that these species are subject to density dependence, following a logistic growth curve. These institutions and managers thus focus their efforts on achieving high growth by trying to maintain populations at densities presumed to be at half-carrying capacity (K/2). This would enable them to translocate animals at presumed half-carrying capacity to retain local population densities and to create new populations in areas of suitable habitat, where animals are considered safe against poaching. Such mega-herbivore populations are also increasingly located within intensively managed, fragmented areas. This presents conservation challenges which require an in-depth knowledge of population dynamics, including possible density dependence; because the management is critically dependent on this assumption that populations grow fastest at this presumed ‘half-K’ and that density dependency operates the way it is assumed to do.

This study focussed on the eastern black rhinoceros (*Diceros bicornis michaeli*) to test the idea of density dependence population regulation in a mega-herbivore, and to use the findings to contribute towards possible solutions for conservation challenges facing this species. This species has biological and ecological characteristics that make it suitable for such a study. Firstly, it could have a potentially large impact on its habitat (Owen-Smith 1988, Hansen and Galetti 2009), especially if enclosed at high density. Its little roaming behaviour leaves it with few possibilities to mediate environmental fluctuations. Its hind-gut digestive system can process plants of low nutritional quality or those considered highly toxic such as *Euphorbia* spp. Like other mega-herbivores, it does not have effective predators, apart from man, to regulate or control its population top-down, hence its population is mainly controlled by bottom-up processes through key resources (*e.g.*, Fritz *et al.* 2002). Secondly, this species, on the one hand, like the other rhinoceros species, faces a risk of extinction from an upsurge in poaching and

therefore requires high protection; on the other hand, the species needs to be dispersed more widely to reduce negative effects of poaching. These two situations pose a conservation dilemma in balancing between exposing 'surplus' animals to higher poaching risks in vast areas that are expensive to protect or accommodating slow and possibly population crashes as a consequence of density dependence.

This study was conducted in nine conservation areas for black rhinoceros selected on the basis of different densities, fenced or open, and varying Plant Available Moisture ('PAM' *i.e.*, 'soil moisture') and Plant Available Nutrients ('PAN' *i.e.*, 'soil fertility'). My expectations were that, if density dependence were to take place, (i) increases in population density would result in a decrease in reproductive performance, and (ii) physiological stress levels in animals in populations of high density would be higher than in animals in populations of low density. Long term (1993-2010) measures of reproductive performance were derived from archived data on individual animals. Furthermore, the dietary quality and endocrinal changes (mainly physiological stress through measures of levels of the hormone corticosterone) in the eastern black rhinoceros were measured. Dietary quality and levels of corticosterone were estimated through faecal analysis from field and zoo animals. Woody cover estimates were used as a measure for available browse for black rhinoceros. Two measures of density, *i.e.*, absolute density (animals/km^2) and relative density, *i.e.*, absolute density as a ratio to the estimated maximum safe stocking density (often taken equal with 'carrying capacity' but in truth not based on the concept of density dependency) were used. The effects of edaphic factors (PAM and PAN), and climatic variables (rainfall and temperature) were incorporated and controlled for in testing the expectations.

In **Chapter 2** sex ratios but not population density was shown to influence population growth rate. The exponential and logistic growth models gave similar results and the former were accepted because they better described the actual situation on the ground. The need to interpret population models alongside ground-truthed observations was highlighted. The results underpinned the inference that black rhinoceros population regulation is not density dependent. In **chapter 3**, longer term data on life history traits and one measure of reproductive performance, *i.e.*, yearly percentage of females calving (%FC) were used. %FC is less detailed in its requirements for estimating it, thus inherently making it have higher sample size and statistical power than other measures of reproductive performance. Results showed that %FC was not regulated by population density. Rather, other factors, especially PAM and PAN seem to play a significant role. In other words, both **chapters 2 and 3** pointed in the same direction of lack of density dependence regulation in populations of black rhinoceros that were studied. Quality of dietary browse may be reduced by high density of browsers while macro and micronutrients in diet may limit reproductive performance. These two assertions were tested in **chapter 4** by controlling for the effects of PAM and PAN on quality of forage. Firstly, the faecal and dietary mineral concentrations showed strong correlations in data from feeding trials of 15 black rhinoceroses in zoos. Secondly, faecal mineral concentration in faecal samples from the field increased with increase in PAM lagged by 1 month (*i.e.* to reflect rainfall over the 4 weeks preceding sampling). Areas of high PAM had higher levels of faecal minerals and higher densities of black rhinoceros. PAN did not seem to influence mineral concentration in leaves of browse plants as measured via faecal analysis. We did not find evidence for density dependence on diet quality, *i.e.*, the quality of browse plants of black rhinoceros were not lowered by browsing due to high densities of black rhinoceros. These results suggested that this mega-browser was controlled by bottom-up processes. Further tests for bottom-up control processes were done in **Chapter 5.** Here, PAM, PAN and woody cover were used to explain three measures of reproductive performance, *i.e.*, age at first calving, inter-calving intervals, and yearly percentage of females calving. Reproductive performance measures were better in areas of high PAM, low PAN and sparse woody cover. Here, again, PAM appeared to have a much higher explanatory power than PAN. Age at first calving in black rhinoceros was found to be influenced more by quantity of woody plants as estimated from measures of woody cover than were inter-calving intervals or yearly percentage of females calving. Besides the requirement of sufficient woody plants, findings in this chapter 5 contributed a new criterion for using PAM and PAN to select conservation areas for maximising reproductive performance of black rhinoceros. Under density dependence, high population densities should result in chronic and elevated physiological stress; and stress has been associated with low reproductive success, but **Chapter 6** no significant correlation between density and levels of corticosterone were reported. However, levels of corticosterone were found to increase as constituents of PAM (*i.e.,* rainfall and temperature) increased. Levels of corticosterone were also inversely correlated with the concentration of phosphorus in the faeces. Females showed higher physiological stress than males. These results suggested that secretion of stress hormones were controlled more by climate and diet minerals than by population density.

Generally **Chapters 2, 3, 4 & 6,** which directly evaluated density dependence, did not find density dependent feedbacks, whereas **Chapters 4, 5 & 6** suggested that bottom-up processes through key resources contributed to the reproductive performance of the black rhinoceroses. Finally in **Chapter 7,** these findings are discussed from ecological and socio-economic perspectives, with the specific focus to contributing short and long-term solutions to the conservation challenges facing black rhinoceros. The following six arguments and solutions are discussed: i) that reproductive performance of black rhinoceroses studied could still be higher at higher population densities; ii) that, if top-down control through illegal killing of rhinoceros is halted, then rhinoceros populations would increase and benefit the tourism sector; iii) that black rhinoceros can optimally reproduce on infertile soils with high moisture; iv) that land sparing and not land sharing is ideal for conservation of mega-herbivores; v) that the approach of this study contributes towards standardization for selection of suitable habitats for black rhinoceros; and vi) that, if stressors for females are reduced, reproductive performance may further improve in black rhinoceros populations.

The study concludes that densities of black rhinoceros in the areas under consideration here did neither affect their reproductive performances, nor the quality of browse diet, nor the stress hormone levels. They could

conceivably still reproduce more and at higher densities than those studied. Reproductive performances were better where PAM was high, PAN was low and woody cover was sparse. PAM was found to be directly correlated with the quality of dietary browse. Black rhinoceros populations appeared controlled more by bottom-up processes through key resources, eventhough their densities were perhaps too low to fully support this alternative view**.** It is apparent that the density dependence concept and the concept of bottom-up control deserve more investigation. In the meantime, deliberate efforts should be made to secure high PAM – low PAN – sparse woody cover areas for conservation of black rhinoceros and to encourage wildlife tourism. Conservation managers are advised to consider set percentage translocation as opposed to translocating black rhinoceros on the basis of an imaginary 'carrying capacity' and supposed density dependence. Evidence-based conservation is the way ahead instead of following text-book knowledge that was derived from micro-animals in laboratory flasks.

Photo credit: B. Okita-Ouma

Samenvatting (*Dutch*)

Populatiedichtheden van de Oost-Afrikaanse Zwarte Neushoorn: Ontrafeling van de sturende factoren

Het verkrijgen van inzichten in de factoren die leiden tot variatie in populatiegrootte is een centraal thema in de ecologie. Het zijn voornamelijk deterministische processen, beïnvloed door toevallige gebeurtenissen, die de populatiegrootte beperken. Deterministische processen omvatten terugkoppelingen van populatiedichtheid (dichtheidsafhankelijkheid), door geboorte en sterfte, of populatiegroei. Dichtheidsafhankelijkheid is het gevolg van competitie binnen de populatie. Deze processen interageren en leiden daardoor tot complexe patronen van dichtheidsvariatievan dieren over tijd en ruimte. De beperkende factor in populatiedichtheden van grote zoogdieren, die niet gestuurd worden door top-down predatie, is voedsel; met andere woorden, dergelijke populaties worden gestuurd door bottom-up processen. Voedselschaarste als gevolg van hoge populatiedichtheden leidt dan ook tot minder geboortes en meer sterftegevallen binnen de populatie. Echter, tot op heden is er weinig bewijs voor dichtheidsafhankelijkheid gevonden in grote tot zeer grote herbivoren (ofwel, mega-herbivoren). Tegelijkertijd wordt het voortbestaan van veel mega-herbivoren ernstig bedreigd, van waaruit de dringende noodzaak voortvloeit om tot snelle populatiegroei te komen, om zo ook de negatieve effecten van bijvoorbeeld stropen tegen te gaan. Veel organisaties en beheerders die zich inzetten voor het behoud van deze mega-herbivoren in het wild, nemen zonder veel bewijs aan dat populaties van deze mega-herbivoren onder invloed staan van dichtheidsafhankelijkheid en dat ze als gevolg daarvan een logistische groeicurve vertonen. En dus streven dergelijke instanties ernaar om mega-herbivoorpopulaties op de geschatte helft van het ecologische draagvermogen van de populatie te houden (K/2), om zo op een maximale populatiegroei te komen en te blijven. Dit stelt hen in staat om dieren uit een populatie die op de helft van het ecologisch draagvermogen zit te herplaatsen in nieuwe gebieden waar leefomstandigheden gunstig zijn en waar het risico op stropen minimaal is, om zo tegelijkertijd de bestaande populatie op het huidige niveau te houden. Dergelijke populaties van mega-herbivoren vindt men meer en meer in intensief beheerde en gefragmenteerde gebieden, wat zorgt voor uitdagingen op het vlak van beheer en waarvoor een gedegen kennis van populatiedynamica, en mogelijk ook dichtheidsafhankelijkheid, nodig is. Dit is vooral het geval omdat het beheer bijna volledig afhankelijk lijkt te zijn van de aanname dat populaties het snelst groeien als deze zich op de helft van het ecologisch draagvermogen bevinden en dat dichtheidsafhankelijkheid werkt zoals i.h.a. wordt aangenomen.

Deze studie richtte zich op de Oost-Afrikaanse Zwarte Neushoorn (*Dicerosbicornismichaeli*), om het idee te testen dat populatieregulatie in mega-herbivoren gestuurd wordt door dichtheidsafhankelijkheid, en om een bijdrage te kunnen leveren aan het formuleren van oplossingen voor de uitdagingen rondom het behoud van deze soort. Deze soort heeft zowel biologische als ecologische eigenschappen die het geschikt maken voor een dergelijke studie. Ten eerste kunnen neushoorns een grote impact uitoefenen op het habitat (Owen-Smith 1988, Hansen and Galetti 2009), met name als ze in hoge dichtheden ingesloten zijn in afgesloten gebieden. Door hun beperkte mobiliteit hebben ze weinig opties om fluctuaties in het milieu het hoofd te bieden. Door hun systeem van achterdarmverteringzijn zij in staat tot het verteren van planten met een lage nutritionele waarde alsook planten die gezien worden als zeer giftig, zoals *Euphorbia* spp. Zoals andere mega-herbivoren hebben deze neushoorns geen natuurlijke predators, althans geen predators die de populatie kunnen reguleren, behalve de mens. Als gevolg hiervan wordt de populatiedynamiek grotendeels gedreven door bottom-up processen (*e.g.*, Fritz *et al.* 2002). Ten tweede wordt deze soort, net als de andere neushoornsoorten, ernstig met uitsterven bedreigd door de toename in stroperij en dus is er een noodzaak tot bescherming van de soort, maar ook tot verspreiding van het leefgebied van de soort zodat de negatieve effecten van stroperij kunnen worden verminderd. Deze situaties vormen echter een beheersdilemma, omdat enerzijds ‘surplus’ individuen blootgesteld worden aan hogere risico’s op stroperij in grote, moeilijk te beschermen, gebieden, en anderzijds omdat populaties als gevolg van de effecten van dichtheidsafhankelijkheid minder hard gaan groeien of zelfs instorten.

Deze studie heeft plaatsgevonden in negen beheersgebieden voor zwarte neushoorns, welke geselecteerd waren op basis van variatie in dichtheid van dieren, aan- of afwezigheid van afrasteringen, en variatie in “Plant AvailableMoisture” (‘PAM’, ofwel voor planten beschikbaar bodemvocht) en “Plant AvailableNutrients” (‘PAN’, ofwel voor planten beschikbare bodemvruchtbaarheid). Mijn verwachtingen waren dat, als dichtheidsafhankelijkheid een rol speelt in dit systeem, (i) een toename in populatiedichtheid zou moeten leiden tot een afname in reproductie, en dat (ii) het fysiologisch stress niveau in individuen uit populaties van hoge dichtheden hoger zouden zijn dan in individuen uit populaties van lage dichtheden. Lange-termijn gegevens (1993-2010) van reproductie werden verkregen uit archiefdata over individuele neushoorns. Daarnaast werden de kwaliteit van het dieet alsook de endocriene veranderingen (voornamelijk de mate van fysiologische stress door het meten van corticosteron niveaus) in de neushoorns gemeten. Kwaliteit van het dieet en corticosteron niveaus werden geschat door analyse van de ontlasting van zowel dierentuindieren als in het wild levende individuen. Schattingen van de dichtheid aan houtige vegetatie in het leefgebied van de neushoorns werden gebruikt als maat voor de beschikbaarheid van ‘bladbiomassa’. Twee maten van dichtheid werden gebruikt; absolute dichtheid (aantal individuen / km^2) en relatieve dichtheid (ofwel de absolute dichtheid als ratio van de geschatte maximale bezettingsdichtheid (welke regelmatig gelijk gesteld wordt aan de draagkracht van het systeem, maar wat in werkelijkheid niet gekoppeld is aan het concept van dichtheidsafhankelijkheid)). De effecten van edafische factoren (PAM en PAN) en klimaatvariabelen (regenval en temperatuur) werden meegenomen en voor gecontroleerd tijdens het testen van de verwachtingen.

In **hoofdstuk 2** werd duidelijk dat wel de geslachtsverhouding maar niet de populatiedichtheid invloed bleek te hebben op de groei van de populatie. Exponentiële en logistische groeimodellen gaven vergelijkbare resultaten, maar het exponentiële groeimodel werd gekozen omdat deze de actuele gemeten populatieontwikkeling beter omschreef. Het belang van het interpreteren van groeimodellen naast de daadwerkelijke metingen van populatieontwikkeling werd daarmee duidelijk. De resultaten onderstrepen de conclusie dat populatieregulatie van de zwarte neushoorn niet dichtheidsafhankelijk is. In **hoofdstuk 3** werden de lange-termijn gegevens over levensloopkarakteristieken en een maat van reproductiviteit (namelijk het jaarlijks percentage vrouwelijke individuen dat een kalf kreeg (%FC)) gebruikt. In tegenstelling tot enkele andere maten voor reproductiviteit, is %FC een maat die vrij eenvoudig te verkrijgen is en daardoor was het mogelijk om hiervoor een grotere steekproef te verzamelen, wat de statistische kracht van de analyse vergrootte. De resultaten lieten echter zien dat %FC niet gereguleerd werd door populatiedichtheid. Andere factoren, voornamelijk PAM en PAN leken echter wél een significante rol te spelen. Met andere woorden, zowel **hoofdstuk 2** als **hoofdstuk 3** wijzen in de richting van een gebrek aan populatieregulatie door middel van dichtheidsafhankelijkheid in de populaties van zwarte neushoorns die hier bestudeerd worden.

De kwaliteit van bladbiomassa in het dieet van de neushoorns zou kunnen afnemen door de hoge dichtheid aan bladeters terwijl de aan- of afwezigheid van bepaalde macro- en micronutriënten in het dieet de reproductiviteit van de soort zouden kunnen verlagen. Deze twee voorspellingen werden getest in **hoofdstuk 4** waarin voor de effecten van PAM en PAN op voedselkwaliteit werd gecontroleerd. Ten eerste bleek uit testmetingen bij 15 zwarte neushoorns in dierentuinen dat de concentratie aan mineralen in de ontlasting en in het dieet van de neushoorns sterk gecorreleerd waren. Ten tweede bleek dat de concentratie aan mineralen in ontlasting van verschillende individuen uit het veld toenam met een toename in PAM (hoewel 1 maand vertraagd, dus de effecten van regenval in de 4 weken voorafgaand aan de verzameling van de ontlasting waren zichtbaar). In gebieden met hoge PAM niveaus werden ook hogere concentraties aan mineralen in de ontlasting teruggevonden en waren de dichtheden van zwarte neushoorns hoger dan in gebieden met lage PAM niveaus. PAN daarentegen leek geen effect te hebben op de concentratie aan mineralen in de ontlasting van neushoorns. We vonden ook geen bewijs voor effecten van dichtheidsafhankelijkheid op kwaliteit van het dieet; met andere woorden, de kwaliteit van planten gegeten door de zwarte neushoorn werd niet verlaagd door het voeden aan deze vegetatie door een hoge dichtheid aan zwarte neushoorns. Deze resultaten suggereren dat de populatiedynamiek van deze mega-herbivoor gestuurd wordt door bottom-up processen. Verdere tests met betrekking tot deze bottom-up processen werden beschreven in **hoofdstuk 5**. Hier werden PAM, PAN en de dichtheid aan houtige vegetatie gebruikt om drie verschillende maten van reproductiviteit te verklaren, namelijk leeftijd waarop voor het eerst gekalfd werd, de lengte van het interval tussen het kalveren, en het jaarlijks percentage aan vrouwelijke individuen dat kalfde. Reproductiviteit was hoger in gebieden met hoge PAM, lage PAN en een schaars voorkomen van houtige vegetatie. Ook hier leek PAM een veel groter verklarend effect te hebben dan PAN. De leeftijd waarop voor het eerst gekalfd werd bleek meer beïnvloed te worden door de hoeveelheid houtige vegetatie dan het interval tussen het kalveren of het jaarlijkse percentage aan vrouwelijke individuen dat kalfde. Naast de noodzaak van voldoende houtige vegetatie, lieten de bevindingen van **hoofdstuk 5** zien dat het ook belangrijk is om PAM en PAN in ogenschouw te nemen wanneer nieuwe beheersgebieden met maximale reproductiviteit voor zwarte neushoorns geselecteerd dienen te worden.

Onder de theorie van dichtheidsafhankelijkheid zou een hoge dichtheid aan dieren moeten leiden tot chronische en verhoogde niveaus van fysiologische stress. Daarnaast is stress gerelateerd aan reproductief vermogen. Echter, in **hoofdstuk 6** vonden we geen significante correlatie tussen dichtheid van dieren en corticosteron stress niveaus. Corticosteron niveaus namen toe als PAM variabelen (regenval en temperatuur) toenamen. Corticosteron niveaus waren ook negatief gecorreleerd met de fosfor concentratie in de ontlasting. Vrouwelijke individuen lieten hogere fysiologische stress-niveaus zien dan mannelijke individuen. Deze resultaten suggereren dat de afgifte van stresshormonen meer gereguleerd wordt door het klimaat en het dieet dan door de populatiedichtheid.

Over het algemeen bezien, werd in de **hoofdstukken 2, 3, 4 & 6**, welke dichtheidsafhankelijkheid direct evalueerden, niet gevonden dat dichtheidsafhankelijkheid een rol speelt in populatiedynamiek, terwijl in **hoofdstukken 4, 5 & 6** de suggestie gewekt werd dat bottom-up processen door middel van hoofdbestanddelen in de voeding invloed uitoefenden op de reproductiviteit van zwarte neushoorns. Tot slot, in **hoofdstuk 7**, worden deze uitkomsten bediscussieerd vanuit een ecologisch en sociaaleconomisch perspectief, met specifieke aandacht voor het bijdragen aan korte- en lange-termijn oplossingen voor de uitdagingen die er zijn rondom het behoud van zwarte neushoorns. De volgende zes argumenten en oplossingen worden bediscussieerd: (i) reproductiviteit van de zwarte neushoorn zou nog hoger kunnen zijn onder hogere populatiedichtheden; (ii) als top-down regulatie van populatiedichtheden door bijvoorbeeld illegale jacht zou worden gestopt, dan zouden neushoorn-populaties toenemen en zou de toeristische sector profiteren; (iii) zwarte neushoorns kunnen optimaal reproduceren op onvruchtbare gronden met een hoge bodemvochtigheid; (iv) het scheiden van land voor productie en land voor natuurbeheer is beter dan het integreren van land voor productie en natuurbeheer voor het behoud van mega-herbivoren; (v) de aanpak van deze studie draagt bij aan een standaardisatie voor het selecteren van geschikte habitats voor zwarte neushoorns; en (vi) een verlaging in stressfactoren voor vrouwelijke individuen zou de reproductiviteit van zwarte neushoorn populaties verder kunnen verhogen.

De conclusie van deze studie is dat de dichtheden aan zwarte neushoorns in de door ons bestudeerde gebieden geen effect hadden op reproductiviteit, op kwaliteit van het dieet en op de niveaus van stress-hormonen. Het is mogelijk dat de neushoorn populaties in deze studie nog meer kunnen reproduceren en onder nog hogere dichtheden zouden kunnen leven dan we hier gevonden hebben. Reproductiviteit was beter in gebieden waar PAM hoog was, waar PAN laag was en waar houtige vegetatie schaars was. PAM bleek rechtstreeks gecorreleerd te zijn aan de kwaliteit van het dieet voor de neushoorns. Zwarte neushoorn populaties leken vooral gereguleerd te worden door bottom-up processen, ook al waren hun dichtheden misschien te laag om deze alternatieve zienswijze volledig te kunnen onderbouwen. Het is zonder meer duidelijk dat de concepten van dichtheidsafhankelijkheid en bottom-up regulatie meer onderzoek verdienen. In de tussentijd zou er veel energie gestoken moeten worden in het veiligstellen van gebieden met hoge PAM, lage PAN en weinig houtige vegetatie voor het behouden en beheren van zwarte neushoornpopulaties en voor het aanmoedigen van wildtoerisme.

Beheerders worden geadviseerd om vaste percentages van de populatie te herplaatsen in andere gebieden, in tegenstelling tot het herplaatsen van zwarte neushoorns op basis van een denkbeeldige ‘draagkracht’ en aangenomen dichtheidsafhankelijkheid. Bewijs-gedreven natuurbeheer is de weg vooruit, in plaats van het overnemen van tekstboekkennis welke is verkregen middels het bestuderen van micro-dieren in erlenmeyers.

Photo credit: Swaib Okita

Résumé (*French*)

Démêler les facteurs contrôlant les densités de population du rhinocéros noir oriental

Comprendre les forces qui provoquent la variabilité dans la taille des populations est un thème central en écologie. Les facteurs limitants comprennent, dans une large mesure, les processus déterministes façonnés par des événements stochastiques. Les processus déterministes comprennent des rétro-contrôles de densité de population (densité dépendance) dus aux naissances et décès qui induisent le taux de croissance de la population. La densité dépendance résulte d'interactions compétitives au sein d'une population. Ces processus interagissent et génèrent des patrons spatio-temporels complexes de l'abondance des animaux. Le facteur limitant des populations de grands mammifères qui ne sont pas contrôlés par la prédation est la disponibilité de la nourriture ; ces populations sont ainsi contrôlées par des processus ascendants. La pénurie de nourriture induite par une forte densité se traduit par de faibles taux de natalité et de forts taux de mortalité. Cependant, peu d'études ont mis en évidence une densité dépendance chez les grands herbivores. Beaucoup de grands herbivores sont en danger critique d'extinction et il apparaît urgent d'augmenter leur taille de population aussi vite que possible en contrant, par exemple, les effets dévastateurs du braconnage. De nombreuses institutions et gestionnaires de l'environnement responsables de la gestion et de l'élevage de grands herbivores in situ supposent, sans beaucoup de preuves, que ces espèces sont soumises à la densité dépendance, suivant une courbe de croissance logistique. Ces institutions et gestionnaires concentrent donc leurs efforts sur une croissance élevée en essayant de maintenir les populations à des densités présumées à la moitié de la capacité de charge (K/2). Cela leur permettrait la translocation d'animaux afin de préserver des densités les populations locales et de créer de nouvelles populations dans les zones d'habitat favorable où les animaux sont considérés comme à l'abri du braconnage. Ces populations de grands herbivores sont également de plus en plus souvent situées dans les zones fragmentées gérées de manière intensive. Cela pose des défis de conservation qui nécessitent une connaissance approfondie de la dynamique des populations, y compris une éventuelle densité dépendance, car la gestion repose de façon critique sur l'hypothèse que les populations croissent le plus rapide à cette supposée demi capacité de charge et que la densité dépendance se produit de façon prévisible.

Cette étude sur les rhinocéros noirs orientaux (*Diceros bicornis michaeli*) teste l'hypothèse de la régulation par la densité dépendance de populations de grands herbivores et utilise les résultats afin de contribuer à des solutions possibles pour remédier aux problèmes de conservation de cette espèce. Cette espèce possède des caractéristiques biologiques et écologiques qui la rendent appropriée pour une telle étude. Tout d'abord, elle pourrait avoir un impact potentiellement important sur son habitat (Owen-Smith 1988, Hansen et Galetti 2009), surtout lorsqu'elle est contrainte dans de fortes densités. Son comportement peu itinérant lui laisse peu de possibilités de médiation suites à des fluctuations de l'environnement. Son système digestif lui permet de consommer des plantes de faible qualité nutritionnelle ainsi que celles considérées comme hautement toxiques, tels que les euphorbes. Comme d'autres grands herbivores, il n'a pas de prédateurs, en dehors de l'homme, pour contrôler sa population ; ainsi, sa population est principalement contrôlée par des processus ascendants dépendants de ressources clés (e.g. Fritz et al. 2002). Ensuite, cette espèce, d'une part, comme les autres espèces de rhinocéros, fait face à un risque d'extinction dû à une recrudescence du braconnage et nécessite donc une protection élevée et, d'autre part, les espèces doivent être réparties plus largement afin de réduire les effets négatifs du braconnage. Ces deux situations posent un problème de conservation dans l'équilibre entre l'exposition des animaux ”excédentaires” à des risques plus élevés de braconnage dans de vastes zones coûteuses à protéger ou accepter une croissance faible susceptible de subir des crash de population en raison de la densité dépendance.

Cette étude a été menée dans neuf zones de conservation pour rhinocéros noirs sélectionnés sur la base de différentes densités, en enclos ou en liberté, et de différentes humidités disponibles pour les plantes (de dire “HDP”, i.e. l’humidité du sol) et différentes quantités d’éléments nutritifs disponibles pour les plantes (“EDP”, i.e. la fertilité de sol). J’ai fait l’hypothèse que, s’il y avait densité dépendance, (i) l'augmentation de la densité de la population se traduirait par une diminution de la reproduction, et (ii) les niveaux de stress physiologiques chez les animaux dans les populations de forte densité seraient plus élevés que chez les animaux des populations de faible densité. Des mesures sur le long terme (1993-2010) du succès reproducteur ont été calculées à partir des données archivées sur différents individus. En outre, la qualité du régime alimentaire et les changements endocriniens (stress physiologique déterminé par des mesures de niveaux de l'hormone corticostérone) dans les rhinocéros noirs ont été mesurés. La qualité de l'alimentation et les niveaux de corticostérone ont été estimés par l'analyse des excréments d’animaux en liberté et de zoo. Les estimations de la couverture arborée ont été utilisées comme une mesure de navigation disponible pour le rhinocéros noir. Deux mesures de la densité, c'est à dire, la densité absolue (animaux/km^2) et la densité relative, c'est à dire, la densité absolue divisée par la densité de peuplement maximale estimée (souvent considérée comme la “capacité de charge”, mais en fait ne se basant pas sur la notion de densité dépendance) ont été utilisées. Les effets des facteurs édaphiques (HDP et EDP) et les variables climatiques (précipitations et température) ont été intégrés et afin de tester mes hypothèses.

Dans le **Chapitre 2**, j’ai montré que le sex-ratio, et non la densité de la population, influençait le taux de croissance des populations. Les modèles de croissance exponentielles et logistiques ont donné des résultats similaires et les premiers ont été choisis parce qu'ils correspondaient mieux à la situation sur le terrain. La nécessité d'interpréter les modèles de population avec des observations de terrain a été soulignée. Les résultats s'appuient sur la conclusion que la régulation des populations de rhinocéros noirs n’est pas dépendante de la densité. Dans le **Chapitre 3**, les données à long terme sur les traits d'histoire de vie et une mesure du succès reproducteur, c'est à dire, le pourcentage annuel des femmes qui mettent bas (%FMB), ont été utilisées. %FMB est moins détaillé dans ses exigences d’estimation, augmentant ainsi intrinsèquement la taille de l'échantillon et la puissance statistique comparé à d’autres mesures du succès reproducteur. Les résultats ont montré que %FMB n'était pas régulé par la densité de la population. Au contraire, d'autres facteurs, notamment HDP et EDP, semblent jouer un rôle plus important. En d'autres termes, les deux **chapitres 2 et 3** vont dans le même sens, insistant sur l'absence de régulation de densité dépendance des populations de rhinocéros noirs étudiées. La qualité de la prospection alimentaire peut être réduite par une forte densité d’animaux et les éléments nutritifs peuvent limiter le succès reproducteur. Ces deux constatations ont été testées dans le **Chapitre 4** en contrôlant les effets de la HDP et EDP sur la prospection alimentaire. Tout d'abord, les concentrations des minéraux présents dans l’alimentation et les excréments ont montré de fortes corrélations avec les données d'alimentation de 15 rhinocéros noirs de zoos. Ensuite, la concentration minérale des excréments prélevés sur le terrain augmentait avec l'augmentation de HDP avec un décalage d’un mois (reflétant ainsi les précipitations au cours des quatre semaines précédant l’échantillonnage). Les zones de forte HDP avaient des niveaux plus élevés de minéraux présents dans les excréments et des densités plus élevées de rhinocéros noirs. L’EDP ne semble pas influencer la concentration des minéraux dans les feuilles des plantes, telle que mesurée par analyse des excréments. Nous n'avons pas trouvé d’effet de densité dépendance sur la qualité de l'alimentation, à savoir, la qualité des plantes consommées par les rhinocéros noirs n’a pas été diminuée par de fortes densités de rhinocéros noirs. Ces résultats suggèrent que ce grand herbivore est contrôlé par des processus ascendants. D'autres tests sur les processus ascendants ont été réalisés dans le **Chapitre 5**. HDP, EDP et la couverture arborée ont été utilisés pour expliquer trois mesures du succès reproducteur, c'est à dire, l'âge au premier vêlage, les intervalles entre les vêlages et le pourcentage annuel de femelles vêlant. Les mesures du succès reproducteur étaient meilleures dans les zones de forte HDP, bas EDP et de couverture arborée clairsemée. Ici encore, HDP semble avoir un pouvoir explicatif beaucoup plus élevé que EDP. L’âge au premier vêlage était plus influencé par la couverture arborée que ne l'étaient les intervalles entre les vêlages et le pourcentage annuel de femelles vêlant. Outre la nécessité d’une couverture arborée suffisante, les résultats de ce Chapitre 5 contribuent à un nouveau critère pour l'utilisation de HDP et EDP à sélectionner des zones de conservation afin de maximiser le succès reproducteur des rhinocéros noirs. Sous l’influence de la densité dépendance, de fortes densités de population devraient se traduire par un stress physiologique chronique et élevé, et le stress a été associé à un faible succès reproducteur, mais le **Chapitre 6** montre qu’il n’y a pas de corrélation significative entre la densité et les niveaux de corticostérone. Cependant, les niveaux de corticostérone augmentaient avec les composants de HDP (i.e. précipitations et température). Les niveaux de corticostérone étaient également en inversement corrélés à la concentration en phosphore des excréments. Les femelles présentaient un stress physiologique plus élevé que les mâles. Ces résultats suggèrent que la sécrétion d'hormones de stress était plutôt contrôlée par le climat et les minéraux alimentaires que par la densité de population.

En règle générale, les **Chapitres 2, 3, 4 et 6**, qui ont directement évalué la densité dépendance, n'ont pas trouvé de rétro-contrôle dû à la densité dépendance, alors que les **Chapitres 4, 5 et 6** ont suggéré l’existence de processus ascendants induits par des ressources clés qui contribuent au succès reproducteur des rhinocéros noirs. Enfin, dans le **Chapitre 7**, ces résultats sont discutés des points de vue écologique et socio- économique, en mettant l'accent sur les solutions aux problèmes de conservation des rhinocéros noirs, à court et à long termes. Les six arguments et solutions suivants sont abordés : i) que le succès reproducteur des rhinocéros noirs étudiés pourrait être encore plus élevé à plus forte densité de population ; ii) que, si le contrôle descendant par abattage illégal de rhinocéros est arrêté, les populations de rhinocéros augmenteraient et le secteur du tourisme en bénéficierait; iii) que les rhinocéros noirs peuvent parfaitement se reproduire sur des sols infertiles à forte humidité ; iv) que des réserves totales, plutôt qu'un partage des terres, sont idéales pour la conservation des grands herbivores ; v) que l'approche de cette étude contribue à la normalisation de la sélection d'habitats appropriés pour le rhinocéros noir et vi) que, si les facteurs de stress des femmes sont réduits, le succès reproducteur peut encore être améliorer.

L'étude conclut en affirmant que les densités de rhinocéros noirs dans les domaines considérés ici n'ont aucune incidence sur leurs succès reproducteur, ni sur la qualité de l'alimentation et les niveaux d'hormone de stress. Les rhinocéros noirs pourraient ainsi se reproduire davantage et à des densités plus élevées que celles étudiées. Le succès reproducteur étaient meilleur là où HDP était élevée, EDP était faible et la couverture arborée clairsemée. HDP était directement corrélée avec la qualité de la nourriture. Les populations de rhinocéros noirs semblaient plus contrôlée par des processus ascendants, dus à des ressources clés, bien que leurs densités étaient peut-être trop faibles pour soutenir une telle affirmation. Il est évident que les concepts de densité dépendance et de contrôle ascendant méritent une étude plus approfondie. Dans le même temps, des efforts délibérés doivent être déployés pour garantir une forte HDP, une faible EDP et des zones de couverture arborée clairsemées pour la conservation des rhinocéros noirs et encourager le tourisme. Les gestionnaires sont donc invités à étudier ensemble un pourcentage translocation par opposition à la translocation des rhinocéros noirs basée sur une “capacité de charge” imaginaire et une densité dépendance supposée. Une conservation basée sur les faits scientifiques est la voie à suivre au lieu de se fier aux connaissances acquises d'études sur des microorganismes dans des flacons de laboratoire.

Muhtasari (*Swahili*)

Idadi ya faru weusi wa mashariki kwa eneo: Ng'amua vidhibiti

Kuelewa nguvu zinazosababisha tofauti katika ukubwa wa idadi ya wanyama ni mada ya msingi katika ikologia. Mambo kikwazo kwa kiasi kikubwa huundwa na taratibu zinazoeleweka na kupewa muelekeo na matukio yasiofahamika. Taratibu zinazoeleweka ni pamoja na mrejesho unaotegemea uwiani tegemezi (idadi ya wanyama kwa eneo) kwa kuangalia vizazi na vifo kwenye uongezekaji wa idadi ya wanyama. Mrejesho wa uwiani tegemezi (au unaotegemea idadi ya wanyama kwa eneo) hutokea unapokuwepo mwingiliano wa ushindani wa wanyama kwenye eneo husika. Mwingiliano huu hutengeneza mgawanyiko tata wa wingi wa wanyama kwa muda na eneo. Kikwazo katika idadi ya wanyama wakubwa ambao hawadhibitiwi kutoka juu-chini (top-down) kwa kuliwa ni chakula; kwa maneno mengine , idadi ya wanyama wakubwa hudhibitiwa na taratibu zinazotoka chini -juu (bottom-up). Kikwazo cha chakula kinachotokana na wingi wa wanyama kwa eneo kinasababisha kupungua kwa kuzaliana na kuongeza kiwango cha vifo. Hata hivyo kuna ushahidi kidogo unaonyesha kikwazo cha chakula kutokana na wingi wa hebivora wakubwa na wakubwa zaidi. Hebivora wakubwa zaidi wengi wako hatarini kutoweka hivyo basi kuna hitaji muhimu la kuwafanya waongezeke kwa kasi iwezekanavyo ili kufidia janga la uwindaji haramu. Asasi nyingi za uhifadhi na wahifadhi wenye jukumu la kusimamia na kuzalisha hebivora wakubwa zaidi kwenye mapori hudhani bila ushahidi wa kutosha kuwa spishi za hebivora wakubwa zaidi huadhiriwa na uwiani tegemezi ukifuata ukuaji wa kilogistiki (logistic growth curve). Taasisi hizi na wahifadhi wake huweka juhudi zao kufanikisha ukuaji wa juu kwa kujaribu kudhibiti idadi ya wanyama kwa uwiano unaofikiriwa ni nusu ya uwezo wa kuhifadhiwa (half carrying capacity ~ K/2). Hii itawawezesha kuhamisha wanyama kwa idadi inayodhaniwa ni nusu ya uwezo wa kuhifadhiwa na kubakiza kiasi huku wakitengeneza kundi jipya la wanyama kwenye makazi yanayofaa ambapo wanyama watakuwa salama dhidi ya ujangili. Kiasi kwamba idadi ya hebivora wakubwa zaidi imekuwa ikiongezeka kwenye maeneo yaliogawanyika na kutunzwa zaidi. Hii inatoa changamoto za uhifadhi ambazo zinahitaji elimu ya kina kuhusu mienendo ya idadi ya wanyama ikiwa ni pamoja na uwiani tegemezi, kwa sababu wahifadhi hutegemea zaidi katika dhana hii kwa ongezeko la haraka la idadi ya wanyama katika dhana ya nusu uhifadhi " ½ K", na hii inalingana na dhana ya uwiano tegemezi.

Utafiti huu umelenga faru weusi wa mashariki (*Diceros bicornis michaeli*), ili kujalibu wazo la uwiani tegemezi unaoweza rekebisha idadi ya hebivora wakubwa zaidi na kutumia majibu yatakayopatikana kutoa suluhisho la changamoto za uhifadhi. Spishi hii inatabia za kibiologia na kiikologia zinazofanya kufanya utafiti huu kufaa. Kwanza, inaweza kuwa na athari kubwa kwenye makazi yake (Owen-Smith 1988, Hansen and Galetti 2009), hasa kama watakuwa kwa idadi kubwa sehemu moja. Vile vile tabia yake ya kutembea eneo dogo inapunguza fursa za kubadili mazingira anayoishi. Mfumo wake wa chakula unaotumia kidole tumbo una uwezo wa kumeng'enya chakula chenye ubora wa chini hata vile ambavyo hufikiriwa vina viwatilishi vya sumu kama vile spishi za Euphobia.

Kama hebivora wengine wakubwa zaidi, faru hana mnyama mahususi kando na binadamu anaeweza kurekebisha idadi ya wanyama toka juu-chini; hivyo idadi ya faru zaidi inarekebishwa na taratibu zinazotka chini-juu kwa kupitia rasilimali muhimu (*e.g.*, Fritz *et al.* 2002). Pili aina hii ya mnyama kwa upande mwingine; kama spishi zingine za faru iko hatarini kutoweka kufuatana na ujangili usionakipimo; hivyo huuitaji ulinzi wa hali ya juu; kwa namna nyingine spishi hii inahitaji kuhifadhiwa maeneo mengi ili kupunguza madhara hasi ya ujangili. Hizi hali mbili zinaweka uhifadhi wa faru njiapanda namna ya kuwianisha kati ya kuwaweka hatarini faru wa ziada kwa jangili kwenye maeneo makubwa yanayohitaji gharama kubwa ya kuyalinda au idadi ya faru kupungua zaidi kwa ajili ya uwiani tegemezi (idadi ya wanyama kwa eneo).

Utafiti huu ulifanyika katika maeneo tisa yanayohifadhi faru weusi yalichaguliwa kwa misingi ya idadi kwa eneo, yenye uzio au wazi, na Unyevu Mimea Uliopo “yaani unyevu wa udongo” (PAM) na Virutubisho Mimea Vilivyopo yaani “rutuba ya udongo” (PAN). Matarajio yangu ni kwamba , kama wiani tegemezi utachukuwa uchukua nafasi yake, (i) kuongezeka kwa idadi ya wanyama kwa eneo kutasababisha upungufu uwezo wa uzazi, na (ii) Kiwango cha msongo wa kifiziolojia utakuwa mkubwa kwenye eneo lenye msongamano mkubwa wa wanyama kuliko kwenye msongamano mdogo. Takwimu za muda mrefu (1993-2010) zinazoonyesha uwezo wa kuzaliana zilipatikana toka kwenye kumbukumbu za mnyama mmoja moja. Zaidi ya hapo, ubora wa chakula na mabadiliko ya endokrinol (hasa msongo wa kifiziolojia kupitia vipimo vya kiwango cha homoni za kortikosterone) yalipimwa kwenye faru weusi wa mashariki. Ubora wa chakula na kiwango cha kortikosterone zilikadiriwa kupitia kinyesi cha faru wa mwituni na kwenye bustani za wanyama. Mtandazo miti (majani ya mimea kwa eneo) yalitumika kama kipimo cha uwepo wa chakula cha faru weusi. Vipimo viwili vya wingi wa faru kwa eneo vilitumika ambavyo ni idadi kamili ya wanyama kwa eneo (wanyama/km2); na idadi ya uwiano kwa eneo ambayo ni uwiano wa idadi kubwa inayotakiwa kwa eneo (kwa kawaida huchukuliwa kama kiasi sawiya kinachotakiwa kwa eneo lakini ukweli sio toka dhana ya uwiani tegemezi). Athari zitokanazo na uoto wa asili (PAM and PAN) na mabadiliko ya hali ya hewa (mvua na joto) yaliingizwa na kudhibitiwa wakati wa majaribio ya mategemeo.

Sura ya 2 imeonyesha kuwa uwiano wa jinsia na sio msongamano wa wanyama wenye ushawishi wa uongezekaji wa idadi ya wanyama kwa eneo. Hesabu za ukuaji kilogistiki (logistic growth models) na kilogarithmu (exponential models) zilitoa majibu yanayofanana hasa hesabu za kilogarithmu ambazo zilielezea hali halisi. Haja ya kutafsiri idadi za wanyama inayotokana na hesabu (models) sambamba na uhakiki wa wanyama ardhini (ground thruthing) imegusiwa. Matokeo yanadhihirisha kuwa wingi wa faru weusi haudhibitiwi na uwiani tegemezi (yaani idadi ya wanyama kwa eneo). **Sura ya 3** imetumia takwimu za muda mrefu za viashiria vya historia ya maisha ya faru na kipimo kimoja cha uwezo wa kuzaliana yaani asilimia ya idadi ya faru waliozaa (%FC). Kipimo cha asilimia ya faru walio zaa %FC hakihitaji vitu vingi kwa hiyo husaidia kufanya kuwa na idadi kubwa ya takwimu ambazo zina nguvu kitakwimu. Matoke huonyesha kuongezeka (%FC) haitegemei uwian tegemezi wa wanyama. Badala yake sababu zingine hasa PAM (*Unyevu Mimea Uliopo*) na PAN (*Virutubisho Mimea Vilivyopo*) kuwa za muhimu zaidi. Kwa maneno mengine **sura ya 2 na 3** zimeonyesha mwelekeo sawa uliokosa ushahidi na unaoonyesha idadi ya faru weusi inadhibitiwa na uwiani tegemezi wa msongamano wa faru kwenye utafiti huu. Ubora wa chakula unaweza kupunguzwa kwa wingi wa wanyama wakati virutubisho vinaweza kupunguza ufanisi wa uzazi. Haya madai mawili yalijaribiwa kwenye **sura ya 4** kwa kudhibiti madhara ya PAM na PAN kwenye ubora wa malisho. Kwanza, kiwango cha madini kwenye kinyesi na chakula kilionyesha mahusiano makubwa katika takwimu za majaribio 15 ya chakula cha faru weusi toka kwenye bustani za wanyama. Pili, kiwango cha madini kwenye sampuli za kinyesi toka porini ziliongezeka kadri PAM ilivyoongezeka hasa mvua ilipoanza wiki 4 kabla ya kuchukua sampuli. Maeneo ya PAM kubwa yalikuwa na kiwango kikubwa cha madini kwenye kinyesi na msongamano mkubwa wa faru weusi. PAN hata hivyo haikuonyesha uwezo wa kuathiri kiasi cha madini kwenye majani ya miti kama ilivyopimwa kupitia kinyesi. Hatukupata ushahidi wa uwiani tegemezi wa wanyama kwenye ubora wa marisho yaani ubora wa majani ya miti ya faru weusi haukupungua kutokana na wingi wa faru. Haya majibu huonyesha faru walidhibitiwa na michakato ya kutoka chini-juu. Majaribio zaidi ya mchakato wa chii-juu ulijaribiwa **sura ya 5**. Hapa PAM, PAN na mtandazo wa miti ulitumika kuelezea vipimo vitatu vya ufanisi wa uzazi yaani umri wa uzazi wa kwanza, muda baina ya uzazi, na asilimia ya uzao kwa mwaka. Vipimo vya ufanisi wa uzazi vilikuwa bora kwenye maeneo ya PAM kubwa, PAN ndogo na maeneo ya mtandazo miti uliotawanyika. Hapa tena PAM ilionekana kuwa na maelezo yenye nguvu kuliko PAN. Umri wa uzazi wa kwanza wa faru weusi uliathiriwa na kiasi cha miti kama ilivyopimwa toka kwenye mtandazo wa miti, kuliko muda baina ya uzazi na asilimia ya uzao kwa mwaka. Mbali na mahitaji ya kutosha ya miti, matokeo ya **sura ya 5** yamechangia kigezo kipya cha PAM na PAN katika kuchagua maeneo ya uhifadhi na kuongeza ufanisi wa uzazi wa faru weusi. Chini ya uwiano tegemezi, msongamano mkubwa wa wanyama ulitakiwa usababishe kiasi kikubwa cha msongo wa kifizilogia ulio sugu; na msongo umeoanishwa na ufanisi mdogo wa uzazi, lakini **sura ya 6** imeonyesha hakuna uhusiano wowote kati ya msongamano na kiasi cha kortikosterone. Hata hivyo kiwango cha corticosterone kilionekana kuongezeka kadri sehemu ya PAM (yaani*,* mvua na joto) ilivyoongezeka. Kiasi cha kortikosterone kilikuwa kinyume cha uhusiano na kiasi cha phosphorus kwenye kinyesi. Majike yalionyesha kiasi kikubwa cha msongo wa kifiziologia kuliko madume. Matokeo haya hupendekeza kuwa utolewaji wa homoni za msongo hudhibitiwa na hali ya hewa na madini yaliyomo kwenye chakula na sio msongamano wa wanyama.

Kwa ujumla, **sura 2, 3, 4 & 6** zilizotathmini uwiani temezi wa wanyama hazikuona mrejesho wa uwiani tegemezi ambapo **sura ya 4, 5, & 6** zimependekeza mchakato wa chini-juu (botton-up) kupitia rasilimali muhimu umechangia ufanisi wa uzazi wa faru weusi. Hatimaye **sura ya 7**, matokeo haya yamejadiliwa kwa taswira ya kiikolojia, kijamii na kiuchumi huku lengo maalum ni kuchangia ufumbuzi wa muda mfupi na muda mrefu kwenye changamoto za uhifadhi zinazowakabili faru weusi. Hoja sita na ufumbuzi wake ulijadiliwa kama ifuatavyo: i) kwamba ufanisi wa uzazi wa faru weusi uliofanyiwa utafiti unaweza kuwa wa juu kwenye msongamano wa juu. ii) kwamba, kama udhibiti wa juu-chini kupitia ujangili utakomeshwa idadi ya faru itaongezeka na itafadisha sekta ya utalii; iii) kwamba faru weusi wanaweza kuzaliana vizuri kwenye ardhi isiyo na rutuba yenye unyevu mkubwa; iv) kwamba kuachia ardhi na sio kuchangia ardhi ni bora kwa uhifadhi wa herbivora wakubwa zaidi; v) kwamba njia ya utafiti huu imechangia vigezo vitakavyotumika kupanga vingezo sawiya vya kuchagua maeneo ya uhifadhi wa faru weusi na vi) kwamba, kama msongo wa faru jike utapunguzwa ufanisi wa uzao kwenye makundi ya faru weusi utakuwa bora.

Utafiti unahitimisho kwamba msongamano wa faru weusi kwenye eneo zingatifu haujathiri ufanisi wa uzazi wala ubora wa malisho hasa majani ya miti wala kiwango cha homoni za msongo. Huenda faru weusi wanaweza kuzaa kwenye eneo la msongamano mkubwa kuliko hata kwenye eneo la utafiti huu. Ufanisi za uzazi ulikuwa bora kwenye maeneo ambapo PAM ilikuwa kubwa, PAN ilikuwa ndogo na mtandazo miti ulikuwa umetawanyika. PAM ilifahamika kuhusina moja kwa moja na ubora wa malisho ya majani ya miti. Idadi ya faru weusi huonekana kudhibitiwa na michakato ya chini-juu zaidi kupitia rasilimali muhimu ingawa msongamano wa faru ulikuwa mdogo kuunga mkono mtizamo huu. Ni dhahiri kwamba dhana ya uwiani tegemezi na udhibiti wa chini-juu unahitaji utafiti zaidi. Kwa sasa, nguvu za makusudi zichukuliwe kupata maeneo ya PAM kubwa, PAN ndogo na mtandazo wa miti uliotawanyika ili kuhifadhi faru weusi na tuhimize utalii wa wanyamapori. Wahifadhi wanashauriwa kupanga asilimia ya kuhamisha faru kinyume na kuhamisha faru weusi kwa msingi wa kufikirika “uwezo wa eneo kubeba kiasi fulani” na udhanio wa uwiani tegemezi. Uhifadhi utokanao na ushahidi ndio njia bora iliyo mbele yetu kuliko kufuata ujuzi tuliosoma kwenye vitabu vilivyofanya utafiti wa wanyama wadogo kwenye chupa za maabara.

Acknowledgements

The PhD journey

My PhD journey in Wageningen started in 2007, but did not properly commence until 10th February 2009. Cattle incursion into our protected conservation areas in Kenya was a nuisance then as it still is today. I was keen to find a scientific solution to this nuisance. During a visit to Ol Pejeta Conservancy with a delegation from Asia and Europe, whom I was taking around the country to have a first-hand experience of Kenya's rhino conservation work, we had an opportunity to hear from the Conservancy's management, about how they sustainably and mutually integrated wildlife and cattle. It was very impressive to hear. I initiated a discussion on this wildlife-cattle integration with Dr. Rajan Amin, Dr. Richard Pettifor and Professor Richard Kock, all then affiliated to the Zoological Society of London and were part of the visiting delegation. They were all very supportive and offered a lot of enlightening ideas. I got fired-up to study this as a PhD topic, with the aim of using my findings to harmonise cattle-wildlife interactions in wildlife conservation areas. I was encouraged by my work colleagues Drs. Erustus Kanga and Shadrack Ngene to undertake the PhD in the Netherlands where they were studying. On mentioning to Richard Pettifor my desire to study in the Netherlands, he immediately introduced me via email to his friends and colleagues, Professors Herbert Prins and Han Olff of Wageningen and Groningen Universities respectively. I quickly picked up communication with Herbert, discussed my topic via emails, and in 2008 he asked me to put together a proposal. Herbert encouraged me to apply for University funding towards my tuition and living costs in the Netherlands. He guided me through the admission processes and appointed Dr. Ignas Heitkönig to be my day-to-day supervisor. Professor Nigel Leader-Williams of University of Kent then but now of Cambridge University, Richard Pettifor and Rajan Amin wrote strong recommendations to Wageningen University, where I was enrolled as a PhD candidate in February 2009. I thank you all very much.

On 10th February 2009, I arrived in the Netherlands, it was late in the night and I could not access the email instructions about my accommodation that Patricia Meijer, our ever-super-efficient secretary at the Resource Ecology Group had sent. On alighting from bus No 88 at Droevendaalsesteeg with a heavy suitcase, I looked "lost" in a winter night, in front of Lumen building, that later turned to be where our Resource Ecology Group offices were located, at least for the period I lived in Wageningen. Luca Siahano, a student cycling past Lumen "found" me. He took me into the Gaia building from where he made reservations of a hotel Bennekom from a borrowed computer, after we still failed to access Patricia's email with arrangements for my accommodation! To cut the long story short; on our way to Bennekom, one of Luca's friends, Mathilde, offered me accommodation at her place. We cancelled the hotel reservation for Mathilde's offer. What wonderful people with such great sense of community in Wageningen! Thank you guys and my very best wishes in all your undertakings wherever you are. Patricia, your super-efficiency in administration is top-notch!

On day 1 of registration, the ever jovial Marion Rodenburg was very helpful. She continued to be so in all subsequent registrations as required for sandwich PhD students. Then there was the ever-lively and charming Gerda Martin, our finance and administrative manager. She was the first to receive and introduce me to the Resource Ecology Group, including to Herbert and Ignas who I was meeting for the first time! Gerda remained very supportive throughout my stay in Holland. Thank you very much Marion and Gerda.

The week grey hairs grew!

So my first appointment with my PhD promotor, Professor Herbert Prins and my daily supervisor Dr. Ignas Heitkönig was set up. We familiarised ourselves with each other and extensively discussed my cattle-wildlife proposal. It felt like an interview, with the first question being why I wanted to do a PhD. I was well prepared for it given I had received a series of warnings from many people who knew Herbert's renowned intellectual acumen. The initial bits of our discussions went well and I felt good that I could sustain close to an hour of academic discussion... but then the shockers came, like bombshells, they were dropped! First it was Herbert. He remarked that as much as my cattle-wildlife proposal – which we had worked on together for most of 2008 – was excellent, it was not contributing much to a cutting-edge science. This meant more work! In his own words he said "...*I want to see you contribute to scientific knowledge that will still be talked about even 50 years from now*..." Then was Ignas, with the next bombshell.... He smiled, turned my printed proposal upside down and said; "*assume this proposal never existed and let us start fresh thinking*". He nevertheless gave me the option of reconsidering the cattle-wildlife proposal if I still strongly wanted to pursue the topic. Both Herbert and Ignas had convictions that my strengths were in field experiments. Herbert was keen to strengthen my knowledge in rhino conservation. He said "*I want to mentor and shape you into a much better rhino conservationist*". Both Herbert and Ignas then urged me to think about novel research on rhinoceros. They gave me a few leads on density dependence and endocrinology and set the next appointment in two weeks' time. It was during those two weeks of thinking of a novel research project that I noticed presence of a few scattered grey hairs adjacent to my left ear! Over time though, I came to appreciate my Herbert's and Ignas' judgements on where my strengths lay. They had actually thrown "*the turtle back into its waters*". I thereafter enjoyed my PhD studies and now already feel a much better rhino conservationist as was Herbert's desire. More grey hairs continued to pop-up but not at a fast pace as they did in those two weeks. I wish to also add here that the subsequent very insightful comments and discussions with Ignas, and who was always available for my academic support. Herbert's continued emphasis on the need to first understand science before making far reaching conservation decisions, was deep, and is very much appreciated and treasured. Thank you Herbert and Ignas; I owe my first two grey hairs to you :-)

PhD study is indeed a cooperative work. As much as it trains one to be an "independent" scientist, it also trains one to be a "cooperative" scientist. I reckon the latter applies more. I wish to therefore thank my other supervisors at the university, Dr. Sip E. van Wieren, and at home country Dr. Rajan Amin. The time you dedicated

and support you offered during the period of this study are invaluable. Other than the supervisors, I also thank the other co-authors of the chapters in this thesis. They include; Dr. Frank van Langevelde, Dr. Gerrit Gort, Prof. Joshua Millspaugh, Prof. Marcus Clauss, Prof. Nigel Leader-Williams, Mr. Peter Maina and Dr. Richard Pettifor. Your contributions towards this thesis are very much appreciated.

Institutional/organizational supports

I undertook this PhD study at a time when rhino poaching was rampant and at an all-time high. It was psychologically very difficult for me to turn my back and just focus on a PhD alone without actively contributing to providing possible solutions to this crisis. Balancing national and international responsibilities with PhD studies as National Rhino Coordinator and later Head of Conservation Programmes at Kenya Wildlife Service (KWS) and Deputy Chairman of the IUCN's SSC African Rhino Specialist Group was challenging. I wish to appreciate the deep understanding, support and permission by my employer; Kenya Wildlife Service, IUCN's SSC African Rhino Specialist Group and the Resource Ecology Group that enabled me to balance these responsibilities. In this regard I wish particularly thank Mr. Julius Kipng'etich and Mr. William Kiprono who were directors of Kenya Wildlife Service during the period of my study. I also thank Mr. Patrick Omondi and Dr. Samuel Kasiki who were my immediate superiors at KWS. Joe Onyango, John Karenju, Linus Kariuki, Lynette Muganda and Sharon Kesire of KWS were also very supportive in this regard. Dr. Simon Stuart, the Chairman of IUCN-Species Survival Commission, and Dr. Mike Knight, the Chairman of the IUCN's SSC African Rhino Specialist Group are thanked too in this regard. Prof. Herbert Prins, the Chairman of the Resource Ecology Group and also my Grand supervisor, and Dr. Ignas Heitkönig my daily supervisor were very understanding and supportive.

Financial and in-kind support

Wageningen University provided the Scholarship to pursue this PhD and supported my living expenses while in the Netherlands. In addition to in-kind support, Kenya Wildlife Service provided a vehicle during data collection, financed analyses of mineral nutrients and costs of freeze-drying samples and kept me as its employee. WWF ESARPO Nairobi office and The Mohamed bin Zayed Species Conservation Fund financed field data collection and shipment of faecal samples to the USA for laboratory analyses. Paul Pearce-Kelly facilitated financial support from the Zoological Society of London towards field data collection, whereas Michel Louis of Zoo D'Amnéville provided support towards some field equipment and air travels – *merci mon ami* Michel. Professor Joshua Millspaugh and his laboratory staff assayed faecal samples for corticosterone at the University of Missouri, USA. Cathy Dean of Save the Rhino International is thanked for her in-kind support during one of my field visits in Kenya. Mark Pilgrim provided financial support from Chester Zoo (North of England Zoological Society) towards public defence of this thesis. Staff at Geology and Mines Laboratories in Nairobi especially Edward Mwangi, Joram Wambua, Micah Obondi and John Munyao analysed faecal mineral nutrients while Sophie continuously explained the analytical procedures. Field freezers were financed by the Resource Ecology Group, Wageningen University, except for Ol

Jogi Ranch that offered a freezer onsite. Ngulia Safari Lodge offered a freezer for samples from Ngulia Sanctuary and Tsavo West National Park Intensive Protection Zone. James Kimotho, Peter Kaiguri and particularly Samuel Muchiri of Kenya Medical Research Institute in Nairobi cordially supported freeze drying and storing the faecal samples prior to shipment. Hesbon Odongo and his laboratory staff in Chiromo Campus, University of Nairobi and Paul Okwach of Pathcare Kenya Laboratories helped with initial hormonal assay trials in Kenya and trained me on radio and enzyme immunoassay techniques. Frank Basiye Laboratory Technical Advisor at the Centres for Disease Control and Prevention in Kenya and Professor George Owiti-Otiang', the Principal of Kenya Wildlife Service Training Institute recommended reputable laboratories and contact persons for freeze drying and trials for hormonal assays. My little daughter, Hawi Jane and three student attachés at KWS helped with sorting stored faecal samples. Dominic Mijele and Abraham Sangula facilitated testing of samples for Foot-and-Mouth Disease at the disease' Laboratory in Embakasi, Nairobi prior to shipping to USA. Aguno, Macharia, Maingi, Njoroge and Pamela of Kenya Soil Survey; Mutero of KWS; Okoth of ICRAF; and van Oostrum of ISRIC-Wageningen provided information on soils and plant available nutrients. Kevin Davey and Rajan Amin in conjunction with Zoological Society are thanked for developing the KIFARU rhino information system and guiding in efficient extraction of demographic data.

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The Kenyan rhino monitoring team helped with collection and storage of long-term demographic data. I want to particularly thank the following for assisting with collection of faecal samples and or facilitating or providing data for this study: Alice, Andanje, Anyona, Boit, Bundotich, Cedric, Chelal, Chemirmir, Christine, Ekile, Ekitela, Elimlim, Elsie, Etyang', George-Paul, Hamisi, Jammie, Juma, Karanja, Kenana, Kiampi, Kimanta, Kimanzi, Kipish, Kiplimo, Kosgey, Kuyuoni, Lucas, Luvati, Magena, Manegene, Micheni, Mishuko, Mulama, Mureithi, Musyoki, Mutegi, Muthama, Mutisya, Mutua, Muya, Mwadime, Ngene, Ochuodho, Okoth, Opanga, Owago, Oyugi, Plimo, Robert, Rotiken, Saitoti, Sangawe, Sgt. Mureithi, Shannon, Wafula and Wa-Njeri. Peter Maina and Kayijamahe are thanked for support with GIS. Adrian Shrader, Eduardo Barbosa, Evans Mwangi, Farshid Ahrestani, George Anyona, Keryn Adcock, Marcus Clauss, Martin Brooks, Martin Mulama, Noah Sitati, Phillip Muruthi, Richard Emslie, Rob Brett, Robert Chira, Richard Kock, Sophie Nam, Shadrack Muya, Shadrack Ngene, Shelley Waterland, Steve Stratakos, Thomas Hildebrandt, Yoganand Kandasamy and Yussuf Wato are acknowledged for either proof reading or providing helpful comments on drafts of some of the chapters or proposal of this thesis. I thank Frank van Langevelde, Gerrit Gort, Marcus Clauss and Richard Pettifor for spending many hours with me going through complex statistical procedures and analyses. Edward Kohi, Lennart Suselbeek and Vincent Comor are acknowledged for translating the summary of this thesis into Swahili, Dutch and French languages. My son Bady Mboga-Okita is thanked for the design and drawing on cover page of this thesis.

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Fred de Boer, Eduardo and Priya Koduganti provided useful insights and reference materials on statistics, digestive physiology and plant physiology while Kyle Tomlinson tweaked my English here and there. I wish also to thank Anne-Marie, Christine, Herman, Milena, Patrick and Pim for their encouragements. Alfred, Daniel, Edson, Eduardo, Emmanuel, Farshid, Helen, Henjo, Inge, Iris, Jasper, Jente, Jooste, Kohi, Kraus, Lennart, Mikhail, Ralf, Rudi, Tessema, Tim, Tom, Tsewang, Vincent, Yolanda, Yong and Zheng are thanked for various discussions we held and light moments in our group. Our tea/coffee and lunch breaks were always enjoyable and enticing since there was much to learn from our amalgam of cultures and interests. I am forever indebted to the Resource Ecology Group and wish to also thank Claudius van de Vijver of PE&RC, the "fire man" for his support in the training and supervisory planning.

Kenyans in Wageningen

Kenyans in WUR, you were amazing! You made my stay in Wageningen feel like home away from real home. Our occasional *nyama na mahindi choma* (meat and maize roasts) went down really well with cold Grolsch or Tusker beers especially after a month of hard work. Great ideas emerged during these times and we knitted well as a Kenyan community. You are all very special and I continue to hold you dear; Adero, Ayuke, Dennis, Hellen, Hongo, Inge, Irene, Jamleck, Jimmy *Mtu wa Kawaida,* Kilelu, Martin, Menge, Moses, Morgan, Muhonja, Muturi, Imbahale, Jessica, Ondiaka, Oyake, Susan, Treazah, Virginia and Wickama, it is now time to cancel all those accumulated "*Giant Panda Credits*" – remember our joke? Thanks to my jogging mates; Agogo, Chepkemoi, Faith, Winnie and Yussuf for being part of our fitness club. – "*mimi ni yule yule ... tangu shule shule"* – remember our song? Thanks to Farai Maphosa for inspiring us every Sunday.

Family support

Long periodic-breaks from my family as a parent and a husband were psychologically very challenging. I always missed my wife Sophie and our children, Hawi, Rajan, Swaib and Bady. My daily phone calls and or skypes are treasured. Sophie, you did marvellously well taking care of our children during my physical absence. Your perseverance, understanding and love are very much appreciated. To my parents, Jane and James, and the entire Jo-ka Mboga and Ouma's families, I thank you very much for your love, encouragement and continued support. My mother in law, Monica, your continued encouragement is very much appreciated. Richard Pettifor and your family including our friend Codie; you are amazing and thank you for hosting me in London as you spent many hours helping with statistics! Janak Amin and Raj Amin and family, you are part of family; our discussions about this study at your residence in London were very inspiring. Tim Oloo; you and Elizabeth Amayo are always thanked for welcoming and introducing me into the arena of rhinoceros conservation!

...and so, LONG LIVE RHINOS in high population densities -☺!

Photo credit: Maandoi Rotiken

Curriculum *vitae* and list of publications

Benson Okita Ouma, MBS was born on 9th July 1973 in Homa Bay, Kenya. He has been involved in the conservation of rhinoceros in Kenya since 1996. Rhinoceros was declared a special species in Kenya through a Presidential decree in 1985 following a decline in its numbers from 20,000 to less than 350 animals within 20 years. Poaching rhinoceros for illegal trade in it horn was the main reason for the drastic decline. Today, through collaborative efforts on security, technology, science and finance, rhino numbers in Kenya are gradually recovering. However, serious threats to rhinos remain. Recently, rhino poaching has escalated in Africa and Asia, while in Europe and America rhinoceros horns are being stolen from museums and private collections. Okita has been at the forefront of national and international efforts to protect this iconic species and to provide and implement long-term solutions to avert the poaching crisis.

Benson is currently the Senior Scientist and National Coordinator of Kenya's Rhinoceros Conservation Programme at KWS; a position he has held since 2006. He has been the Chairman of the East African Community Rhinoceros Management Group since 2009. He has been a member of the IUCN's SSC-African Rhinoceros Specialist Group since 2003 and was appointed to the position of Deputy Chairman of this IUCN's group in 2011. He joined the editorial board of the Pachyderm journal in 2012. He has been lead negotiator for the Kenyan Government on rhinoceros matters at the 14th (2007), 15th (2010), and 16th (2013) Conference of the Parties meetings of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). During these CITES meetings, CITES Resolution Conf. 9.14 (Rev. CoP 15) was amended as championed by Kenya to place more obligations and accountability on States whose citizens are implicated in illegal trade in rhinoceros horn. Stricter rules and Decisions were also enacted to help reduce rhinoceros poaching, reduce illegal demand for rhinoceros horn and curtail illegal exports and re-exports of rhinoceros horns and hunting trophies.

Apart from wildlife conservation work, Okita continues to be actively engaged in mobilizing resources towards improving the welfare of members of his community in Homa Bay and improving the education of young children and youth groups. Between 2004 and 2012, he championed mobilization of financial resources and in-kind support amounting to approximately US $250,000 for community development. The Kenyan government and an amalgam of institutions and friends jointly provided these funds. The funds were used to: reconstruct dilapidated classrooms and a library in a primary school currently hosting about 600 pupils aged between 5 and 15 years. Other projects included construction of a new village youth polytechnic and provision of associated tools and equipment for vocational training of youth groups; establishment of an open-air market; provision of rural grid-line electricity; provision of a greenhouse; provision of two boreholes for clean drinking water; and opening up of a new 2-km access road. He is currently part of a partnership championing establishment of a primary school to provide good quality education to young children in Nakuru, Kenya.

In 2008, President Mwai Kibaki, CGH, of the Republic of Kenya conferred on Okita the highest citizen accolade – The Moran of the Order of the Burning Spear (**MBS**) – in recognition of his distinguished service to the Nation.

Education

Okita schooled at St. Brigid's & Arina Primary Schools, and Kisumu Boys High & Homa Bay High Schools between 1980 and 1991. He proceeded to graduate in 1997 with a Bachelor of Science degree (Honours) in wildlife management from Moi University Eldoret, Kenya. His dissertation on '*the relationships between the age of the black rhinoceros and its faecal texture*' was supervised by Professor Jethro Odanga.

In 2004, Okita graduated with a Master of Science degree (Distinction or *Cum Laude*) in conservation biology from University of Kent in Canterbury, United Kingdom. His dissertation on 'population dynamics and performance of black rhinoceros in Kenya' was supervised by Professor Nigel Leader-Williams.

In 2009 he enrolled as PhD candidate at Wageningen University, Netherlands. This thesis '*Population densities of eastern black rhinoceros: unravelling the controls*' is a product of his research as a PhD candidate. His lead supervisor for this research was Professor Herbert H. T. Prins.

List of publications

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25+ articles in conference proceedings

Photo credit: Rajan Okita

PE&RC Training and Education Statement

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)

- Density dependent reproductive performance of black rhinoceros (*Diceros bicornis michaeli*)

Writing of project proposal (4.5 ECTS)

- Density dependent reproductive performance of black rhinoceros (*Diceros bicornis michaeli*)

Post-graduate courses (6 ECTS)

- Getting to the Bottom of Mount Kenya: analysis of land dynamics and sustainable development in an interdisciplinary perspective; PE&RC (2009)
- Linear mixed models; PE&RC (2009)
- Advanced reproductive physiology at North Carolina Zoo, USA; Leibniz Institute for Zoo and Wildlife Research, Germany (2011)
- Introduction to R; PE&RC (2011)
- Bayesian statistics; PE&RC (2011)

Laboratory training and working visits (3 ECTS)

- Radio and enzyme immunoassays techniques; School of Biological Sciences, Chiromo Campus, university of Nairobi (2010/2011)

Deficiency, refresh, Brush-up courses (2 ECTS)

- Ecological methods - I (2011)

Competence strengthening / skills courses (1.8 ECTS)

- EndNote X5 demonstration; Wageningen Library (2011)
- Techniques for writing and presenting a scientific paper; PE&RC (2013)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.2 ECTS)

- PE&RC Weekend (2009)
- Tropical Ecology symposium; University of Amsterdam (2011)

Discussion groups / local seminars / other scientific meetings (6.9 ECTS)

- PE&RC Ecological theory and application (2010-2013)
- Three meetings of wardens and scientists of Kenya Wildlife Service (2010-2013)
- Seminar on *in situ* and *ex situ* rhino conservation and Kenyan rhino strategic planning; North of England Zoological Society (2013)

International symposia, workshops and conferences (8.4 ECTS)

- 15th and 16th Conference of Parties Meeting on the Convention of international trade in Endangered Species (CITES); Qatar and Bangkok (2010 & 2013)
- International elephant and rhino conservation and research symposium; Rotterdam (2011)
- 10th and 11th Meetings of the IUCN's SSC-African Rhino Specialist Group; South Africa and Kenya (2011 & 2013)
- African rhino emergency summit co-hosted by KWS and AWF; Nairobi Kenya (2012)
- The Rhinoceros, what is the future; Thiory conference, France (2013)
- The Sumatran Rhino Crisis Summit; Singapore (2013)

Lecturing / supervision of practical's /tutorials (3 ECTS)

- Strategic planning in conservation of Critically Endangered species (2013)
- Tactical and strategic options to address illegal trade in rhino horn; Manchester Metropolitan University, UK (2013)
- Supervision MSc student in Wild Animal Biology at Royal Veterinary Collage, UK (2010)

Supervision of MSc Student (3 ECTS)

- Non-invasive assessment of glucocorticoid concentrations in the evaluation of adrenal activity in wild eastern black rhinoceros (*Diceros bicornis michaeli*); Kenya

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