The Ecology and Conservation Biology of the Endangered African Wild Dog (*Lycaon pictus*), in the Lower Zambezi, Zambia.

by
Kellie A. Leigh

A thesis submitted in fulfillment of the requirements for the degree of
Doctor of Philosophy
Faculty of Veterinary Science,
University of Sydney.
September, 2005

STATEMENT OF RESPONSIBILITY

This thesis is my own original work, except where specifically acknowledged.

September, 2005

ABSTRACT

The African wild dog (*Lycaon pictus*) is one of Africa's most endangered carnivores. Previous research into this species has focussed on the largest extant populations in Africa. However, there are a large number of relatively small populations (20 to 50 dogs) distributed across Africa, which represent an important component of the diversity of the species and its remaining habitat. This study investigated the status of a small population of wild dogs in the Lower Zambezi area in Zambia. Objectives focussed on assessing population dynamics and identifying causes of decline. Research was carried out over a broad range of topics in an effort to provide comprehensive information for conservation management of the population.

The scope of the project was divided into five sections:

1) Demography and pack dynamics were assessed to identify the structure and status of the population, and the main causes of mortality.

2) An assessment of habitat types and related ecological factors was carried out to determine wild dog habitat utilisation in relation to vegetation type, prey densities and hunting success in each area.

3) The effects of interpredator competition on wild dog population dynamics was investigated, specifically, the effects of lions (*Panthera leo*) and spotted hyaenas (*Crocuta crocuta*).

4) Genetic analyses were carried out to assess the historic and contemporary genetic variability of the population, and to define patterns of geographic structuring and population differentiation.

5) Results were combined to assess the viability of the population and recommend conservation management strategies.

Snaring was identified as the most important cause of adult mortality, and a threat to wild dog population persistence. Inbreeding avoidance led to the emigration of adult males and females from the area and appeared to be a substantial contributor to population decline. Limited mate selection corresponded with neither sex displaying philopatry and large dispersal distances effectively removed adults from the

population. This result has important implications for the management of small populations, whereby lack of mate choice may increase dispersal distances and thereby increase edge effects on populations, regardless of home range sizes.

Home range sizes were related to den locations in remote areas of the Zambian Escarpment, which was used as a breeding refuge area. The Zambezi River and Zambian Escarpment appeared to be effective barriers to wild dog home range movements. The study area contained a diversity of habitats on the alluvial terraces of the river valley floor. There was a high density of impala (*Aeypceros melampus*), which formed the main prey base for the wild dog population.

Studies of other populations have found that wild dogs often avoided areas with high competing predator densities, which corresponded with high prey density areas. In contrast to those findings, the Lower Zambezi wild dog population showed a strong preference for high prey density areas. This population also showed only temporal avoidance of high lion density areas. Low lion density areas were preferred during breeding periods, while moderate to high lion density areas were preferred during non-breeding periods. Direct predation of adult wild dogs by lion and spotted hyaenas was rare. Kleptoparasitism of wild dog kills by either competing predator species was also rare. Predator competition was not considered to be an important determinant of population decline.

The Lower Zambezi population suffered from a loss of heterozygosity, low allelic richness, and there was significant evidence of a recent population bottleneck. The population did not contain any new mtDNA haplotypes, nor any unique alleles on the commonly used microsatellite loci, but was differentiated from African wild dog populations in other regions. There was evidence of historical and recent gene flow between the Lower Zambezi and the neighbouring southern African populations of Hwange and Okavango. This was the first study to show a loss of genetic variability in a free-ranging African wild dog population. Although more immediate anthropogenic and demographic factors were the critical determinants of population

decline, the loss of genetic variability has important implications for the conservation of the remaining small and fragmented wild dog populations in Africa.

Results showed that due to its small size the population is likely to have suffered from inverse density dependence and Allee effects on dispersal and reproductive success. Management recommendations focussed on mitigating anthropogenic causes of mortality, and improving connectivity with a larger, potential source population to increase the probability of successful dispersal and to restore genetic diversity. The high density prey base, small home range sizes and low levels of interpredator competition detected in this study suggest that the area has the capacity to support a large and potentially viable population of wild dogs if appropriate management strategies are implemented.

ACKNOWLEDGEMENTS

I am grateful to the Zambia Wildlife Authority for granting the permits necessary to carry out this research. The research was funded by a wide range of generous donors, who made the project possible. For providing research grants I would like to thank; Mads Sandau-Jensen and the Danish International Development Agency (DANIDA), and The Wallace Research Foundation, USA. Several Zoological Parks provided support for the project, including: Sedgwick County Zoo, Kansas, USA; The Cincinnati Zoo and Botanical Garden, USA; Zoos Victoria, Melbourne, Australia; and the Western Plains Zoo, Dubbo, Australia. I would also like to thank the private individual and corporate donors who provided ongoing support for this project: Yancey Walker Productions USA, Dwight Hibbard, Arthur Vorys, Afrikeye UK, Pamela Riley, Neil Hardie, Elefriends Australia, Old Mondoro Camp Zambia, Kanyemba Lodge Zambia, Chongwe River Lodge Zambia, Conservation Lower Zambezi Zambia, Airwaves Charters Zambia, Philip and Julia Leonard, and particularly Julie McIntosh of the Classic Safari Company in Sydney for introducing me to Zambia and the Lower Zambezi.

Many people provided support for my fieldwork in the remote environment of the Lower Zambezi National Park. Thank you to the managers and guides of the safari camps in the study area that assisted with reporting wild dog sightings, and after much harassment, with the collection of wild dog faecal samples. There was always a race to collect the faeces before the hyaenas or vultures could get them. Conservation Lower Zambezi (CLZ) assisted with numerous field activities, including making their anti-poaching aircraft available for charter for aerial tracking, and donating the use of their darting equipment for wild dog immobilisations. Thanks go to Ian Stevenson and Leanne Edwards of CLZ, for their friendship and company, especially those long nights counting spotted hyaenas, and the den walks through the adrenaline grass. Special thanks to Ian for assisting with snare removals. Thank you to Riccardo Garbaccio of Kanyemba Lodge who generously donated assistance with servicing and

maintaining the project vehicle and equipment, and also provided an oasis of fine Italian food, friendship and mains electricity when it was most needed.

I am grateful to veterinarians Ian and Noeleen Parsons who donated their time to help with wild dog immobilisations and collaring, provided support and encouragement throughout the project, and also raised financial support from the Mazabuka community. Veterinarian Sally Shiel also donated considerable time, assistance and equipment for wild dog immobilisations and pathology tests, and offered logistical support in Lusaka, as well the warm hospitality of her family and home.

Many thanks to John Murphy for those high speed aerial tracking sessions through the window of the Cessna 206, and to Airwaves Charters for transporting everything from veterinarians through to wild dog necropsy samples. I am grateful to the safari operators in the South Luangwa and Kafue National Parks who collected wild dog sightings reports and faecal samples for the project, and to all the camps that agreed to store faecal samples in their kitchen freezers. Thanks also to the many ZAWA Wildlife Police Officers for their interest, enthusiasm and support in reporting sightings, particularly in the more remote areas of the National Park.

There are many people at the University of Sydney whom I would like to thank. My supervisor Tony English, who not only accepted me as a PhD candidate, but also supplied endless encouragement, moral support, and understanding of the difficulties associated with working in a remote environment in Zambia. My co-supervisor Herman Raadsma provided advice on genetic analyses, feedback on the final thesis drafts, a sense of humour and a bar-fridge in the office. Kyall Zenger kindly introduced me to the world of population genetics and the enormous number of statistical programs involved. Imke Tammen provided advice and extensive training to familiarise me with genetic laboratory techniques. To everyone in the Shute Building who offered help and advice in the laboratory, and patiently tolerated me returning from field work in the bush each year to ask the same questions all over again, thank you; Natasha Ellis, Julie Cavanagh, Marilyn Jones, Gina Attard, Marie Wildridge and Luke Chappel. Peter Thomson, Mat Crowther, Scott King and Neil

Hardie all provided helpful comments and advice on the statistical analysis used in this thesis. Thank you also to Eleanor Bruce and David Chapman from Geosciences who provided advice and assistance with GIS mapping techniques. The Faculty of Veterinary Science provided scholarships and grants-in-aid in support of this research, for which I am grateful.

Lastly I would like to thank my family, for encouraging me to follow my dreams, even though those dreams took me far from family and friends. Thank you for appreciating the novelty of having a “canine faecal collector” in the family.

TABLE OF CONTENTS

LIST OF FIGURES **PAGE**

LIST OF TABLES **PAGE**

CHAPTER 1: GENERAL INTRODUCTION

1.1 Natural History of African Wild Dogs.

1.1.1 Status and Distribution

The African wild dog (*Lycaon pictus*) is one of Africa's most endangered carnivores, and is Red Listed as an endangered species by the International Union for the Conservation of Nature *(IUCN 2002)*. The wild population of *Lycaon pictus* has been reduced dramatically over the last 50 years, and population estimates range from 2500 to 5000 animals left in Africa (Fanshawe et al. 1991; Woodroffe et al. 1997).

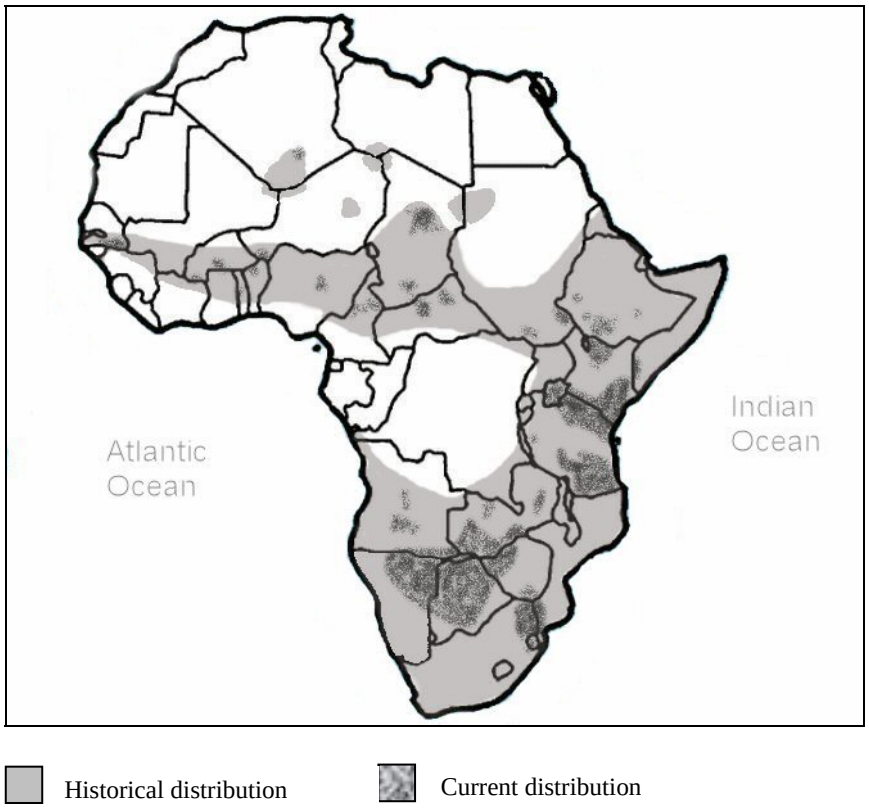


Figure 1.1 Historical and current distribution of Lycaon pictus in Africa. Current distribution indicates general regions inclusive of both vagrant and resident populations. Data drawn from Woodroffe et al. (1997), Ginsberg (1993), and Fanshawe et al. (1991).

Wild dogs were considered to be vermin by colonial governments who attempted to eradicate them in many areas. In Zambia alone vermin control units killed approximately 5000 wild dogs between 1945 and 1959 (Buk 1995).

It was believed that wild dogs suppressed antelope numbers and in some African countries persecution of wild dogs was official National Parks policy as late as 1979, and was carried out in some areas until the mid 1980's (Creel & Creel 1998; Woodroffe et al. 1997).

African wild dogs were historically distributed all over sub-Saharan Africa, but their range has decreased and become fragmented over recent decades (Figure 1.1). From the 34 sub-Saharan countries in which they used to exist, few countries are now thought to hold potentially viable populations. The Republic of South Africa, Namibia, Zimbabwe, Zambia, and Ethiopia are all estimated to hold populations of 400 dogs or over, while the largest extant populations reside in Botswana and Tanzania, estimated at 800 and 1800 dogs respectively. (Fanshawe et al. 1991; Woodroffe & Ginsberg 1999a; Woodroffe et al. 1997; Woodroffe et al. 2004b).

Long-term studies on wild dogs have been carried out in several African countries, which have identified the main causes of mortality for each population (Creel et al. 1997b; Creel & Creel 1996; Fuller & Kat 1990; Maddock & Mills 1994; Malcolm & Marten 1982; McNutt 1996; Van Heerdan et al. 1995; Woodroffe et al. 1997). Many of these causes were found to be linked with the encroachment of human populations into wild dog areas. The commonly cited causes of mortality observed in most study populations were road kills from fast moving traffic, illegal game snaring, shooting and poisoning, diseases from domestic dogs, and competition from other large carnivores. Each of these is discussed in greater detail in Chapter 2.

1.1.2 Description and Taxonomy

The African wild dog is a highly social group-living canid. The specific Latin name, *Lycaon pictus* (Temminck 1820), literally translates as “painted wolf-like canid”, which describes the unique tri-coloured pelage of black, white and tan. Another distinctive feature of the species is the unusually large, rounded black ears (Estes 1991). The average wild dog measures 60-75cm tall at the shoulder, and weight ranges from 18-34kg (Estes 1991; Smithers 1983; Woodroffe et al. 2004b). The species has only four toes on the foreleg and is missing the

vestigial dewclaw found in other canid species (Estes 1991).

Domestic dogs, wolves, jackals, and dingoes all belong to the genus *Canis*, however the African wild dog diverged from this group several million years ago into the genus *Lycaon* (Chen et al. 2000; Girman et al. 1993; Wozencraft 1989). The species therefore represents a unique line. Chen et al. (2000) recently used mitochondrial cytochrome b DNA sequence to classify foxes, wolves, raccoon dogs, domestic dogs and African wild dogs. The resulting molecular phylogenetic tree (Figure 1.2) suggested that African wild dogs were the earliest divergent. The genus *Canis* then diverged earlier than the raccoon dog and red and blue foxes.

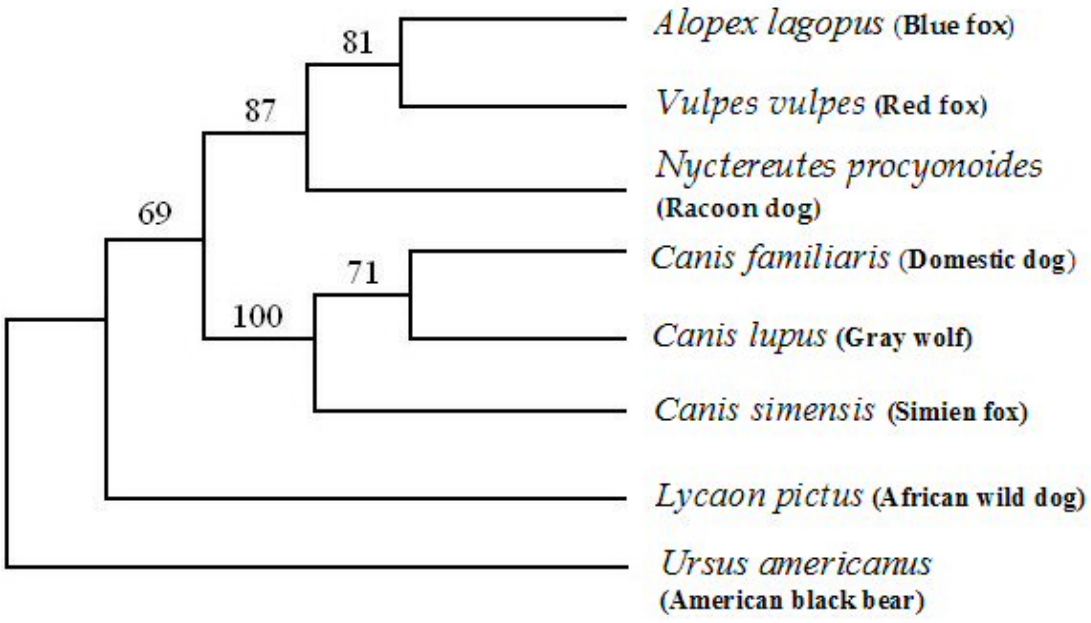


Figure 1.2 Neighbour joining phylogenetic tree of African wild dog and other Canidae species, generated from 372 bp sequence of cytochrome b mtDNA. The American black bear was included as an outgroup. Values at each node show percentage from 1000 bootstraps. Figure from Chen et al. (2000).

The African wild dog has evolved physiological adaptations which suit it to its specialised niche as a highly carnivorous cooperative hunter. In experiments on captive bred wild dogs, *Lycaon* maintained a higher rectal temperature than the domestic dog when running (by 1-2°C), and lost a smaller percentage of heat production through respiratory evaporation (Taylor et al. 1971). This increased

tolerance for high body temperature and low evaporation rate may increase the wild dog's pursuit distance, and thus increase its success as a coursing predator.

Van Valkenburgh and Koepfli (1993) grouped canid species based on dental adaptations towards predation. They grouped four canid species including the African wild dog, in a group of canids which regularly take prey larger than themselves by up to a factor of ten; the other species being the grey wolf (*Canis lupus*), the dhole (*Cuon alpinus*) and the rare bush dog (*Speothus venaticus*). All of these species possess cranial and dental adaptations to their highly predatious diet which include: reduced grinding areas in their dentition, larger canines and incisors, larger second moments of area of the dentitiary relative to its length, broader snouts, wider occiputs and increased mechanical advantage of the temporalis and masseter muscles. In comparison to predators of smaller prey, van Valkenburgh and Koepfli (1993) suggest that the wild dog group's dental adaptations may be the result of selection for increased bite capacity. The reduced post-carnassial grinding surfaces would bring the canines closer to the jaw joint, increasing the mechanical advantage of the jaw closing muscles. Wild dogs, dholes and the bush dog all possess a modification of the first lower molar known as a trenchant heel, which is only partially developed in the grey wolf (Van Valkenburgh & Koepfli 1993). The tooth is modified so that a normally basin-like structure is converted to a blade like cusp. This lengthening of the tooth's blade structure enhances its meat slicing capabilities and would enable the dog to consume meat more quickly. Ecologically, this adaptation would allow the African wild dog to gorge its prey quickly, before the arrival of larger competitive predators, which often attempt to steal kills (Creel & Creel 1996). Within the wild dog species Kieser and Groeneveld (1992) found that females have relatively larger postcanine tooth sizes to cope with the higher masticatory demands of lactation and pregnancy.

1.2 Purpose and Scope of the Study

1.2.1 General Objectives

This research project was aimed at the collection of strategic technical data to assess the status of Lower Zambezi National Park wild dog population, and to deliver practical conservation strategies to conserve the population and those in adjacent areas. The conservation of any species in-situ requires a site-specific assessment, to identify and prioritise the threats to the population. Most species show a degree of adaptation to prevailing environmental conditions, and wild dogs have shown themselves to be highly intelligent carnivores that adjust their behaviour to suit conditions (Fuller & Kat 1990; Kruger et al. 1999; Rasmussen 1996).

There is a lack of data on the Zambian African wild dog populations, from both an ecological point of view and also from a genetic perspective. Therefore this study incorporated ecological research for immediate identification and amelioration of threats, as well as the collection and analysis of genetic samples for longer term conservation measures, such as translocation and reintroduction, which may become necessary in the future.

The most extensive research on the African wild dog has come from large study populations; the Serengeti (Frame et al. 1979), Kruger National Park in South Africa (Kruger et al. 1999; Maddock & Mills 1994; Mills & Gorman 1997; Reich 1981), northern Botswana (McNutt 1996; Woodroffe et al. 1997) and the Selous in Tanzania (Creel & Creel 2002). With the exception of the Serengeti, these areas still contain viable populations of wild dogs and are generally viewed as stronghold populations for the conservation of the species (Woodroffe et al. 2004b). In contrast, the Lower Zambezi wild dog population is small and fragmented. However, because of this structure it is more representative of a large number of the remaining wild dog populations throughout Africa. Small populations are known to be more sensitive to the effects of increased mortality near reserve borders or “edge effects” (Caughley 1994; Ginsberg et al. 1995a; Woodroffe & Ginsberg 1998; Woodroffe et al. 2004b), and are more likely to require active management. Although small population dynamics have been modelled extensively, there is a lack of

empirical data on small population dynamics in wild dogs. For an endangered species where every remaining wild population is of value, it is vital that research and subsequent management of extant populations are carried out before more local extinctions occur.

In addition to its endangered status, the wild dog plays a role as an umbrella species, and is useful for identifying conservation needs for large areas of habitat. Umbrella species are those "species whose conservation confers protection to a large number of naturally co-occurring species" (Roberge & Angelstam 2004), usually through the size of the areas conserved and the amelioration of common threats. The umbrella concept is useful in the conservation of biodiversity where time and resources are limited, and is particularly relevant for the Lower Zambezi area where little ecological research has been carried out. Conserving minimum area requirements for a population of a highly mobile species such as the African wild dog would provide a protected area that benefits a range of species with smaller range requirements. Umbrella species management has also been used as an extended concept, in habitat connectivity (Roberge & Angelstam 2004; van Langevelde et al. 2000), whereby protected area networks are created by determining the area an umbrella species requires to allow for successful dispersal. By conserving the African wild dog and establishing a network of protected areas that allow for its successful dispersal, again it could be assumed that many other species would benefit. There is continued discussion on the effectiveness of the use of single species or taxa as umbrella species (Andelman & Fagan 2000; Caro 2003; Linnell et al. 2000; Roberge & Angelstam 2004; Simberloff 1998), but although further evaluation of the level of biodiversity conserved by managing areas using the African wild dog as an umbrella species may be required, in an underdeveloped country such as Zambia resultant reductions in general threats such as indiscriminate poaching by wire snare, diseases in nearby domestic animals, and habitat loss, could benefit numerous wild species, particularly large mammals. Umbrella species have been used effectively in a multi-species process for determining habitat protection, with each species selected according to sensitivity in various categories (Andelman & Fagan 2000; Lambeck 1997; Poiani et al. 2001; Watson et al. 2001). The African wild dog

alone would be appropriate for two out of four categories according to Lambeck (1997); by being area-limited in that each pack within a population requires a large home range for day to day activities, and dispersal-limited in that both sexes disperse over great distances (sometimes hundreds of kilometers) to establish new packs (Fuller et al. 1992a; McNutt 1996).

The wild dog is particularly sensitive to anthropogenic threats since its nomadic behaviour often takes it to the edge of protected areas where it encounters hazards such as illegal poaching, high speed roads, and direct persecution from livestock farmers (Woodroffe & Ginsberg 1998; Woodroffe et al. 1997; Woodroffe et al. 2004b). The species may not be representative of many other species' level of sensitivity to poaching due to differences in population densities and range movements. However, in aiming to conserve the most sensitive species any conservation plan will err on the side of caution, particularly as snares are arguably not a natural variable to which any populations within a protected area should be subjected.

1.2.2 Scope

The African wild dog Status Survey and Action Plan (Woodroffe et al. 1997) recommended that the first priority for conservation of the African wild dog should be to conserve those populations that remain in the wild, due to the limited success of programs aimed at re-establishing populations (Fanshawe et al. 1991; Scheepers & Venzke 1995). This study was aimed at identifying the threats to the extant Lower Zambezi African wild dog population, both anthropogenic and non-anthropogenic, long and short term. Research was therefore carried out over a broad range of topics, in an effort to provide comprehensive information to maximise the accuracy of assessment and subsequent recommendations.

The scope of the project was divided into five sections:

i) Demography and pack dynamics – aimed at identifying the structure and status of the population, and the main causes of mortality.

ii) Ecology and habitat utilisation – aimed at assessing the impact of ecological factors on the population and its home range movements.

iii) Interpredator competition – aimed at assessing the effects of lions and

spotted hyaenas on the population.

iv) Genetic analysis – to assess the historic and contemporary genetic variability of the population, and to define patterns of geographic structuring and population differentiation.

v) General discussion and implications for management.

1.3 Study Area

Zambia may be able to sustain viable populations of African wild dogs due to its large conservation areas. There are several clumps of adjacent protected areas that measure over 10,000 km^2 each, which has been estimated as the most effective area required to sustain a viable population of large carnivores such as the African wild dog (East 1981). Few countries contain protected areas of this size.

Zambia has three basic types of wildlife conservation areas, National Parks, Game Management Areas (GMA) and Open Areas. The National Parks and GMAs makeup almost 33% of Zambia's total land area (Fanshawe et al. 1991; Jachmann 2000; Scheepers & Venzke 1995). The National Parks are wildlife sanctuaries, under the control of the Zambia Wildlife Authority (ZAWA). Hunting takes place in some areas of the GMAs and in the Open Areas, and human settlement and agriculture are permitted in both. ZAWA's jurisdiction is limited to the wildlife in these areas. GMAs and Open Areas are located around the National Parks, providing a buffer zone against human impacts, depending on the level and type of settlement.

The study area overlapped two wildlife areas, the Lower Zambezi National Park (4092 km^2) and the adjoining Chiawa GMA (2344 km^2) on the south-eastern border of the country (Figure 1.3). Within the context of this thesis the study population is referred to as the Lower Zambezi population.

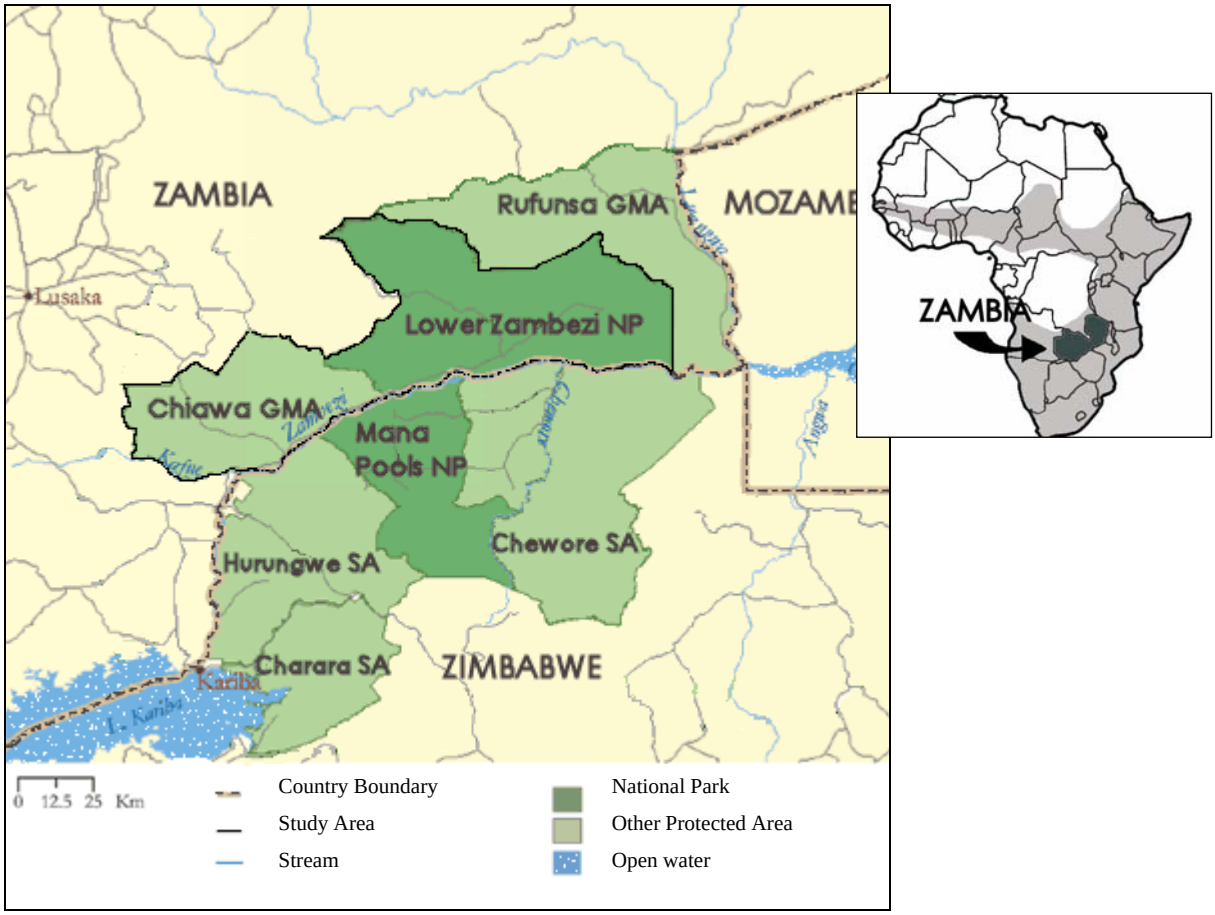


Figure 1.3. Map of general study area (modified from AWF, 2004).

UTM (WGS84) coordinates for the study area were Zone 35S, between 711683.6 and 826054.8 mE, and between 8245105.8 and 814443.1 mN. The borders of the core study area and delineation of the wild dog population's home ranges are defined in detail in Chapter 2. The valley floor between the Zambezi River to the south and the Zambian Escarpment to the north ranges between 1.9km and 19.2km wide, and the game is concentrated in this area. It is plausible to suggest that the ranges of many species are restricted by the river and the escarpment. The Zambezi River ranges from approximately 300m wide at its deepest points, to 1.5km wide in other areas, and has a fast and constant flow. The Zambian Escarpment rises steeply from the valley floor by approximately 600m, then to 900m on the plateau. Anecdotal reports on sightings and spoor frequency from Zambian Wildlife Authority patrol members suggest that game numbers appear to be low in the escarpment. Low prey numbers may reduce the everyday movement of predators, including wild dogs.

Zambia has a mild climate, with three distinct seasons; cool dry from May to August, hot dry from September to November, and warm wet from December to April. The hottest month is usually November (mean maximum temperature 39.6), July is the coldest (mean minimum temperature 10.7) and annual rainfall averages 758mm (Dunham 1994). The elevation of the area ranges between 347 -1200m.

The Lower Zambezi National Park and adjoining GMAs form part of the southern end of the East African Rift system. Soil types in the Lower Zambezi fall into the category of the Central African Rift Geomorphological region, and contain soils derived from basalts from past volcanic activity, plus fertile alluvial soils deposited by the river systems (Jachmann 2000). A preliminary report by Du Toit (1982) describes the basic geology of the area: the mountains and escarpment to the north of the river are formed from the oldest rocks in the region, from the Precambrian Basement; descending into the valley floor, ancient alluvial terraces are made up of post-Cretaceous sediments, while recently deposited alluvium from the Zambezi river appears within approximately 5-10km of the river. The flooding of the river valley has been regulated since the completion of Kariba Dam in 1958, which lies approximately 90km upstream to the southwest of the study site.

The escarpment vegetation is dominated by miombo woodland, containing *Brachystegia*, *Julbernardia* and *Isoberlina* species (Jachmann 2000). Gorges cutting down from the escarpment to the valley floor support richer vegetation due to seepage, and include species of *Ficus*, *Commiphora marlothii*, *Afzelia quanzensis*, *Kirkia acuminata*, *Terminalia sambesiaca* and *Albizia zimmermannii* (Bingham 1998). The alluvial terraces are further broken up by drainage lines and river channels, and support a variety of vegetation, depending on soil type and depth. The ancient alluvial terraces support a variety of mixed riverine woodland, including *Kigelia Africana*, *Philenoptera violacea*, *Trichilia emetica*, *Combretum imberbe* and *Ficus zambesiaca*, while the herbaceuous layer is dominated by annual forbs and grasses (Dunham 1994). The lower

recent alluviums are dominated by *Acacia* woodlands, particularly *Acacia albida* and *A. tortilis*. Dispersed throughout the valley are various other vegetation types: areas of thickets near to the escarpment containing deciduous *Combretum* species; *Colophospermum mopane* scrub and woodland on the sodic soils between the thickets and alluvium (Dunham 1994); small areas of termitaria vegetation on the edges of the floodplains; open plains of short grasses and stands of Hyphaenae palm. Most of the available data for the region is from studies on the Mana pools side of the Zambezi River in Zimbabwe, which has a wider valley floor and some differences in dominant vegetation types. Due to a lack of published data on the study area, a preliminary vegetation survey was carried out in this study to further describe and classify habitats for wild dog and related species (see Chapter 3).

CHAPTER 2: DEMOGRAPHY AND PACK DYNAMICS

2.1 INTRODUCTION

Effective conservation of any species *in-situ* requires information on population demographics and knowledge of relevant life-history attributes. This chapter investigates the status of the Lower Zambezi African wild dog population by assessing survival, breeding and dispersal rates, and population structure. The following chapter (3) addresses range movements and the ecological factors affecting wild dog behaviour and population viability.

2.1.1 Density

African wild dogs are usually found in low density populations due to their wide ranging behaviour. Susceptibility to local extinction from both stochastic events and edge effects may be exacerbated in relatively small populations such as the Lower Zambezi (Caughley 1994; Ginsberg, Mace et al. 1995; Woodroffe and Ginsberg 1998).

Wild dog density is low compared to other sympatric carnivores. The highest estimate of wild dog density to date is 4 adults per 100km^2 in the Selous in Tanzania (Creel and Creel 1995a; Creel and Creel 2002), while more typically densities in wooded areas range between 1.5 and 3.3 adults/100km^2 (Fuller and Kat 1990; Maddock and Mills 1994; Creel and Creel 1996b; Woodroffe, Ginsberg et al. 1997). Densities in the east African open plains area of the Serengeti were recorded at approximately 0.5 to 1.5 adults/100km^2 over two decades (Frame, J.R. Malcolm et al. 1979; Woodroffe, Ginsberg et al. 1997; Creel and Creel 2002). In comparison, spotted hyaenas and lions occur at much higher densities; lion densities across a wide array of study sites ranged from 3.5 -14 adults/100km^2, and spotted hyaenas from 4.5 - 82 adults/ 100km^2 (Mills and Biggs 1993; Creel and Creel 1996b; Woodroffe, Ginsberg et al. 1997).

2.1.2 Pack Structure and Breeding

A “pack” is defined here as any group containing a potential breeding male and female, while “group” is used to refer to a single sex group of dogs. Wild dog pack or group size varies considerably, and may consist of as few as two dogs through to a pack of more than 50 dogs (including pups), which has been observed in Mana Pools

in Zimbabwe (N. Monks personal communication). In the Zimbabwean Zambezi Valley, the Serengeti National Park Tanzania, Selous Game Reserve Tanzania, Hwange National Park Zimbabwe and Kruger National Park South Africa, average adult group sizes ranged between 8 and 11 dogs (Frame, J.R. Malcolm et al. 1979; Childes 1988; Fanshawe, Frame et al. 1991; Mills and Gorman 1997; Creel and Creel 2002). An earlier photographic survey in the Kruger National Park put mean adult pack size at a larger 13.7 dogs (Maddock and Mills 1994).

Wild dog packs generally consist of an unrelated, dominant (or "alpha") breeding pair, subordinate same-sex relatives, and offspring of the breeding pair (Frame, J.R. Malcolm et al. 1979; Girman, M.G.L. Mills et al. 1997). Wild dogs breed seasonally; Reich (1981) found that 90% of births in Kruger fell between May and July and proposed that this corresponded with prey gathering into higher densities around water sources, and that dens were generally located nearby, making for easier hunting and reduced travel distances for wild dogs while breeding. Other studies have since found the same pattern in southern Africa (Maddock 1993?; Maddock and Mills 1994; McNutt 1996a). In the Selous in Tanzania births occured from June to October, also peaking at the driest time of the year (Creel and Creel 2002). In other areas of east Africa wild dogs were found to den in February to June, which coincides with the wet season (Schaller 1972; Frame, J.R. Malcolm et al. 1979; Malcolm 1979). However, this is also linked to an increased density of prey which occurs during mass migrations at that time of year.

Wild dogs dig out dens, often using old hyaena dens with several entrances, or sometimes the side of river valleys in amongst rocks (Creel and Creel 2002; Malcolm unpublished report.). Pups remain in the den for the first three months of life (Malcolm and Marten 1982; Courchamp, Rasmussen et al. 2002). Litter sizes are large. In the Kruger National Park litter size in 1988/89 averaged 11.9 and ranged from 7-16 (Maddock and Mills 1994), but more recently average litter size was estimated at 9.4 pups (±SE 0.7, n=57; (Creel, McNutt et al. 2004). In the Serengeti average litter size was recorded as 10.1 (Frame et al. 1979), and the average over 38 litters in the Selous Game Reserve was 7.5 pups (±SE 0.56; Creel et al. 2004).

Pups are fully weaned at approximately eight weeks of age except in times of food scarcity, when they may nurse into the tenth or eleventh week (Malcolm and Marten 1982). Adults begin to regurgitate meat to the pups from the age of three weeks until they are ready to leave the den (Courchamp, Rasmussen et al. 2002). At nine to eleven months old pups begin killing easy prey, but they are not proficient hunters until around twelve to fourteen months old (Frame, J.R. Malcolm et al. 1979; Reich 1981).

Generally only the dominant pair in a pack will breed whilst non-breeding pack members assist to care for the pups, making the wild dog an obligate cooperative breeder (Frame, J.R. Malcolm et al. 1979; Malcolm and Marten 1982; Stephens and Sutherland 1999). Occasionally subordinate animals will breed. However, the alpha male's hostility to other males and his behaviour of maintaining close proximity to the dominant female suggests that he would usually sire the dominant female's pups (Malcolm and Marten 1982). Genetic data supports this observation; in the Kruger National Park subordinate reproduction occurred at similarly low levels in males (10%) and females (8%) (Girman et al. 1997). In a study in the Selous 76% of litters were produced by alpha females, and 81% of litters in the Kruger National Park (Creel, N.M. Creel et al. 1997c), while only 6-17% of subordinate females gave birth compared to 82% of dominant females. When a subordinate female does breed, often her pups will not survive to one year of age. In Kruger, at ten of twenty five dens more than one female produced pups. However, microsatellite data showed that only 8% of pups 1 year of age were produced by subordinates (Girman et al. 1997). In the Serengeti six litters of subordinate females were observed and only one litter was raised successfully (Frame et al. 1979). The successful litter was born at a different time to that of the alpha female's litter which would have limited resource competition.

Hradecky (1985) suggested that overall suppression of marking and reproductive activity in subordinates influences their endocrine function. In a behavioural study, Hradecky (1985) found subordinate animals contributed little to territorial scent marking, and "double marking" with urine intensified between the alpha male and female in the breeding season. This behavioural pattern has been explained as a means to hide the reproductive status of the female from other males.

Creel et al. (1997c) carried out an endocrine and behavioural study in the Selous and found that during non-mating periods subordinate females had higher oestrogen levels and oestrogen/progestin ratios than dominant females, which apparently prevented ovulation. In mating periods subordinate females' oestrogen levels dropped lower than the alpha females, and the subordinates mated less often and were less aggressive. Subordinate males also mated less often and were less aggressive, and had lower testosterone levels than dominant dogs. Beta males were similar to the alpha males in behaviour and testosterone levels, and are therefore more likely to share paternity. If this is the case then shared paternity would give beta males more incentive to remain with the pack. In contrast to the theory that reproductive suppression is caused by social stress in subordinates, Creel et al. (1997c) found basal corticosterone levels were higher in dominants.

Although the dominant role of the alpha male and female has been primarily observed in breeding, dominance in other roles has also been observed. In the Serengeti only the alpha pair regularly urine marked and they also determined most of the movements of the pack (Frame et al. 1979). Leadership of which dog leads the pack can be variable; often when the alpha female is breeding she will lead the hunts and pack movements (Courchamp and MacDonald 2001); Malcolm unpublished report). Malcolm and Marten (1982) observed that dominant dogs chased predators away from the den more often than subordinates, and the dominant male led the highest number of hunts.

2.1.3 Survivorship

Adult mortality rates in study populations across Africa have been reviewed and ranged between 57% and 20% (Creel and Creel 2002). Causes of mortality across study sites have been classified into natural and anthropogenic causes; where predation, diseases, accidents and death caused by other wild dogs were considered natural causes, and human causes include road kills, snaring, shooting and poisoning (Woodroffe, Ginsberg et al. 1997; Woodroffe, McNutt et al. 2004). The proportion of deaths caused by anthropogenic factors ranged from only 7% in the remote area of northern Botswana (n=15), to 88% in south-western Zimbabwe (n=116), an area fringed by human development and high speed roads (Woodroffe, McNutt et al.

2004). Both adult mortality rates and their causes varied greatly, and were not strongly correlated with wild dog population density (Creel and Creel 2002). For conservation management purposes this suggests that populations need to be assessed on a case by case basis.

Wild dog pup mortality is generally high, but also varies greatly. A recent study of three of the largest populations in Africa showed a range of 65% pup mortality in Kruger, to a low of only 35% in the Selous (Creel, McNutt et al. 2004). The data from Kruger and Botswana were based on approximately 15 years of field study, and the Selous on 6 years. Within populations, pup mortality was recorded to range between 70% to 36.8% in different studies in the Kruger and adjoining Transvaal area (Van Heerdan et al. 1995, n=10 packs; Reich 1981, n=121 pups), and between 76% and 17% in the Serengeti (Frame, J.R. Malcolm et al. 1979; Malcolm and Marten 1982; Burrows, Hofer et al. 1994). Although juvenile survival has been identified as a key demographic variable affecting population persistence in large populations (Creel et al. 2004), it may play a more or less significant role in smaller populations and those where deterministic factors are involved in population decline, hence the need for population specific assessment.

2.1.4 Anthropogenic Causes of Mortality

Anthropogenic factors which affect wild dog populations consist of direct persecution as well as other more indirect factors. Direct persecution has most frequently involved the shooting and poisoning of dogs which enter farming areas, although this is now illegal in many African countries, including Zambia (Buk 1995; Creel and Creel 1998; Woodroffe, McNutt et al. 2004). Although wild dogs are no longer shot within National Parks their unjustified reputation as ruthless killers has remained, resulting in continued persecution of the species outside National Parks (Childes 1988). If the dogs enter farming areas, they are often still eradicated as vermin, and reports of this type of persecution were received during the course of this study. Reports of wild dogs taking goats and commercial livestock do occur, but should be considered the exception rather than the norm (Rasmussen 1999; Creel and Creel 2002; Woodroffe, McNutt et al. 2004).

Indirect anthropogenic factors include road kills and illegal poaching. Wild dogs often adapt to using open areas such as roads to travel and to hunt prey (Fanshawe, Frame et al. 1991), and are thus killed on high speed roads which border protected areas. Illegal poaching by wire snare is generally targeted at antelope species but affects many predator species. For some reason wild dogs seem to be particularly susceptible to snaring and appear to pick up snares more frequently than other predator species (Ginsberg, Mace et al. 1995; Van Heerdan, M.G.L. Mills et al. 1995; Creel and Creel 1998), possibly due to their larger home ranges. Snares have the potential to have a large edge effect on wild dog adult mortality and population persistence, since they are often placed around reserve borders which can be frequently encountered during normal wild dog range movements (Woodroffe and Ginsberg 1999a).

All of these factors combine to have an edge effect on wild dog populations. Wild dog pack home ranges have been recorded as large as 850 – 1500 km^2 (Frame, J.R. Malcolm et al. 1979; Gorman, M.G.L. Mills et al. 1992). Once they leave the protection of National Parks and Game Reserves mortality rates are likely to increase. Woodroffe and Ginsberg (1999a) recorded that more than 60% of adult wild dog mortality recorded in nominally protected populations was directly caused by contact with human activities on or outside reserve borders. Thus wild dogs generally have greater chances of survival in larger reserves (Ginsberg 1994).

2.1.5 The Role of Disease in Mortality

Disease exposure was included for assessment in this study since it is a potentially major cause of wild dog mortality. Diseases which have been isolated from free ranging wild dog populations include rabies, canine distemper, parvo-virus, anthrax, and canine ehrlichosis (Fanshawe, Frame et al. 1991; Gascoyne, M.K. Laurenson et al. 1993; Van Heerdan, M.G.L. Mills et al. 1995; Creel, N.M. Creel et al. 1995a; Woodroffe and Ginsberg 1999a). African wild dogs have also been found to host several protozoal infections, including *Babesia canis*, *Hepatozoon canis*, *Toxoplasma gondii*, Sarcocysts and *Neospora caninum* (Bwangamoi and Richardson 1993; Woodroffe, Ginsberg et al. 1997). Some may cause mortality in pups (*Toxoplasma* and *Neospora*) but it is unlikely that any of these would have a substantial effect on wild dog populations (Pierce, M.K. Laurenson et al. 1995; Van Heerdan, M.G.L.

Mills et al. 1995; Woodroffe, Ginsberg et al. 1997).

Rabies and canine distemper virus (CDV) have both caused local extinctions in wild dog populations, and many other less severe diseases carried by domestic dogs have reduced wild dog numbers (Durchfeld, W. Baumgartner et al. 1990; Fanshawe, Frame et al. 1991; Alexander, P.W Kat et al. 1996; Woodroffe and Ginsberg 1999a). Because of the wild dog's typically low density populations, it is unlikely that wild dogs could sustain such diseases. Evidence suggests that domestic dog populations may serve as a disease reservoir for this species (Alexander, P.A Conrad et al. 1993; Gascoyne, M.K. Laurenson et al. 1993; Ginsberg, Mace et al. 1995; Woodroffe, Ginsberg et al. 1997; Woodroffe and Ginsberg 1999a) and for other carnivores (Van Heerdan 1979; Roelke-Parker M.E., L. Munson et al. 1996). Disease transmission in wild dogs is likely to be rapid for both rabies and CDV, due to the dogs' close social interactions, resulting in high mortality (Fenner, E.P.J. Gibbs et al. 1993; Kat, K.A Alexander et al. 1995; Scheepers and Venzke 1995; Hofmeyer, J. Bingham et al. 2000).

Rabies is widespread in dog populations around the world, particularly in undeveloped countries, in which dogs cause most of the human rabies infections (Fenner, E.P.J. Gibbs et al. 1993). It has been known to devastate populations of endangered wild canids, impacting populations of Ethiopian wolves and Blanford's foxes, as well as wild dogs (Kat, K.A Alexander et al. 1995; Woodroffe, Ginsberg et al. 1997). In the Masai Mara in 1989 twenty of twenty two dogs in a single pack died of rabies (Fanshawe, Frame et al. 1991), and it has been hypothesized that rabies or distemper may have been responsible for the local extinction of the population in the Serengeti-Mara area (Woodroffe and Ginsberg 1999a; Woodroffe 2001). Rabies has also killed wild dogs in the Central African Republic (Woodroffe and Ginsberg 1999a), in Namibia (Scheepers and Venzke 1995), and was believed to be responsible for local declines in Zimbabwe (Kat, K.A Alexander et al. 1995). Wild dog populations have generally tested seronegative for rabies despite neighbouring domestic dog populations with up to 30% seroprevalence (Laurenson, J. Esterhuysen et al. 1997; Creel, N.M. Creel et al. 1997b). This suggests they would be susceptible to infection on contact.

There has been continuing debate about the role of handling (immobilisation, radio-collaring and vaccination) in the disappearance of the Serengeti wild dogs, which was discussed in detail in the IUCN Wild Dog Status Survey and Conservation Action plan (Woodroffe et al. 1997). Burrows et al. (Burrows 1992; Burrows, Hofer et al. 1994) and East (1996) hypothesised that handling and rabies vaccination may have caused high mortality in the population by compromising the dogs' immune systems and reactivating a latent rabies infection. This debate has been continued in more recent years (Burrows 1998; Woodroffe 2001). It is likely that wild dogs carry latent rabies infections (Fekadu, Chandler et al. 1982) rather than suffering high mortality rates following infection, as has been recorded in several populations (Kat, K.A Alexander et al. 1995; Scheepers and Venzke 1995; Woodroffe, Ginsberg et al. 1997). Alexander and Appel (1994) suggested that the disappearance of wild dogs in the Serengeti in 1991 was instead due to a canine distemper outbreak, concurrent with an outbreak in the domestic dog population. The role of canine distemper in the wild dogs' disappearance was debated by Burrows et al. (1995). Overall there has been little evidence that handling causes chronic stress in wild dogs (Creel and MacDonald 1992); in fact there is now substantial evidence to the contrary (Ginsberg, K.A. Alexander et al. 1995; Creel and Monfort 1997a).

CDV has been identified through seroprevalence in wild dog populations and has been associated with confirmed and suspected mortality (Schaller 1972; Van Heerdan, M.G.L. Mills et al. 1995; Alexander, P.W Kat et al. 1996; Woodroffe and Ginsberg 1999a) but also with low pathogenicity in other populations (Creel, N.M. Creel et al. 1997b). Similarly, anthrax has caused deaths in wild dog populations in some areas of Africa (Turnbull, R.H.V Bell et al. 1991), while resistance to the disease has been recorded in other areas (Creel, N.M. Creel et al. 1995a). *Bacillus anthracis* spores can survive in soil and tissues for many years, therefore anthrax is endemic in many areas (Turnbull 1990).

Vaccinations for several diseases have been trialed both in captivity and in free-ranging wild dog populations, and have been the subject of controversy. Wild dog pups have died following vaccination with modified-live canine distemper virus (McCormick 1983; van Heerdan, J. et al. 1989; Durchfeld, W. Baumgartner et al. 1990), and other vaccines have either failed to increase antibody levels or may have

induced immune incompetence and predisposed the animals to disease (Van Heerdan, W.H. Swart et al. 1980; Spencer 1991; Colly and Nesbit 1992). In contrast Spencer and Burroughs (1992) found positive seroconversion from a booster dose of modified-live distemper vaccine with no harmful side effects in seven captive wild dogs. Rabies vaccination in wild dogs to date has also had limited success. Vaccination failure was reported in reintroduction populations in Etosha, Namibia (Scheepers and Venzke 1995) and Madikwe, South Africa (Hofmeyer, J. Bingham et al. 2000). There was recent success with delivery of rabies oral vaccination by bait, but this was limited to a captive pack with artificial social structure (Knobel and Toit 2003). More research is required in captive populations before free-ranging wild dog vaccination programs are implemented. It may be wiser to implement rabies management programs in reservoir populations of domestic dogs and other canids living at higher densities in wild dog areas.

2.1.6 Sex Ratio and Dispersal

There is variation in overall adult sex ratios observed in different wild dog populations under study. Studies in the Kruger National Park region and the Selous Game Reserve in Tanzania found that population sex ratios were not significantly different to parity (Maddock and Mills 1994; Creel and Creel 1995a; Creel, Creel et al. 1998b), while populations in Northern Botswana and the Serengeti in Tanzania were male biased (Frame, J.R. Malcolm et al. 1979; Malcolm and Marten 1982; McNutt 1996a).

A number of explanations have been offered to explain the observed sex bias. Wild dogs disperse in single sex groups, with littermates or siblings (Frame and Frame 1976). In many mammals it is the males that are the dispersing sex, due to intra-sexual competition for mates, and as a possible mechanism for inbreeding avoidance (Tuyttens and MacDonald 2000). However, an early study of the wild dog found that dispersal was actually female biased (Frame and Frame 1976), and subsequent studies have discovered that both sexes disperse (Fuller, M.G.L. Mills et al. 1992; McNutt 1996a; Girman, M.G.L. Mills et al. 1997; Creel, Creel et al. 1998b). If female wild dogs were to emigrate more frequently and therefore suffer higher mortality this would be a feasible explanation for male sex bias in the adult age class, as was hypothesized by Creel et al. (1998b).

In Botswana McNutt (1996a) found that there was a male bias in the adult wild dog population, and that although both sexes dispersed with equal frequency, females dispersed at an earlier age, in smaller groups, and generally established ranges nearby to their natal range. Males had a much greater dispersal distance (n=57 dispersing dogs). This dispersal trend fits the mate competition hypothesis in the case of a male bias in the population, where females would be expected to emigrate more readily, in smaller groups and upon reaching reproductive age (Waser 1996). McNutt (1996a) proposed resource competition may explain dispersal in both sexes, as access to kills is reduced with increasing age for all subordinate dogs. All dogs were found to emigrate in the presence of their opposite sex parent, and inbreeding avoidance may explain why males disperse further. By waiting longer and dispersing with non-litter mates in larger groups the males may counter the associated increased mortality risks of long distance dispersal (McNutt 1996a). McNutt also re-assessed data from Frame et al.'s (1979) study in the Serengeti where an adult male bias was also found, and established that although female dispersal was observed to be more frequent in this population, females did remain close to their natal areas while males disappeared from the study area, and males who did not disperse from their natal packs had lost their probable mother and had access to unrelated females. Therefore the adult sex ratio may well have an effect on sex biases in dispersal by affecting mate competition.

In the Selous Game Reserve in Tanzania where no overall sex bias was detected in the study population, males had higher survivorship after the age of two years old resulting in a sex ratio of 0.55M: 0.45F, although this was only significant after the age of seven years (Creel and Creel 2002). Females were found to disperse significantly more frequently than males (annual probability of dispersal was 0.21 for males, 0.33 for females), the risks of which may explain the lower survivorship in females in this case (Waser 1996). Dispersal was found to have no sex bias in Kruger National Park, where the adult sex ratio was also unbiased (Maddock and Mills 1994; Girman, M.G.L. Mills et al. 1997; Creel, N.M. Creel et al. 1997c).

None of the hypotheses discussed above hold across all wild dog populations. Although emigration may be a source of mortality, particularly given the large dispersal distances and the threat of edge effects to wild dog populations, in the largest study populations displaying a male sex bias the females were found to stay

close to their natal ranges while the males dispersed further (McNutt 1996a, and his re-assessment of Frame et al. 1979). Therefore female emigration patterns do not seem to explain the male bias adult sex ratios, as the females are not removed from the population. Although females were observed to disperse more frequently in the Selous population, no significant male bias was observed in that population (Creel and Creel 1995a) save in age classes over 7 years old (Creel and Creel 2002).

Alternatively, adult male bias may be the cause of female emigration (due to mate competition), rather than the result of failed female emigration. However, there is limited data to support male bias in pups, and only one case in the Serengeti where male bias in pups corresponded to male bias in adults (Malcolm and Marten 1982, n=10 litters). There was no significant male bias in the adult sex ratio in the Kruger National Park or the Selous Game Reserve and no bias in the birth ratio was observed in either population (Maddock and Mills 1994; Creel and Creel 1995a; Creel, N.M. Creel et al. 1997c). Malcolm and Marten (1982) proposed that male bias observed in the pup sex ratio suggested that males contributed more to pup survival, and this has been supported by the observations above where females were found to disperse more frequently and earlier than males, and thus females helped less with communal rearing of pups. However, again, the cases of more frequent female dispersal do not necessarily correspond with the populations which exhibited male bias in sex ratios for pups and/or adults. In fact only the Serengeti population showed more frequent female emigration combined with male sex bias in pups and adults (n=10 litters, Frame et al. 1979; Malcolm and Marten 1982).

Creel et al. (1998b) investigated birth sex ratios in wild dogs and the underlying physiology, based on 18-20 litters in the Selous population. The study found that primiparous females produced litters with a significant male bias, while multiparous females produced more females. A previous study of the same population found increased oestrogen levels in subordinate female wild dogs compared to dominant females, during non-mating periods (Creel, N.M. Creel et al. 1997c). Creel et al. (1998) suggest that increased, albeit slowly decreasing, oestrogen levels in newly dominant females may be associated with male-biased sex ratios in primiparous litters. However, given the low turnover of dominant females in wild dogs (Creel and Creel 2002) one would expect at least an equal, if not higher, proportion of multiparous

litters in a stable wild dog population. Since the proportion of male pups produced by primiparous females was approximately equal to the proportion of female pups produced by multiprimous females (63% vs 64% respectively), this would be more likely to result in an equal or female biased birth ratio rather than a male biased ratio. No overall significant sex bias was observed in the 20 litters monitored in Creel et al.'s (1998) study, although of the 18 litters where the mothers parity was known, 12 were multiparous. Therefore, the information on male bias in birth ratios resulting from primiparous females would fail to explain the overall male bias observed in some wild dog populations, unless these populations had an unusually high turnover of alpha females.

Two main hypotheses have been discussed extensively in the literature to explain secondary sex ratio bias in mammals (sex ratio bias at birth); the Trivers and Willard hypothesis and the local resource competition hypothesis. The Trivers and Willard hypothesis predicts that mothers in good condition produce offspring biased towards the sex that has the highest variation in reproductive fitness, and thus increase the chances of highest future reproductive success (Trivers and Willard 1973; Maynard-Smith 1980). The second hypothesis predicts the opposite, that mothers in poor condition will produce the sex most likely to disperse, based on the principal that these offspring are less likely to compete with the parents for resources (Clark 1978). However, there are often complex interactions between environmental stochasticity and population density, which may result in inconsistent sex ratio trends and affect the fit of any proposed sex ratio models (Kruuk, Clutton-Brock et al. 1999; Post, M.C. Forchhammer et al. 1999; Bradshaw, Harcourt et al. 2003). Thus details on demographic, sociobiological and environmental factors are required for an understanding of apparent sex bias in any population.

2.2 OBJECTIVES

The objective of this section of the study was to determine the present status of the Lower Zambezi wild dog population, its structure and dynamics, and assess any causes of decline and potential threats to the population.

Specifically, the aims were to:

1) Determine population size and density in the study area

2) Determine population fecundity and rates of survivorship in adults and pups, and identify the primary causes of mortality. This included an assessment of natural and anthropogenic causes previously identified in other wild dog populations

3) Assess pack dynamics and dispersal patterns and compare these to previous studies of larger populations

4) Determine the presence or absence of secondary sex bias (sex bias at birth) or adult sex bias in the population, and test for effects of mate selection on dispersal.

2.3 METHODS

2.3.1 General Tracking Methods

Observations of the Lower Zambezi African wild dog population were carried out over a 5 year period, from May 1999 to October 2003. Field work seasons fell between April 1st and 30th November of each year. No ground tracking was carried out during the Zambian wet season (December to March) since the study area was cut off from vehicle access by high river levels and impassable areas of mud. Sporadic data were collected from December through March by occasional aerial tracking and records from staff at safari camps within the study area. An average of 208 days per year was spent in the field, a total of 1040 field days. This included days that were spent on vehicle and camp logistics, and surveying the other related species for the purposes of this study; lion (*Panthera leo*), spotted hyaena (*Crocuta crocuta*), prey counts and vegetation surveys. Wild dogs were located on a total of 388 days. Some days included sightings where more than one pack was observed; the inclusion of this data from all pack observations gave a total of 440 "pack days".

Although largely restricted to the dry season, the field study season included the wild dog breeding and denning period plus 3 to 4 months of nomadic movements either side. The data therefore covered the period with the largest variations in wild dog home range behaviour according to previous studies (Reich 1981; Gorman, M.G.L. Mills et al. 1992; Burrows 1995). No major seasonal migrations are known to occur in the study area, possibly due to biogeographical boundaries (Figure 2.2), therefore wild dog movements during the study period were likely to be representative of pack annual movements.

Due to their large home ranges and nomadic habits the dogs were collared and tracked using radio telemetry. At least one wild dog per pack was fitted with a radio-collar wherever logistically possible. Wild dogs rarely separate from their pack for more than a few hours during hunting (McNutt 1996a; McCreery and Robbins 2001), therefore radio-collaring one dog allowed for accurate tracking of the entire pack. Telemetry was used to aid direct observations; no remote tracking data were collected.

Dogs were individually identified using their unique pelage patterns. Left and right side photographs were taken of each dog using a Digital Hi-8 Video Camera Recorder

(Sony® Australia Ltd, Sydney) and entered into a computer database file. Figure (2.1) below provides an example of the distinct patterning of black, gold and white typical of the species. Each dog was given an identification number, beginning with the pack number for the area (P2), followed by the month and year that the pack was first identified (1099) and the sex and number of the individual dog (F3). The Universal Transverse Mercator (UTM) quarter degree squared coordinate (QDS) was also recorded for the area in which each pack was discovered (1529D1).

ID Code: P21099F3 Left side ID Code: P21099F3 Right side

Figure 2.1 Examples of wild dog identification records and corresponding identification number.

Once dogs were sighted the location coordinates were recorded using a Global Positioning System (GPS); either a Garmin 12XL or later a Garmin Etrex model (Garmin International Inc, Olathe, KS, USA). Coordinates were imported into the Geographical Information System software package ArcGIS 8.1 (1999-2001, ESRI™ Inc., USA) for spatial analysis.

Permission was granted by the Zambia Wildlife Authority to drive off road within the National Park for research purposes. The existing vehicle roads and tracks provided a linear network of 255.4km for tracking. Given a signal range of approximately 3km, this network covered 83% of the valley floor within the study area. Radio telemetry signal range is restricted by vegetation and other line-of-sight barriers and therefore the remaining areas were covered by using high points in the foothills of the escarpment which gave increased signal range. These were accessed by vehicle or on foot. When no signal was obtained via ground telemetry aerial telemetry was used. Once the dogs were located via aerial telemetry the vehicle was driven to within sighting distance, usually 20-100m. Binoculars (8x30) were used for identification and behavioural observations.

The central study area was defined by the home range movements of the monitored packs and biogeographical boundaries. Ground tracking covered approximately 790km^2. Aerial tracking was generally carried out in the escarpment area and limited to linear transects within 10-15km north of the valley floor, increasing the total search area to over 1500km^2.

Figure 2.2 illustrates the core study area. Remote areas of the escarpment outside the study area boundary were accessible by aerial tracking but were rarely surveyed. Records of wild dog GPS locations are clustered in the valley floor area and indicate that the study area gave good coverage of wild dog home range movements. Village settlements begin immediately to the west of the study area boundary; these areas were easily accessible but rarely entered by wild dogs and therefore did not form part of the core study area. The Zambezi River formed the southern boundary of the study site, and the Zambezi Escarpment can be seen on the satellite image as a pale green area occupying a large portion of the northern section of the study area.

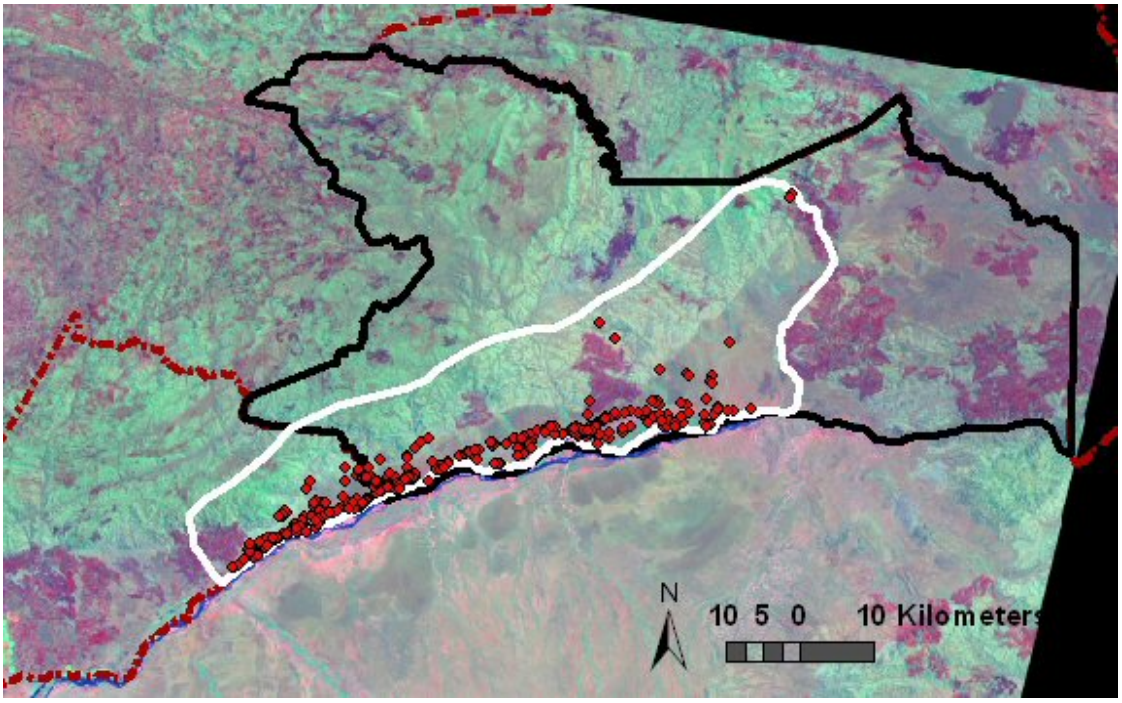


Figure 2.2 Map of protected area boundaries and study area boundaries imposed over a Landsat-7 satellite image of the area (Intec America Corp. USA). The Lower Zambezi National Park is outlined by solid black line, a solid white line borders the core study area. The Chiawa GMA boundary is indicated by a dashed red line to the west of the National Park. GPS locations of wild dog records from 1999 to 2003 are indicated by red dots.

2.3.1.1 Immobilisation

A Zambian-registered veterinarian was called in to assist with immobilisation, as stipulated by the requirements of the Zambia Wildlife Authority. Non-veterinarians holding a recognized qualification in Wildlife Chemical Immobilisation were permitted to dart and immobilize animals to remove snares with the permission of the Lower Zambezi Area Warden. Due to the logistical difficulties and costs of locating and transporting an available veterinarian to the study area, in the case where a wild dog was immobilized for snare removal authorisation was given for a collar to be fitted if the dog was of suitable age and fitness and from an uncollared pack. Male dogs were preferentially darted due to their larger body size and strength.

Each animal was darted using a combination of approximately 6.6mg/kg of ketamine hydrochloride (Ketaject, 100mg/mL, Dopharma B.V., Zalmweg 24, 4941 VX Raamsdonksveer, Netherlands) and1mg/kg xylazine hydrochloride (Rompun, Bayer

Laboratories, RSA), depending on the dog's condition, age and if it had recently eaten (based on belly size). Dosages ranged from 150mg to 200mg ketamine hydrochloride and 30mg to 40mg xylazine hydrochloride per animal.

Dogs were darted in the shoulder muscle mass whilst standing, or preferably resting in a sternally recumbent position if the dogs were not habituated to the vehicle. Standing dogs often heard the dart rifle when it was fired and evaded the dart. The shoulder was chosen over the muscle mass of the hind leg because it was easier to obtain a perpendicular angle for darting in the shoulder when the dog was seated, generally with its hind legs lying to one side. The target muscle mass was roughly the same size in shoulder and upper hind leg. Darting was carried out from a distance of 24m or less, using a plastic 1.5 mL dart and 20mm needle, projected from a Dan-inject 7-JMSPEC-16 dart rifle (Dan-Inject^{TM}, Denmark), with pressure calibrated to distance (to a maximum of 6 bar for 24m).

Anaesthesia induced sternal recumbency was achieved between 3 to 20 minutes after darting. Occasionally a top-up dose was required by dart rifle or hand syringe if the dart dosage had not fully discharged intra-muscularly, or if the initial dose did not allow sufficient time to fit the collar and take biological measurements and samples. The dog was treated with a 2mL dose of long-acting penicillin (Megapen) by intramuscular injection after dart removal. The dog's eyes were covered and cotton wool placed in the ears. Pulse, respiration rate and temperature were monitored regularly throughout the procedure. Where time permitted, standard body measurements were taken including height from longest toe to shoulder; length from nose to tail; girth around the widest part of the rib-cage; and weight.

Two 10mL blood samples were drawn from the saphenous vein, or if blood pressure was depressed by the sedative effects of xylazine hydrochloride, from the jugular vein. 10ml aliquots were stored in vacutubes containing EDTA and Heparin as preservatives, then frozen for storage until analysis. A further 5mL to 10mL of blood was drawn and allowed to stand for 6-12 hours, then the serum was drawn off by syringe and frozen in cryotubes. The blood serum samples were sent to the University of Pretoria, either within 48hrs stored cold, or frozen then transported in liquid nitrogen and stored at -75˚C until analysed for antibodies to a range of pathogens.

Tissue samples were taken as a 2x4mm ear-notch which was halved, and then one sub-sample was frozen while the other sub-sample was preserved in 80% ethanol as a backup. The frozen tissue samples were transported to the University of Pretoria in liquid nitrogen and stored at -75˚C until DNA extraction (see Chapter 4).

The dogs were monitored until fully recovered and reunited with their pack, which occurred between 55 and 190 minutes after darting. No reaction to carrying the collar was observed on any dog, and most were observed hunting normally within a few hours of the procedure. Occasionally other pack members would show interest and chew the collar antenna for the first few days.

2.3.1.2 Disease tests

Disease testing was carried out opportunistically when samples could be obtained from immobilised dogs. Pathogens tested for had been previously identified in wild dog populations (Woodroffe, Ginsberg et al. 1997) and consisted of: canine distemper virus, canine parvovirus, canine adenovirus, canine herpes virus, and canine para-influenza virus. All but canine para-influenza virus are known to have severe effects on either adult or juvenile wild dogs or, where wild dog data is lacking, on domestic dogs (Woodroffe, Ginsberg et al. 1997). Serology tests were carried out using indirect fluorescent antibody tests (Dept of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, RSA). Rabies was not included due to the logistics and expense of testing at the time of the study.

2.3.1.3 Radio-collars and telemetry equipment

Radio-collars were manufactured to design specifications by Sirtrack Ltd (Havelock, New Zealand). The transmitters were micro-controlled, with a base pulse of 50 pulses per minute (ppm), which changed to 30ppm after 15 seconds of inactivity and then returned to 50ppm after subsequent movement. Mortality was indicated by a pulse rate of 15ppm and occurred after 24 hours of inactivity. Two AA lithium cells gave a minimum transmission life of approximately two years. Stainless steel plates with protruding rivets were fitted to the collar as an anti-snare device (see Figure 2.3). Wild dogs typically catch snares around the neck (see Results 2.4) and the purpose of this device was to trap the wire snare in the rivets on the collar where it would either

break during the dog's struggles, or fasten onto the metal plate of the collar between the rivets, thus protecting the dog's neck. The dog would generally break free from the tree or branch the snare was fastened to, and the snare could later be removed. The collar and rivet design was modified from collars originally designed by G. Rasmussen (Painted Dog Research Project, Zimbabwe). Each collar was custom fitted to the appropriate neck size for each animal, fastened with locknuts and any excess trimmed off.

Figure 2.3. Adult male wild dog fitted with an anti-snare radio-collar.

Each collared dog was tracked using a Telonics TR-4 receiver (Telonics Inc., Mesa, A.Z. USA) and a directional hand-held 3 element yagi antenna (model FANT150, Sirtrack Ltd Havelock, New Zealand).

2.3.1.4 Data collection

i) Direct Observation

Tracking generally began half an hour before first light and continued until following became impossible due to terrain or loss of signal. Tracking sessions ran from a minimum of one day to usually several consecutive days. The dogs were followed until they settled for the night, then again at first light the next morning when any change in position during the night was recorded. The use of a spotlight appeared to disturb them, particularly when hunting, so this was kept to a minimum. Moonlight was used for night tracking when conditions were suitable. Numerous other studies have found wild dog activities fell between the times of 5.00 to 9.00 hours and 17.00

to 20.00 hours (Fuller and Kat 1990; Mills and Biggs 1993; Creel and Creel 2002), within the tracking periods of this study.

When a signal was obtained but the dogs were inaccessible their position was estimated by triangulation, using direction and signal strength from two or more points. This method took into account the effects of different densities of vegetation and signal bounce caused by the escarpment, and from experimental trials was found to be accurate to within 100-400m. Error in GPS readings taken prior to May 2000 was approximately 30m due to intentional signal degradation by the US Department of Defence. Later readings taken on the Etrex model GPS were accurate to <15m. For this analysis a consistent error of 30m is assumed. Although triangulation error was greater, the dogs were only inaccessible when within thicket or escarpment vegetation as these were the only physical barriers to access, therefore the increased error would not alter the recorded habitat type for that location. Habitat type was recorded directly from all observations, rather than from mapping.

For each observation the following data were recorded; date, time, location details, GPS coordinates, number of adult males and females present (2 years old or more), number of yearling males and females present (12 to 24 months old), number of male and female pups present (1 year old or less), identification codes, belly score, whether the dogs hunted, whether they killed, prey species and sex, the presence of spotted hyaenas or lions, vegetation type, and details of film footage or photos taken. Additional behavioural notes were also taken. More details on definitions of these behaviours and their analysis is given in the following chapter (3). GPS locations were taken either side of a period of movement as either resting sites or kill sites. If no kill was confirmed and/or the pack was only seen on the move locations were recorded as either hunting or travelling.

Intrapack and interspecific interactions and behaviour were recorded. The alpha pair of each pack was identified by dominance behaviour and the incidence of increased double urine marking during the breeding season (Hradecky 1985), and additionally for females by signs of pregnancy, lactation and parturition. Relationships were confirmed with microsatellite analysis where DNA samples were available. Faecal

samples were collected opportunistically in and around known den areas, or when following a pack.

ii) Survey Forms

Booklets containing photographs of identified wild dogs, pack composition information, and sightings questionnaire forms were compiled at the beginning of each field season and distributed to all the safari operators in the study area. Sightings were collected only from qualified safari guides employed by the camps or Zambian Wildlife Police Officers on patrol, through collection of the forms and also direct reports via VHF radio. Data collected by this method included: vegetation type, pack composition at each sighting, presence or absence of the competitive predators, lion and/or spotted hyaena, prey species and sex, belly scores if known, and any observed activities. Photographic identification kits of recorded wild dogs were distributed to safari guides at the beginning of each field season to increase reliability. Faecal samples were occasionally collected and submitted by guides, as were photographs of new litters or immigrant dogs. Submission rate increased when a reward of a bottle of whiskey was offered in reward for a sample and/or accompanying identification photograph of new dogs.

There were some irregularities in the quantity of wild dog data for the 1999 and 2001 field seasons as no radio-collars were fitted during these years. This was due to a difficult organisational transition period for the Zambia Wildlife Authority which resulted in failure to renew permits for immobilization and radio-collaring. In these years the dogs were tracked by spoor, and via reports from the safari camps. Although field work ceased at the end of 2003, photographic identification records were collected remotely to update population status until July 2005.

2.3.2 Data Analysis

Visual contact was frequent enough to document changes in pack composition through photographic identification of all study animals. Detailed life history records for each dog were obtained and used in demographic data analysis. Frequent annual sightings of all individuals in the study area indicated that data is representative of the true population within the study area.

2.3.2.1 Survival analysis

Survival analysis was carried out on the population based on life history records which provided age at death for analysis. Updates on pack status during 2004 were obtained by remote collection of sightings forms and confirmation photographs submitted by safari guides and were included in this section of analysis. Survival analysis takes into account right-censored values, for example dogs that emigrated and left the study area, and those that survived beyond the term of the study. This method prevents the underestimation of survivorship which may otherwise result from excluding censored values in a small study population. Using the program GenStat 8.1 (2005) Kaplan-Meier estimates of survival curves were plotted using data grouped by sex and area, the latter to test whether survival differed inside or outside of the National Park. Time was specified by age at death, using an entry time of 0 age, and analysis was carried out according to the methods of Kalbfleisch and Prentice (1980). Non-parametric log-rank tests were run to test differences in survivorship for males and females, and to compare between survivorships in different areas; this test ranks all cases equally rather than weighting early events, and gives a test statistic with a χ^2 distribution (Collett 1994). Effects of rank were not analysed here due to small sample sizes.

Annual survivorship was based on each year ending on May 30th to coincide with the annual breeding cycle. Data were grouped by age class and graphed to compare annual survival rates. Yearlings are largely independent of adult care and have been pooled with adults in other studies (Fuller, P.W Kat et al. 1992a; Burrows, Hofer et al. 1994) so they were pooled in this comparison to increase sample size. Differences in average pup and adult survivorship were compared using a Students unpaired t-test.

Pups remained underground at the den until approximately three weeks of age, so pup survivorship was difficult to estimate accurately. Some litters were not counted until after the pups had left the den and moved to an accessible area at approximately two to three months of age. Data therefore represents a minimum estimate of litter size, and may underestimate pup mortality. In the case of the GMA pack litter of 2001, pups were first seen at five months of age. Survival for this litter was conservatively based on a litter size of seven pups, the smallest litter size observed in this study and also in 18 litters studied in Kruger NP (Maddock and Mills 1994).

For classification of survival for animals of unknown fate, animals which disappeared were assumed to be dead under the following criteria:
1. Dominant (alpha) dogs that disappeared, except if pack dissolution or same sex immigration had occurred.
2. Pups less than 8 months of age which disappeared.
3. Dogs of five years of age or older who disappeared alone, with no preceding behavioural indications of conflict or changes in pack hierarchy, and who were not seen after separation from their pack.
4. Dogs last observed with neck snares that disappeared before the snare could be removed.

Other studies have adjusted survival data for undetected emigration by assuming the number of undetected emigrants of a given age, sex, and rank was equal to the number of previously unknown immigrants of the same age, sex and rank (Creel and Creel 2002). However, for a small population with limited samples of different age, sex and rank classes this method was unreliable. Additionally, this method assumes no edge effects are acting on the population, which if present could result in under-estimation of emigrants as few immigrants enter the study population. In this study, observed immigration was substantially less than observed emigration, suggesting edge effects, therefore mortality rates were based on the available descriptive data.

Where unknown, ages were estimated by pelage, overall condition, tooth wear and social rank. With the exception of 5 adults which disappeared in the first year of study, a dog from each unknown age cohort was darted and closely examined at sometime during the study, so age estimates were considered accurate up to 2 years of age and accurate to within 2 years in older dogs. Ages were estimated for a total of 18 out of the 69 dogs. Removal of these individuals would have removed a substantial amount of adult mortality and dogs of alpha rank, so the data were included.

2.3.2.2 Pack dynamics

A pedigree tree was constructed for the population using observational field data for birth, death, rank and dispersal for each individual. Rather than using a traditional pedigree tree program which is limited to sire and offspring information, the tree was

drawn over a timeline incorporating the duration of the study. This format provides details on seasonal survival as well as data on immigration and emigration which did not necessarily result in successful pack formation and breeding.

2.3.2.3 Dispersal and sex ratios

Definitions of emigration in African wild dogs have differed to fit behavioural variations observed across study sites (Frame, J.R. Malcolm et al. 1979; Fuller, P.W Kat et al. 1992a; McNutt 1996a; Creel and Creel 2002). Emigration was defined here as the movement of individuals or groups of same sex individuals out of an established pack. In the case of pack dissolution, only the individuals which moved to a new home range were counted as emigrants (although home ranges often overlapped), as these individuals bear the risks associated with moving to a new area to find new mates. In this study emigration was not defined as dependent on the successful establishment of a new pack because this would have excluded individuals who dispersed and then either died, left the study area, or remained as a single sex group until observations were censored at the end of the study. Emigration was recorded if: i) Same sex siblings separated from the main pack and were subsequently observed as an independent group for 48hrs or more. If the group later returned to the natal pack this was recorded as attempted emigration. ii) Individuals or a group separated from their natal pack before disappearance from the study area. iii) If dogs were aged between 18 months and 4 years old and disappeared along with, or within 7 months of, other same sex siblings. Although these criteria are not comprehensive they covered all observed situations for the study population.

Immigration was recorded where previously unidentified same sex groups appeared in the study area, regardless of whether they formed a successful breeding pack. Pack dissolution here is the same as defined by Reich (1981), the breakdown of an extant pack through combined adult and juvenile age classes permanently splitting into same sex groups.

Sex ratio data were tabulated and graphed according to age class. Annual means and standard errors were calculated for the adult/yearling age classes for comparison with other studies. Annual adult sex ratio data was not independent as adults often contributed data to more than one year, therefore data could not be pooled.

Contingency table analysis was used to test for any significant annual bias. The program GPOWER (Erdfelder, Faul et al. 1996) was used to assess power in analysis. Pup litters were independent samples, therefore data was combined and tested for bias using a non-parametric Wilcoxon matched-pairs signed-ranks test.

Because wild dogs are obligate cooperative breeders, the pack is the important ecological unit, therefore general analysis was conducted at the pack level rather than the individual level to avoid pseudo-replication.

2.4 RESULTS

2.4.1 Demography

The study population consisted of 69 African wild dogs over the duration of the study, 47 of which obtained yearling or adult status. Data were collected for 12 pack years. Mean annual density of adults in the study area was 1.7 dogs/100km^2 (±SE 0.1) ranging from a maximum of 2.2 dogs/100km^2 to a minimum of 1.5 dogs/100km^2 in any one year.

Data collected during immobilisation showed slight sexual dimorphism in the Lower Zambezi wild dogs, with mean weights of 27.42kg (±SE=1.29, n=7) in males and 23.0kg (±SE=1.52, n=3) in females.

2.4.1.1 Survival analysis

Figure 2.4a and 2.4b below show Kaplan-Meier survivorship function estimates for the Lower Zambezi African wild dog population between October 1998 and May 2004. Decline in survivorship was steepest during the first year, the survivorship function stabilised from yearling age through to almost 4 years old, then steadily declined over time. No dogs over the age of 8.5 years were observed. The probability of survival to adulthood (2 years) in the population was approximately 60%.

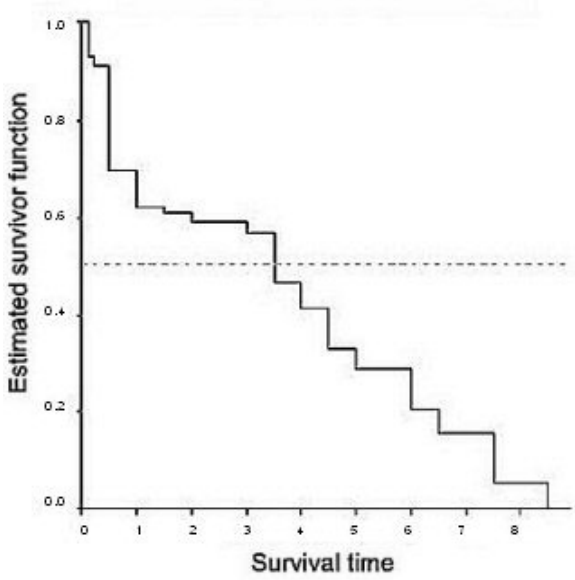


Figure 2.4a. Kaplan-Meier estimate of the survivor function for the Lower Zambezi population. Survival time is in years.

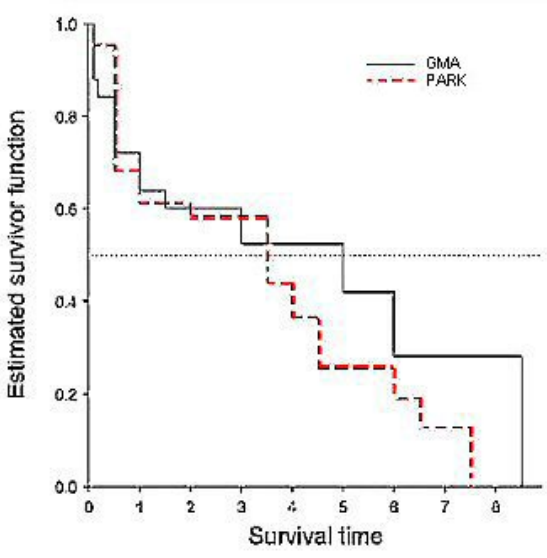


Figure 2.4b. Kaplan-Meier estimate of survival by area grouping. Survival time is in years.

There was no significant difference in survivorship between males and females (Log-rank, χ^2 =0.196, d.f.=1, P=0.658). Survivorship within the National Park was not significantly different from that in the GMA (Log-rank, χ^2 =0.574, d.f.=1, P=0.449). However, as shown in Figure 2.4b, the survivorship curve is steeper for the National Park after the age of about three years. Under the Kaplan-Meier model, dogs had a 50% probability of survival until the age of 3.5 years in the National Park, compared to a 50% probability of survival until 5 years in the GMA.

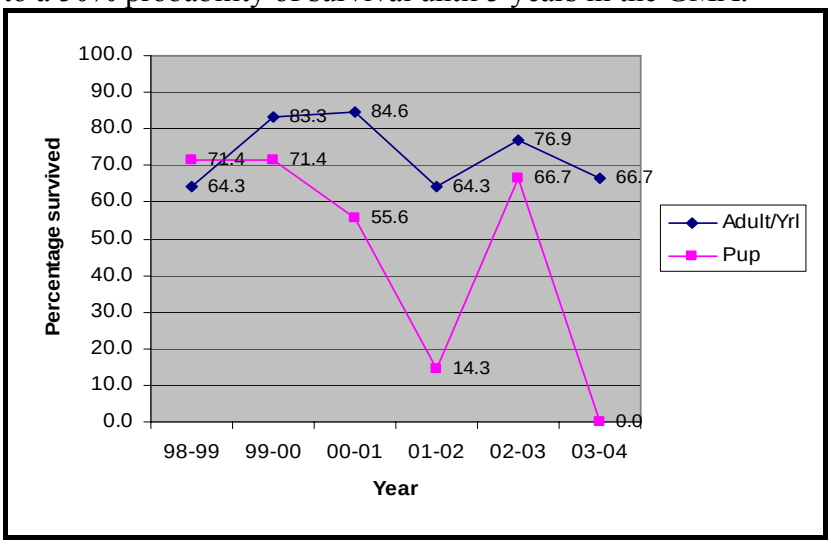


Figure 2.5 Annual survivorship by age class. N ranged from 12 to 18 adults/year, and 5 to 18 pups/year.

Figure 2.5 shows annual survivorship of pup and adult age classes across the study population. Mean survivorship of adults was high compared to pup survivorship, at 73.4% (±SE 3.9) and 47.0% (±SE 12.6) respectively. The difference was not statistically significant (Students t-test; t=2.0, df=10, p=0.073) Pup survivorship was highly variable, ranging from 0.0% to 71.4%.

2.4.1.2 Causes of mortality

Confirmation of the causes of mortality was restricted to cases where death was actually observed or the carcass was found before consumption by other predators. Given the dogs' nomadic movement patterns, only carcasses of collared dogs could be located soon after death. In this study the cause of death was confirmed for only 5 wild dogs. However, there was often a good indication of the cause of death from circumstances observed during sightings just prior to death or disappearance. Table 2.1 contains a summary of data of confirmed and suspected causes of mortality in

adults and yearlings. Mortality data was omitted for dogs that did not fall into any of the following categories:

1) Predation was suspected if disappearance occurred during a period when the pack was observed to be harassed by lions or spotted hyaenas and/or the dog suffered injuries characteristic of an encounter with predators. In two cases dogs with injured legs were killed and consumed by predators; these were included here since predators were the final cause of death, although the cause of original injury was unknown.

2) Natural causes were suspected where the dog had been observed to be deteriorating in condition prior to disappearance, with no obvious causes (included dogs over 8 years old). 3) Snaring was suspected as a cause of death when dogs who were not potential emigrants disappeared between December and March and surviving pack members were found carrying snares at beginning of the dry season in April. Due to the number of surviving dogs observed carrying snares during the dry season (see below, Section 2.4.1.2.i.) this was considered a reasonable estimate of snaring mortality.

4) Unknown cause of death was recorded whenever a carcass was found with no obvious cause of death, and for any animals that disappeared but did not fit any criteria above. No signs of infectious disease was observed in any age group during the course of the study therefore disease was not included in the causes of death.

Table 2.1. Number of suspected and confirmed causes of mortality in adults and yearlings.

Cause of death	Suspected	Confirmed	Total
Natural	3	2	5
Snare	13	1	14
Predator	4	1	5
Unknown	1	1	2

As shown in Table 2.1 above, snaring accounted for 54% of adult mortality, predators for 19%, and unknown and natural causes combined accounted for 27%.

i) Observed Snaring.

Adult mortality from poaching was reduced by this project which was part of an ongoing conservation program. In addition to the disappearances attributed to snaring in Table 2.1 above, neck snares were observed on 29% (n=14 dogs) of the adult wild dog population, and almost certainly would have had a substantial effect on

population persistence if not removed. Four snares were found on alpha dogs, the death of whom can result in pack dissolution due to inbreeding avoidance (McNutt 1996a; Creel and Creel 2002). Two snares were removed from a two year old female, one of a group consisting of only two sisters, who later became alpha female of the GMA pack. This female produced three litters, one of which had a survival rate of 72% (8 of 11 pups) despite having only four adults in the pack to raise them. It is conservatively estimated that at least nine dogs would have eventually died from severe snare injuries if they had not been treated. This data alone suggests that snaring was the major cause of adult mortality in the adult-yearling population.

There was no difference between the occurrences of snares observed on dogs from packs based inside the National Park compared to those based in the GMA, 64% of snares were observed inside the National Park. The eastern Chiawa GMA is reserved for photographic safari activities and is under the protection of the Zambia Wildlife Authority, therefore all pack ranges fell inside protected areas.

ii) Predators

Adult mortality from predators was rare, however there is strong circumstantial evidence of competing predator interference at some dens. Signs of large predators (spoor and droppings) were observed within the immediate vicinity (<30m) at 3 out of 7 den sites. Packs often moved considerable distances between dens while pups were young. In 2000 the Mushika pack shifted dens when the pups were 6 weeks old, to an initial distance of 11.6 km over two days, then another 16 km over the following week. Despite the long-distance moves, five out of nine pups survived to yearling age. In 2003 the GMA pack shifted dens continuously; with pups at approximately 4 weeks of age the pack moved 4.5 km, then averaged 2.4 km every two days as they moved east along the escarpment ridge for another 15 days, to a total of 22.5 km from the original den site. Dens were accessible only on foot or by aerial tracking since they were located in the escarpment, and the continuous shifting made access difficult, nevertheless spotted hyaenas were encountered at two separate den sites for this pack. By the age of 3 months only three of the eight pups had survived, and none survived to yearling age. Two yearling males who were left to baby-sit at these dens on separate occasions also died during the same time period, and the carcass of one of these dogs was observed being eaten by a spotted hyaena. These events also coincided

with an increase in observed kleptoparasitism by spotted hyaenas in the same year (2003). The impact of competing predators on wild dog population dynamics is discussed further in Chapter 4.

iii) Infectious Disease

Table 2.2 Titre results for indirect fluorescent antibody tests for a range of canine pathogens.

Animal ID	Pack	Canine distemper virus	Canine parvovirus	Canine adenovirus	Canine herpes virus	Canine parainfluenza virus
P21099F6	Mushika	negative	1:10	1:40	positive	1:40
P21099M3	Mushika	1:10	1:20	1:40	positive	1:20
P10498F3	Jeki & GMA	1:40	1:10	1:40	positive	1:40
P30602M3	Simwenzenze	1:20	negative	1:20	positive	1:20
P41201M4	GMA	negative	negative	1:40	positive	1:20

Results from indirect fluorescent antibody tests (Table 2.2) suggest previous population exposure to all pathogens tested for, but low titre levels (1:10 to 1:40) imply infections were not recent prior to sampling. The test for canine herpesvirus was only run at a single serum dilution so no conclusions can be drawn about the level of infection or timeframe involved.

Individual P41201M4 was a yearling when tested; all the other individuals were adults. All packs had previous exposure to canine distemper virus, including a male immigrant to the area (P30602M3). No symptomatic evidence of current infectious disease was observed in either pups or adults in the Lower Zambezi study population.

2.4.1.3 Breeding

Breeding was seasonal, with all litters born between 1st May and the first week of September. The latest litter was that of a subordinate female who became dominant immediately after the death of the alpha female, who had been pregnant. Excluding this late litter all breeding fell between May and July. Mean litter size for the population was 8.0 (±SE 0.58, n=7) and ranged from 7 to 11 pups.

Table 2.3 Litter size, survival to one year of age, and associated number of adults and combined adults-yearlings in the pack.

Litter size	Proportion survived	Adults	Adults & Yearlings
7	0.7	5	5
11	0.7	4	6
8	0.0	4	10
7	0.7	8	9
9	0.6	6	11
7	0.6	8	8
7	0.1	3	3

Table 2.3 summarises the data on litter size, survival rates and the number of adult and yearling carers in the pack. There was a moderate positive correlation between the number of adults and pup survivorship to yearling age (r= 0.59, df=6, p=0.16) although it was not significant. There was no association between the number of combined adults and yearlings against pup survivorship (r= 0.067, df=6, p=0.87). There was no strong association between litter size and the number of adults or litter size and the number of adults and yearlings (r=-0.33, df=6, p=0.47; and r=0.12, df=6, p=0.79 respectively).

Only one subordinate female was observed to breed during the course of the study. This female reached parturition two weeks after the alpha female and denned less than 50m away. The fate of her litter is unknown but all pups had disappeared by 3 weeks of age, when she returned to the alpha female's den to help care for the pups with the rest of the pack.

2.4.2 Pack Dynamics

Mean annual adult pack size was 7.2 (±SE 1.7, range 3 to12, n=12 pack years). Appendix 1 contains a figure showing the life history of each individual and pack dynamics for the duration of the study period. Two matriarchal lineages over four packs were followed throughout the study. In this study population, females showed higher philopatry; in two of two cases of new pack formation (GMA pack and Simwenzenze pack, Appendix 1) females remained in their natal home range and males immigrated into the area.

The first pack observed was the Jeki pack, which originally consisted of 7 adults and 5 new pups. During the first year of study, this pack was dramatically reduced to 5 orphaned pups, at the age of 10 months old. The Jeki pack was not collared in 1999 so there is limited hunting data available. It is possible the pups survived on small prey items, although they were observed successfully killing impala at regular intervals from the age of twelve months. The Jeki females survived to found the GMA pack in 2001, joined by males from the Mushika pack. The Jeki males emigrated from the study area in two separate groups. When the first alpha female of the GMA pack died her only surviving sister became the new alpha.

The second matriarchal line originated in the Mushika pack. Mushika pack dissolution occurred after the disappearance of the alpha male, whereby the remaining Mushika males joined the Jeki female siblings to form the GMA pack. The Mushika females were joined by new immigrant males to form the Simwenzenze pack. When the Simwenzenze alpha female died one of her daughters became the new alpha. No inbreeding was observed in either pack.

Although field work ceased at the end of 2003, photographic sightings records were collected from the safari operators until the end of July 2005. These data indicate that the three females from the GMA pack emigrated from the study area. The remaining GMA pack in 2004 raised only 1 pup to yearling age from a minimum litter size of 7 (first observed after they left the den), and failed to breed in 2005. By the end of data collection this pack consisted of; the alpha female, 3 adult males, one of which became the new alpha and bred successfully in 2004, and one yearling male.

2.4.2.1 Dispersal

Pack home ranges overlapped extensively in the Lower Zambezi population (see Chapter 3, section 3.4.2.1), which made the extent of movement away from the natal home range by emigrants difficult to quantify. Where pack dissolution occurred, the emigrating sex was defined as the sex that moved away from the core areas occupied by the natal pack that year.

Seven dispersal events were observed, summarised in Table 2.4. Four single sex groups and two individual males were observed to emigrate. Two of the emigrant

groups included dogs of more than one age cohort (Mushika 2001, 3 males; and Mushika 2001, 4 females). One group of males immigrated into the area in 2002.

Table 2.4 Summary of dispersal events and group composition.

Year	Natal Pack	No./Sex	Ages (yr)
2000	Jeki	2 M	1.4
2001	Jeki	1 M	2.5
2001	Mushika	1 M	5
2001	Mushika	3 M	6,2.5,1.4
2001	Mushika	4 F	2,2,2,2.2
2002	Simwenzenze	4 M	3.5
2004	GMA	3 F	2.1,2.1,3.1

Two of the recorded dispersal events resulted in successful pack formation. After Mushika pack dissolution occurred, 3 males consisting of an adult (6 years), a two year old and a yearling male emigrated and formed the GMA pack with the Jeki females. Four immigrant males joined the Mushika females within 6 months of pack dissolution to form the Simwenzenze pack, and were the only immigrants observed in the population. A single subordinate male emigrated from the Mushika pack and joined the Jeki females, nine months prior to Mushika pack dissolution. The male was observed with a badly broken leg and died weeks later. The remaining four dispersal events involved emigrants who left the study area and were not seen again, including the two female groups.

2.4.2.2 Sex ratios

Figures 2.6a and 2.6b summarise the results for age sex ratios in different age classes. There was a consistent trend of female sex bias in all age classes. Average sex ratios for different age classes are shown in Figure 2.6a (n=6 years data). Since individuals generally contributed data points for several years of data, samples were not independent and means were not compared statistically. Although there was a higher mean proportion of females in adult, yearling and pup age classes (1.3F:M, 2.3F:M, 2.4F:M respectively), 95% confidence intervals suggest there was no significant difference between mean values.

Previous studies have pooled yearlings into the adult age class to investigate population sex ratios (Frame, J.R. Malcolm et al. 1979; Maddock and Mills 1994; McNutt 1996a; Girman, M.G.L. Mills et al. 1997) therefore yearlings and adults were

also pooled here for analysis. For a total of 47 dogs which were observed to reach 1 year of age or more, 44% were male. Figure 2.6b shows a consistent female bias in adults throughout the study, with the exception of one year where there was no bias (2002). Contingency table analysis showed no significant difference between expected and observed sex ratios (χ^2=2.47; df=5, p=0.78).

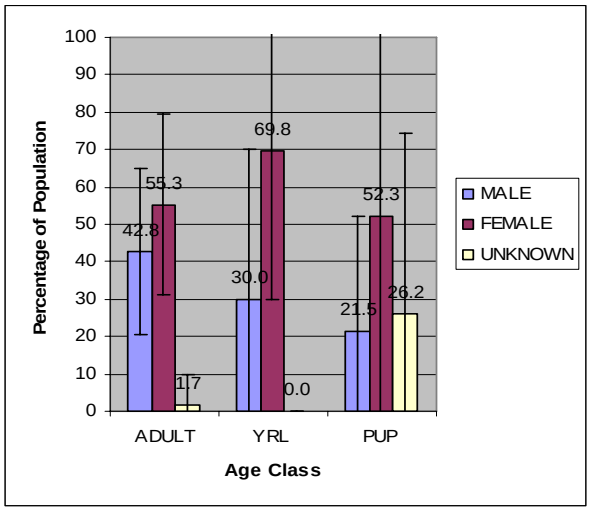


Figure 2.6a Mean sex ratio of age classes over six years. (error bars =95% ranged confidence interval)

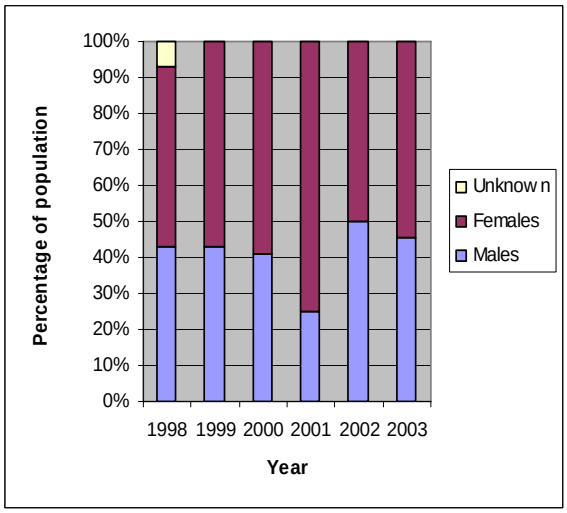


Figure 2.6b Annual sex ratio of combined adults and yearlings (N from 14 to 22).

For five of seven litters the mothers' parity was known, three of which were primiparous. Due to limited accessibility and visibility at den sites, which were inevitably located in thick vegetation in the foothills of the mountains, each litter contained some pups which died/disappeared before they could be sexed. Assuming equal probabilities of these unsexed pups being male or female, four litters of five litters contained more female pups, and the remaining multiparous litter contained equal numbers of male and female pups (see Appendix 1). There was no significant difference in sex ratios over seven litters (Wilcoxon matched-pairs signed-ranks test; p= 0.0625, W+ = 1.50, W- = 19.50, n = 6). However, out of 56 pups, 20 were of unknown sex, 23 were female and 13 were male. Power analysis showed that in order to detect a small effect in sex ratios using a chi-squared contingency table at the 95% confidence level, for six years of data (df=5), 1,979 samples would be required compared to the 96 dog-years used here. A sample of this size would only be obtainable from a very large population and this highlights the problems associated with obtaining reliable estimates of sex ratio bias for a low density predator.

2.5 DISCUSSION

The density for the Lower Zambezi wild dog population (1.7 dogs/100km^2) falls within the lower estimates of wild dog densities observed in other study populations, which ranged from 1.5 to 4 dogs/100km^2 (Fuller and Kat 1990; Maddock and Mills 1994; Creel and Creel 1996b; Woodroffe, Ginsberg et al. 1997). Given the small size of the Lower Zambezi population, density alone should not be used to indicate the robustness of the population. The maximum number of adults recorded in any one year was 18 (31 dogs including pups). At less than 50 adults, the Lower Zambezi population is representative of the majority of extant populations in Africa (Woodroffe, Ginsberg et al. 1997), and particularly susceptible to extinction through environmental stochasticity and genetic factors due to its small size (Gilpin and Soulé 1986).

2.5.1 Survival Analysis

Adult survivorship has been shown to be an important determinant of population persistence, in all populations, but particularly small populations; using the program VORTEX to model populations, Woodroffe et al. (1997) predicted the probability of extinction of various population sizes using demographic data from a wide range of previously studied wild dog populations. More recent studies implicated that pup mortality was the most important factor in determining population persistence (Cross and Beissinger 2001; Creel, McNutt et al. 2004). In a small population any increases in either adult or juvenile mortality will have a detrimental effect on population persistence, since every individual is of value. Adult mortality may reduce pack sizes below the optimum number required to successfully raise a litter, thus affecting juvenile survivorship (Courchamp and MacDonald 2001) and in turn maintaining small pack and population sizes.

Both adult and pup survival rates in the Lower Zambezi population were comparable to rates found in other larger populations. Pup survivorship was highly variable, partly due to small sample sizes; however it has been found to be similarly variable both between and within other wild dog study populations (Reich 1981; Malcolm and Marten 1982; Fuller, P.W Kat et al. 1992a; Van Heerdan, M.G.L. Mills et al. 1995; Creel and Creel 2002). Annual survival rates in the large populations in Kruger National Park in the Republic of South Africa and in northern Botswana were 0.35

and 0.48 respectively (Creel, McNutt et al. 2004), comparable to the mean annual pup survival rate in the Lower Zambezi (0.47). It is often difficult to identify the causes of pup mortality since pups are not collared and tend to disappear from the population. Data collected from three large wild dog populations in Tanzania, South Africa and Zimbabwe found 83% of pups deaths were attributed to natural causes of predation and disease, predation being the most common cause (Woodroffe, McNutt et al. 2004). Regardless of overall survival rates, in a small population with limited ability to recover from demographic stochasticity, pup survivorship declining as low as 14% and 0% (Figure 2.5) could have important implications for population persistence.

The survivorship curves for the Lower Zambezi population stabilised from the age of one year to approximatley 3.5yrs. Yearling survivorship was observed to be stable in other populations, ranging from 64% to 92% over 4 populations (Creel and Creel 2002; Creel, McNutt et al. 2004). This study may provide the first record of orphaned wild dog pups surviving in the wild alone. Based on previous studies, the five 10 month old Jeki orphans should not have been expected to survive without adult support. Age estimates here were conservative because data was limited during the wet season when the pack adults disappeared; the pups may have been orphaned as early as the age of 8 months. Wild dogs are generally still learning to hunt and have priority access to kills made by adults until the age of 10 to 12 months, and then play an increasing role in hunts as yearlings (Malcolm and Marten 1982; McNutt 1996a). Even yearling contributions to hunting appear variable, and they have been generally classified as dependents based on their effects on hunting efficiency, although not on hunting success (Creel and Creel 1995a). However, dispersal events have been recorded from the age of 13 months (McNutt 1996a), which suggests hunting skills are sufficiently developed for survival by this age. As mentioned in results, the orphans may have partially survived off smaller prey, which is likely to be consumed quickly and therefore less likely to be observed.

The probability of survival to adulthood (2 years) in this population was relatively high at 60%. A recent study compared survivorship between three of the largest extant wild dog populations in Africa; the Selous Game Reserve in Tanzania, Kruger National Park, and the study population in northern Botswana (Creel, McNutt et al. 2004). In Kruger and Botswana only 16% and 35% (respectively) of dogs reached

adulthood. Lower Zambezi results are comparable to those of the Selous, which had a much higher rate of survival to adulthood of 63%. At between 64% and 84%, Lower Zambezi annual adult mortality rates were within the range found in other study populations (reviewed in Creel and Creel 2002).

Although there was no significant difference in survival rates between protected areas, comparison of the curves indicates lower survivorship inside the National Park. Although the Chiawa GMA is closer to human settlement than the National Park, the Park is likely to have a higher density of competing predators which may affect wild dog movements and interact with mortality rates. This is further addressed in the following Chapter (3).

Large populations have an increased ability to recover from perturbations, but this is compromised in small populations (Woodroffe, Ginsberg et al. 1997). Identifying key causes of mortality is therefore a management priority, particularly the presence of deterministic threats that can drive a population to decline regardless of vital rates.

2.5.2 Causes of Mortality

Snaring was confirmed or suspected in 54% of mortality (Table 2.1). Although evidence for the impact of snaring on adult and yearling mortality was circumstantial, 50% of adult mortality occurred during the wet season, a four month period of the year (December to March). This is a period where poaching by snares is generally acknowledged by local safari operators and residents to increase; due to weather conditions restricting accessibility and the activities of anti-poaching patrols, including aerial patrols. The impact of snaring on adult mortality was reduced by this study, which was part of an active conservation effort to protect and preserve the local population of wild dogs. The data on the number of snares removed from badly injured dogs confirms that snaring was a major threat to population persistence.

The effect of predators on adult mortality was minor, but data suggests they played an important role in pup survivorship in the last year of the field study (2003) when an entire litter of pups was lost. Behavioural evidence implies that harassment at den sites caused large den shifts in two breeding years. Other studies have found average den moves to be a few hundred meters (Reich 1981), to a maximum distance of 1.2km

in the Selous (Creel and Creel 2002), compared to moves of over 20km observed in this study. Predation is a natural part of pup mortality and not necessarily an avoidable threat to be managed, but nevertheless as a stochastic factor it played an important part in this population's decline between 2003 and 2005.

No outbreaks of infectious disease were observed in the study population. From seroprevalence results it appears that all packs had prior exposure to nearly all diseases tested for (only canine parvovirus was absent from the Simwenzenze pack). Whether or not these diseases impacted on population mortality in the past is unknown, but surviving individuals are likely have some resistance to further outbreaks. Canine parvovirus and canine herpes virus are known to affect pup mortality (Woodroffe, Ginsberg et al. 1997), and may have played an undetected role in pup survivorship in this study, particularly since pups could not be observed or counted for several weeks after birth.

Disease has reduced wild dog populations in other areas (Durchfeld, W. Baumgartner et al. 1990; Fanshawe, Frame et al. 1991; Alexander, P.W Kat et al. 1996; Woodroffe and Ginsberg 1999a) and the presence of domestic dogs at the western edge of the study area indicates that there may be a potential threat to this population, since domestic dogs are likely disease reservoirs (Alexander, P.A Conrad et al. 1993; Gascoyne, M.K. Laurenson et al. 1993; Ginsberg, Mace et al. 1995; Woodroffe, Ginsberg et al. 1997; Woodroffe and Ginsberg 1999a). Future studies in the area should certainly continue to monitor this potential threat.

2.5.3 Breeding

The breeding season observed in the Lower Zambezi coincides with that observed in southern Africa where breeding corresponded with the dry season and increased prey density, particularly around water sources (Reich 1981; Maddock and Mills 1994; McNutt 1996a). The Zambian wet season falls between November and March, and prey densities begin to concentrate during winter. Although the Zambezi river is a constant water source for the area, prey has been observed to disperse during the rains and increase in concentration on the alluvial terraces of the valley floor during winter (Dunham 1994). Impala drop their lambs in November each year following the main rutting season in May {(Estes 1991); personal observation} which provides easy

hunting for the dogs. This would be an additional benefit for the dogs that have increased feeding demands from larger, but still dependent pups at that time of year.

There were no significant correlations between pack size and litter size or pup survivorship. This may be partly a result of small sample size leading to a lack of power to detect a significant result. The results contrast with other studies of larger populations where litter size and pup survivorship have been correlated positively with pack size, based on the number of yearling and adults (Courchamp and MacDonald 2001; Creel and Creel 2002; Creel, McNutt et al. 2004). The benefits of increased pack size on pup survivorship derive from increased hunting efficiency as well as increased vigilance. Larger packs kill more frequently, kill larger prey and have more success defending kills from interpredator competition (Fanshawe and Fitzgibbon 1993; Fuller and Kat 1993; Creel and Creel 1995a; Creel, McNutt et al. 2004). This increased hunting success is likely to influence litter size (Creel, McNutt et al. 2004) as well as the ability to raise pups to independence.

The limited results available here indicated that yearling contributions to pup survivorship in this population were negligible. When yearlings were included with adults in pack size, sample size increased, but correlations weakened further. In fact one of the two packs with the largest annual number of yearlings and adults (maximum of 10) failed to raise any pups to yearling age, and were also observed to suffer from increased kleptoparasitism by spotted hyaenas. Only four adults were present in this pack year, a ratio of 0.5 adults:yearlings. In the second pack of 11 which raised five of nine pups successfully, there was a higher ratio of 1.4 adults to yearlings. Thus the number of more experienced dogs may have played a more important role in breeding success than overall pack size in this population.

2.5.4 Pack Dynamics and Dispersal

With an average adult pack size of 7.2 the Lower Zambezi pack estimates were slightly lower than pack sizes in most studies. Average adult (>1yr old) pack sizes ranged between 8 and 11 dogs for study sites in the Zimbabwean Zambezi Valley, the Serengeti National Park, Hwange National Park, Kruger National Park, and the Selous Game Reserve (Frame, J.R. Malcolm et al. 1979; Childes 1988; Fanshawe, Frame et al. 1991; Mills and Gorman 1997; Creel, McNutt et al. 2004), but ranged between 2 to

50. Constant annual adult mortality from snaring in this population is likely to have limited pack size and population size.

Unsuccessful dispersal appears to be the other most important factor in limiting population size. Woodroffe et al. (1997) discussed an unpublished report which suggested that inbreeding avoidance (rather than inbreeding depression) may halt breeding in small populations. However, Woodroffe et al. (1997) concluded that this had not been demonstrated and that relatives had been observed to breed in captivity. Data here does in fact provide evidence that inbreeding avoidance reduced fecundity in this population. In all four cases involving emigrants of known natal origin, the dogs left the study area when no unrelated opposite sex dogs were available, and were not seen again. This included female groups, which in two other studies with larger home range sizes dispersed mean distances of 19km and 27km (McNutt, 1996a and Creel and Creel 2002 respectively). If females in this study had remained in home ranges adjacent to, or overlapping, their natal home range it is highly likely they would have been detected. In the two cases of successful pack formation observed here, when unrelated mates were available, the females were philopatric and did not leave their natal home ranges.

The loss of adults through dispersal contributed to maintaining the population at low density, and thus to increased difficulty in finding a mate. This suggests the presence of an inverse density dependence and Allee effect in the population (Courchamp, Clutton-Brock et al. 1999). The Allee mechanism occurs when individual fitness is related to numbers of conspecifics in a positive manner (Stephens and Sutherland 1999). Boukal and Berec (2002) gave a definition of a "positive relationship between any measurable component of individual fitness and population size, quantified by the number or density of conspecifics". The most cited cases of Allee effects in many species are based on the limited probability of finding mates in small populations (Boukal and Berec 2002). Courchamp et al (2000) identified three processes through which the Allee effect can lead to extinction in wild dogs. The first is an increased probability of pack extinction through small pack sizes, which in turn increases the probability of population extinction. The second is the effect on dispersal success. Smaller populations suffering Allee effects generally have smaller packs, and therefore smaller dispersing groups which would have less probability of successful

pack formation due to increased mortality risk, and create smaller founder packs which have less chance of success. Therefore, lack of successful dispersal and colonization further reduces the population and increases extinction risk. Larger populations with direct density dependence, in contrast, have better success rates from dispersing cohorts to balance pack extinction. Lastly, less colonisation leads to fewer packs, and again to fewer dispersers, further demonstrating Allee effects at the population level. Empirical data from this study fits this model, with a dispersal group size ranging from only one to four dogs. The impact of Allee effects on population survival and its relevance to conservation management are discussed further in Chapter 6.

Woodroffe and Ginsberg (1998) assessed the impact of edge effects, or increased mortality outside reserve borders, on wide ranging carnivore populations. They suggested that if population size determines extinction probability, then critical reserve size should be related to density, as this determines population size. If edge effects determine extinction probability, then critical reserve size would be related to home range size. The study found critical reserve size was related to home range size and that edge effects play an important part in population persistence. This theory could be further extended to include the Allee effect on dispersal. Although home ranges may lie within reserve borders in larger protected areas, in populations of species which exhibit inbreeding avoidance behaviour dispersal distance may increase if population size is reduced and mate choice becomes limited. If based only on home range size, edge effects may still be underestimated in small populations since dispersal and recruitment are critical to population persistence.

While sex bias in larger populations has been related to mate choice and different dispersal distances between the sexes (McNutt 1996a), in smaller populations inbreeding avoidance may play a key role in determining dispersal distances for both sexes. Evidence from this study is limited since actual dispersal distances and the fate of emigrants are unknown. The Zambian Escarpment provided a barrier to tracking of uncolllared dogs, so detection of emigrants in that area was unlikely. However, range data from study packs suggests that the escarpment also provided a barrier to wild dog home range movements, and dogs would be unlikely to remain in the mountainous areas when high prey densities are available on the valley floor (see Chapter 3, section

3.4.1). Dispersers may travel over it, so this physical barrier could also increase dispersal distances. The effect of inbreeding avoidance in smaller populations is an important area for further study in other small populations.

2.5.5 Sex Ratio

Other wild dog studies have encountered either a male biased adult sex ratio (Frame, J.R. Malcolm et al. 1979; McNutt 1996a), or no sex bias at all (Maddock and Mills 1994; Girman, M.G.L. Mills et al. 1997; Creel, N.M. Creel et al. 1997c). There was no significant sex bias in the Lower Zambezi population, nevertheless all age classes displayed a constant trend of a higher proportion of females. The data from this study must be interpreted with caution due to small sample sizes resulting in lack of power. However, lack of power is likely to be a constantly limiting factor for detecting small changes in populations when investigating the dynamics of small and declining populations.

Alongside a shortage of receptive mate encounters, demographic stochasticity is another main category of the Allee effect in small populations (Courchamp, Clutton-Brock et al. 1999), and this provides one explanation for the unusual trend in sex bias observed in the Lower Zambezi population. Additionally, in long lived species such as wild dogs there may be large temporal autocorrelation in sex ratios (Engen, Lande et al. 2003). Nevertheless, female bias was present in pups here, and 40% of adults only contributed data to the adult age class. There is also evidence of sex bias following maternal lines in other canids (Beketov and Kashtanov 2002) which would be exaggerated in wild dogs by the pack breeding structure limiting the number of maternal lines in a population. The number of pups of unknown sex may mean that the trend in female sex bias observed here is the result of differential juvenile survivorship rather than birth ratios, however this would also be the case for comparative wild dog studies since pups do not emerge from the den for three weeks.

Data from large population studies of wild dogs suggested that males contribute more to raising young and improving natal pack success (Malcolm and Marten 1982), but by emigrating further they also reduce resource competition with the natal pack compared to females. Therefore both local resource competition theory and resource enhancement theory (Hardy 1997) would predict a male bias in stable wild dog

populations. Given the size and instability of the Lower Zambezi population there is no evidence here to contradict these theories.

However, it is plausible to consider that any female sex bias may be an artefact of small population dynamics. Based on small pack size and Allee effects on hunting success, it can be hypothesised that maternal condition might play a role in sex bias. Although sexual dimorphism in wild dogs is not as pronounced as in other species, adult males were found to be approximately 16% heavier than females in two previous studies (Woodroffe, McNutt et al. 2004) and 17% heavier in this study. Myers (1978) adapted the Trivers-Willard hypothesis on maternal condition and birth sex ratios to suggest that the production of more of the least expensive sex allows the mother to maximise the number of offspring produced. Myer's assumption that reducing litter size does not enhance the survival of the mother is likely to hold in this case; in wild dogs larger litter sizes are important for small packs due to expected higher mortality in pups, and due to inverse density dependence in small packs. Nutritional stress has been found to result in production of the least expensive sex in various mammal species (Smith, Robbins et al. 1996; Andersson and Bergstrom 1998; Fisher 1999; Kruuk, Clutton-Brock et al. 1999) and the number of helpers was found to have an effect on maternal health in cooperatively breeding meerkats (Russell, Brotherton et al. 2003). As Creel et al (1998b) suggested, if sex ratio bias was due to one zygote being more susceptible to stress, one would expect a bias in one direction only and an overall population wide bias, a trend which was observed here in all age classes.

In conclusion, inbreeding avoidance and mate competition, proposed as important factors in large wild dog populations, may play an important part in small and fragmented populations by contributing to a migration-mediated Allee effect, which in turn has subsequent impacts on breeding success and population demography. High adult mortality rates from snaring compounded Allee effects on dispersal and pup survival, leading to a continuous state of population decline.

CHAPTER 3: ECOLOGY AND HABITAT UTILISATION

3.1 INTRODUCTION

Loss of habitat continues to affect many species, particularly those with large home ranges. Growing human development has had steadily increasing impacts on the survival of African wild dog populations, due to the species' far ranging, nomadic habits (Woodroffe & Ginsberg 1999a; Woodroffe et al. 2004b). Once they leave the protection of National Parks and Game Reserves wild dog mortality rates are likely to increase. Ecological factors may interact to increase the probability of wild dogs leaving protected areas. In previous studies wild dogs were shown to avoid high densities of lions (Creel & Creel 1996; Mills & Gorman 1997), while lions tended to concentrate in protected areas with high prey density (Creel & Creel 1997; Spong 2002; Stander 1993). Wild dogs may therefore be pushed out of favourable habitats into higher risk areas.

This chapter investigates the home range movements of the Lower Zambezi wild dog population, and their utilisation of habitat in relation to vegetation density, prey density and hunting success. The effects of interpredator competition on wild dog range movements are addressed in the following chapter.

3.1.1 Home Ranges and Habitat Preferences

Wild dogs typically have large home ranges, although range sizes vary considerably in differing habitats. In the free-ranging population in Kruger National Park home ranges averaged 537km^2 (Mills & Gorman 1997), similarly, in other wooded areas such as Hwange National Park and Selous Game Reserve, ranges averaged 423 km^2 and 379 km^2 respectively (Creel & Creel 2002; Woodroffe et al. 1997). In the fenced Hluhluwe Umfolozi Game Reserve in South Africa (96000 ha), wild dog home ranges fell to 242 km^2 (Andreka et al. 1999) and were concentrated away from the highest lion density areas. Average home ranges in the more open habitats of the free-ranging Serengeti and Aitong (Kenya) populations averaged 665 km^2 and 650 km^2 respectively (Fuller & Kat 1990; Schaller 1972). Home ranges often overlap, in some areas by 50-80% (Fanshawe et al. 1991; Mills & Gorman 1997). In Kruger National Park it was thought that packs rarely met (Mills & Gorman 1997), however, in the Serengeti Frame et al. (1979) never recognised one pack deliberately avoiding

another and observed larger packs chasing smaller packs from an area. The thicker vegetation cover and subsequently reduced visibility in Kruger National Park may have reduced the likelihood of observing packs interacting.

Wild dog habitat preferences vary; they appear to be able to utilise a variety of vegetation types. Dogs in the area of the Serengeti and Masai Mara have been observed to prefer short and medium grass habitats for hunting and resting (Fuller & Kat 1990; Maddock 1993), while dogs in southern Africa thrived in closed bush and hilly woodland habitats (Mills & Gorman 1997; Reich 1981). Hunting success has been shown to be just as successful in areas of moderate and low prey density (Creel & Creel 1998; Fanshawe & Fitzgibbon 1993), therefore wild dog habitat selection is not necessarily determined by prey density alone.

3.1.2 Hunting and Prey Preferences

African wild dogs generally prey on medium sized antelope species, often favouring the most abundant species in their area. The dogs hunt in cooperative groups, which allow them to take prey much bigger than themselves (Fanshawe & Fitzgibbon 1993; Fuller & Kat 1993; Woodroffe et al. 1997). The dogs weigh on average 20-25kg but may take prey up to 200kg (Creel & Creel 1995; Frame et al. 1979). Chases often continue over distances greater than 5km and reach speeds of up to 60km/hr (Malcolm & Marten 1982; Woodroffe et al. 1997). Prey species include impala (*Aeypceros melampus*), Thompson's gazelle (*Gazella thompsoni*), reedbuck (*Redunca arundinum*), wildebeest (*Connochaetes taurinus*) and kudu (*Tragelaphus strepsiceros*) (Woodroffe et al. 2004b). In areas of mixed bush habitat in Southern Africa impala play a large part in the dogs' diet as they are generally the most abundant medium sized prey (Creel et al. 2004; Fuller & Kat 1990; Kruger et al. 1999; Mills & Gorman 1997). In a study in east Africa the dogs took mostly adult Thompson's gazelles, but killed more juveniles of larger species, including impala (Fuller & Kat 1990). More male Thompson's gazelles were killed than females in two studies in east Africa, which may reflect the male antelope's reluctance to leave his territory, and decreased alertness in comparison to the female breeding herds (Fanshawe & Fitzgibbon 1993; Fuller & Kat 1990). In southern Africa wild dogs did not favour male or female prey (Kruger et al. 1999).

It should be kept in mind that kill data may be biased toward larger prey species, since smaller prey would be consumed very quickly with little evidence left of kills (Childes 1988). Wild dogs have been observed taking springhare (*Pedetes capensis*) and *Lepus* species in east Africa, and bat-eared foxes (*Otocyon megalotis*) in Zimbabwe (Fuller & Kat 1990; Rasmussen 1996).

While some studies have suggested that the advantages of cooperative hunting may be its evolutionary cause (Estes & Goddard 1967; Kruuk 1972), others have indicated that communal hunting is more a consequence of sociality (MacDonald 1983; Packer & Ruttan 1988b). It has been suggested that communal hunting benefits wild dogs by increasing the prey base available to them (Fanshawe & Fitzgibbon 1993). In a study of one pack in the Serengeti, Fanshawe and Fitzgibbon (1993) found that wild dog's hunting of larger prey such as wildebeest maximised their food intake in groups of three to four, while dogs hunting gazelles did best alone. They concluded that communal hunting was beneficial in increasing the range of prey species that could be hunted, and that hunting in groups reduced interspecific competition from spotted hyaenas. In contrast, Fuller and Kat (1993) studied a single pack and found that in an area of abundant prey and low predator competition wild dog pack size remained large, confounding theories about the evolutionary cause of pack size. In data from 404 kills from six packs Creel and Creel (2002; 1995) found that hunting success, prey mass and the probability of multiple kills increased with the number of adult dogs in a pack. These studies overall suggest that the energetics of cooperative hunting favour group living in African wild dogs.

In addition to studies on the benefits of sociality in wild dogs, one study also focussed on assessing the factors that influence the coursing hunting methods of the dogs, especially in comparison to the stalking cheetah and lion (Fanshawe & Fitzgibbon 1993). Coursing predators approach prey openly, flush it and then give chase often over long distances (Estes & Goddard 1967; Kruuk 1972; Schaller 1972). Fanshawe and Fitzgibbon (1993) found that wild dogs tended to either approach prey slowly with the pack grouped together and heads lowered, or run straight up to the prey in full view. Although hunting success was influenced by pack size, it was not affected by the amount of available cover, the size of prey groups, or the distance at which prey groups fled, in contrast to the hunting of stalking predators.

Hunting success in wild dogs is generally high compared to other large carnivores (Schaller 1972) but actual success rates vary depending on the area in which they are found. In an eastern African study data indicated that the dogs were never unsuccessful on two consecutive hunting sessions, and they hunted twice a day (Fuller & Kat 1990), however this was based on hunting sessions rather than the number of individual hunts within each session. Consumption rates were estimated at 1.7kg prey/dog/day. Success rates over eastern and southern Africa range from 39% to 85%, seem to be similar in high and low density populations, and independent of prey density (Creel & Creel 1998; Fuller & Kat 1993). The exception to this independence from prey density would be when packs are denning and movements are restricted, particularly if they are dependent on migratory prey (Creel & Creel 1998).

Adults and yearlings contribute similar amounts of food to dependent pups at the den. However, yearlings play a smaller role in killing larger prey and tend to be more successful hunting juvenile prey (Fanshawe & Fitzgibbon 1993; Fuller & Kat 1993; Malcolm & Marten 1982). Approximately one third of a meal is fed to pups, even in times of food scarcity (Malcolm & Marten 1982). On rare occasions wild dogs have been observed to cache food. This has only been recorded during food scarcity and when the dogs were returning to a breeding den (Malcolm 1980). In wooded areas the dogs rarely hunt at night, but have been observed to travel on moonlit nights (Creel & Creel 1995).

3.2 OBJECTIVES

The objective of this section of the study was to determine the role of ecological factors on wild dog home range movements and population dynamics. Specifically, aims were to:

1. Assess the vegetation structure and classify the main habitats within the study area.

2. Estimate the density of prey within each habitat.

3. Determine the size and distribution of wild dog home ranges and habitat utilisation.

4. Determine wild dog hunting success rates and prey preferences in relation to habitat characteristics.

3.3 METHODS

3.3.1 Habitat and Prey Species

GPS coordinates for wild dog activities and home ranges were collected using the general methodologies for field work outlined in Chapter 2. Surveys were carried out to provide background ecological information on habitat and prey densities within the study area.

Human settlement areas inside the study area were also mapped using ArcGIS 8.1 (1999-2001, ESRI™ Inc., USA), from GPS field data and additional boundary data provided by Conservation Lower Zambezi (I. Stevenson, unpublished data).

3.3.1.1 Vegetation classification

Due to a lack of information on vegetation within the study area, a preliminary vegetation survey was carried out during the course of the study to classify habitat types. The objective of the survey was to obtain a general classification of habitats by means of field surveys and remote sensing classification. The analysis was not intended to provide a comprehensive vegetation survey of the area.

i) Field surveys

Four habitat categories were surveyed within the African wild dog home range area: 1) grassland, 2) *albida* woodland, 3) ecotone (transitional zone of grassland or woodland to thicket), and 4) thicket. Habitats were sampled by line transect methods. Transects were randomly spaced within each habitat, with the restriction that sites were limited to those within 1km of an existing road or track due to park regulations on walking. Vehicle tracks were little more than game trails in most cases so vegetation was not disturbed more than 10m either side. Tracks were sufficiently distributed throughout each habitat type for equal sampling to be carried out. GPS locations for potential transect areas were identified from satellite maps and preliminary ground observations, given a number, then 4 numbers from each habitat were chosen randomly.

In each habitat four replicate 300m line transects were laid out. A 20X20m quadrat was pegged out on each transect at 100m intervals from 0 to 300m, giving a total of 4 quadrats per transect and 16 per habitat. Within this 20m quadrat all species present were identified. Percentage ground cover for each species within each quadrat was

estimated by two to three individual observers, averaged and then recorded. To ensure consistency in estimates I was principal observer at every site. All species with greater than 1% cover were included in the species description list. Since large trees were often sparsely distributed and fell outside of quadrats, a list of all dominant tree species for each habitat was composed by walking each transect and identifying all observed species within 30m either side of the transect line.

Canopy cover was visually estimated at the centre of each quadrat using a vertical projection method, through a viewing square of 30cm by 30cm, held 190cm (researcher arm height) from ground level. Cover was recorded as a percentage estimate of the area at canopy height, bordered by the viewing square, which was covered by foliage. Ten percentile intervals were used for coverage estimates. Digital photographs of sample canopy areas were taken and used as reference.

Field data were then used to describe the four major habitats by growth form, height class, cover and dominant species, based on methods adapted from Walker and Hopkins (1990). The classification tables used are contained in Appendix 2. Both crown canopy structure and percent cover were used to determine cover class for all growth forms.

Vegetation density in each habitat was estimated based on relative visibility, using truncation distances from impala density surveys (see section 3.1.2 below). The examination of perpendicular distance histograms showed no decrease in the frequency of prey sightings with increasing distance below the chosen truncation distance for each habitat, therefore the truncation distance was judged to be a reasonable scale to use as a measure of vegetation density.

ii) Vegetation classification by remote sensing

The satellite image containing the study area was a Landsat-7 image (P171/R71), taken on 24/09/2001, which was ortho-rectified and pan-fused with Enhanced Thematic Mapper (ETM) bands 7-4-2 (Intec Americas Corp. USA). This raster map of the area was used for all spatial analysis of wild dog home range movements and activities, and competing predator densities. A raw version of the satellite image ("Raw" Fast L7-A.fst) was used to carry out a supervised vegetation classification,

using 7 spectral bands, performed in IDRISI Kilimanjaro (version 14.02, Clarke Labs, Worcester, MA, USA).

To classify vegetation on the satellite image, spectral signatures of each vegetation category were created using calibration sites (training sites). This process correlates pixel distribution and characteristics with vegetation cover, and was based on information obtained from the ground transect locations and at least two other known areas for each habitat. The combined training sites resulted in a training class of a minimum of 200 pixels for each habitat. Classification accuracy was assessed from ground-truthing and extensive knowledge of the study area, rather than In-process Classification Assessment procedures. Based on the spectral signatures created, twenty additional ground-truthed sites (five for each habitat) were used for accuracy assessment and 100% were correctly classified.

The hard classifier method MAXLIKE was used to assign pixels. This method is the most powerful hard classifier method and uses Bayesian probability theory to assign pixels to each class based on training site information. It also accounts for correlation between bands. All pixels are assigned to a category using this method so additional habitat categories were created to allow for areas with spectral properties that were not previously allocated to any habitat. The additional habitat categories were derived from ground-truthing from direct observation, and included categories outside the wild dog home range areas. The additional categories were; miombo woodland, burnt miombo woodland, Zambezi River, and sand bars.

3.3.1.2 Prey density

Prey counts using line transect sampling methods were used to assess relative densities of impala, the wild dogs' main prey species, in each of the four major habitats described above. Transect sampling methods do not effectively sample rare or shy animals with low detection probabilities (Thomas et al. 2003), including species such as bushbuck and kudu (*Tragelaphus strepsiceros*) which made up a small proportion of the wild dogs' diet. These species were recorded during prey counts but due to the low encounter rates and subsequent lack of data they were dropped from density analysis. Due to limited field time the density of these species in each habitat was considered in-line with previously published literature.

Prey counts were carried out during the dry season, between August and October in 2003. Visibility and detection of animals was maximized at this time of year because vegetation had been reduced by annual die off, animal trampling and foraging pressure. Line transect counts were carried out on the existing dirt-track network for ease of access and to minimize vehicle impact and prey disturbance. Transects were randomly allocated along straight sections of road in each vegetation type. Due to spatial fragmentation of habitats, two to four transects of varying length were used in each habitat, which were then pooled to give a total average transect length of 13.7km (SE: 0.31km) in each. Four temporal counts were carried out on each transect, divided into two morning and two afternoon counts, each separated by several days. To avoid double counting within a transect due to prey movements, sampling was restricted to between 5.00-9.30 hours in the morning and 16.00-18.30hrs in the afternoon.

Counts were carried out within a maximum distance of 300m either side of the transect, by one to two observers in a vehicle driven at 10-15km/hour. I was always principal observer to standardise counts. The vehicle was stopped whenever prey was observed, and 8x30 binoculars were used to identify species, sex and herd size. Perpendicular distance from the transect line was calculated by recording distance from the vehicle to the centre of the herd/animal where it was first sighted using laser rangefinders (Yardage Pro Legend, Bushnell, USA), and angle from the road which was estimated using a compass.

Due to lack of independence of temporal samples, a single transect from each vegetation type (average length 14.4km, range 13.5 to 15.2 km) was used to estimate density in each vegetation type. Impala count data was imported into the program DISTANCE 4.1 (Thomas et al. 2003). DISTANCE 4.1 (Thomas et al. 2003) relaxes the assumption found in other strip transect methods that all objects within a pre-defined strip are detected, and can also test and adjust for cluster size-bias; the increased probability of detection of larger groups which would affect density calculations (Thomas et al. 2002). A best fit detection model function was chosen by plotting and examining histograms of recorded distances, to set data filters and truncation distances, and by comparing AIC (Akaike Information Criteria) values between different model definitions. This preliminary assessment investigates the

distribution of the data and trends in detection distances. There was little difference between model combinations, however the half-normal detection function with a simple polynomial expansion model generally resulted in lower AIC's and smaller confidence intervals, so this model was used for most analysis. In the case of the "thicket" sample the uniform detection function with simple polynomial expansion model gave a lower AIC and smaller confidence interval so was used for this stratum, which differed due to low sample size. Plots of group size against perpendicular distance showed no correlation, indicating, for instance, that there was no decrease in detection probability with increasing distance, within the chosen truncation distances. Therefore mean group size was used in density analysis. Default settings in DISTANCE 4.1 (Thomas et al, 2003) were used except where specified.

Coefficient of variation increased with larger sample sizes, so density results were log-transformed for further analysis. Log D was used for density estimates. DISTANCE 4.1 (Thomas et al, 2003) outputs the percentage coefficient of variation in the form of CV=(SE/D*100), therefore SE was transformed by the equation log (1+ (cv/100)). One-way ANOVA was used to test for differences between densities and cluster size in each vegetation type. If ANOVA gave significant results, post-tests were carried out using the Bonferroni test to correct for multiple comparisons between the four vegetation groups.

To estimate overall impala density of the study area, density was calculated in DISTANCE 4.1 (Thomas et al, 2003) using one transect from each vegetation type, entered as different samples within the one stratum. Temporal patterns in impala density within each vegetation type were investigated by calculating density in DISTANCE 4.1 (Thomas et al, 2003) using the detection function parameters as above. For each vegetation type a morning and an afternoon density was calculated using the two replicate morning or the two afternoon counts combined, giving a total sampling effort of between 25km and 30km for each density estimate. Since density figures are output only as summary data (mean, SE, 95% CI) from DISTANCE 4.1 (Thomas et al, 2003), raw densities were calculated, based on the area of each transect within truncation distances, and differences between AM and PM densities were tested using the Wilcoxon Matched-Pairs Signed-Ranks Test

Temporal transects for each vegetation type were pooled to assess the relationship between herd (cluster) size and perpendicular distance from the transect line. Pearson's product moment correlations were used to investigate the relationships between impala density and herd size in relation to vegetation density.

3.3.2 Wild Dog Home Ranges and Habitat Utilisation

GPS locations collected from field observations were used to calculate wild dog pack home ranges and habitat selection. For details of field methodology refer to general methods section on Data Collection, 2.3.1. All wild dog fixes were separated by a period of movement such as hunting or travelling so they are assumed to be independent. A maximum of two data points per pack per day was used in analysis. Triangulation from radio signals (see section 2.3.1.3) was used rarely and only when a later sighting confirmed the dogs had been in the area, for example as they left an inaccessible thicket. For habitat selection analysis triangulation readings were removed and only direct observations were used.

3.3.2.1 Home range analysis

Home ranges were calculated using the Minimum Convex Polygon (MCP) method in the program CALHOME (Kie et al. 1994). This basic method of home range calculation is commonly used and allowed comparison with other wild dog study areas. One limitation of the MCP technique is that home ranges may include areas which are not actually utilised by the study animals. A 95% MCP contour is often applied to compensate for overestimation of home ranges due to outlying points (Lawson & Rodgers 1997), however a 100% MCP contour was retained here. Outliers in this dataset were invariably in thicket and mountainous areas at breeding times. Limited accessibility, signal bounce and resource restrictions on aerial tracking may have resulted in underestimation of the use of these areas, therefore all location points were included in home range calculations.

MCP methods have been shown to have a positive correlation between home range size and the number of data points (Gautestad & Mysterud 1993). The minimum required sample size for accurate home range estimates was calculated by plotting MCP home range size against sample size for the two annual home ranges with the highest number of observations, GMA-2003 and GMA-2002 (n>100). Both curves

appeared to plateau at approximately 40 observations (see results section 3.4.2.1). Four annual home ranges were then plotted based on 40 or more observations. For the remaining six pack-years, two years of observations for each pack were combined to establish three home range areas based on 35 or more observations. A correction was then applied; nonlinear regressions of the GMA-03 and GMA-02 data sets provided an asymptote value where extra data points had little effect on home range size. This value was used to estimate the percentage of total home range area generated by each number of observations, at intervals of 10 observations. The regression curve resulting from the mean percentage values of both datasets was used to adjust all home range area estimates, based on the number of observations. All MCP home range areas were further adjusted for non-utilised areas by removing the Zambezi river from the home range polygons using ArcMap 8.1. Correlations of pack size against MCP home range sizes were carried out in GenStat 8.1 (2005).

MCP methodology was also used to calculate breeding home range sizes for each pack that was observed to den. Data was used over a three month denning period, less where a pack was disturbed by predators (see section 2.4.1.2).

Adaptive kernel density analysis methods were used to provide more detailed information on space use within the annual home range area boundaries. The kernel density function is a robust nonparametric method, which allows the user to avoid assumptions about the distribution of data (Seaman & Powell 1998; Worton 1987, 1989). Bandwidth (equivalent to a histogram's binwidth) is the most important parameter in kernel analysis, and determines the amount of smoothing of the data (Seaman & Powell 1996; Worton 1989). Least-squares cross-validation was initially used here to select optimum bandwidth using the program CALHOME (Kie et al. 1994). However, this method gave poor data fit and overestimated home range area. This is often the case with non-normally distributed data where the study subject is using two or more core areas (Kie et al. 1994). In this case a bandwidth below the optimum is recommended. The least-squares cross-validation method is also inappropriate where there are multiple observations at identical locations (Seaman & Powell 1998; Tufto 1996), which occurred in this data set at den areas.

An alternative technique of selecting bandwidth was used by graphing several densities using different bandwidths and then choosing the most suitable bandwidth for best fit (Mugdadi & Ahmad 2004). Smaller bandwidths are generally more appropriate for revealing small-scale patterns in the utilization distribution (Seaman & Powell 1996), and after several trials an arbitrary bandwidth of 65% of the optimum bandwidth produced in CALHOME was used on all home ranges. Density was calculated and mapped using the Spatial Analyst extension in ArcMap (ArcGIS 8.1), with an output cell size of 200m. The Spatial Analyst kernel density function uses a quartic approximation of a Gaussian kernel. This method produced volume contours that outlined the minimum contours in which the study subject spent a specified proportion of time, based on probability under a bivariate probability surface. Contours were calculated at 50%, 75% and 95% intervals.

3.3.2.2 Habitat utilisation and prey selection

i) Habitat utilisation

Wild dog habitat selection in this study was first analysed at a population level using pooled wild dog GPS locations over the entire study area. Habitat selection was then analysed at an individual pack level, within each pack's home range area. Annual ranges for each pack were combined for this analysis which provided data for three to five years per pack. Analysis assessed whether dogs utilised habitats more than would be expected from the proportion of each habitat available to them. Habitat selection was then investigated in relation to prey density and interspecific competition.

Wild dog habitat selection was analysed using Duncan's (1983) method which gives an index of preference (PI), as previously used for wild dogs by Mills and Gorman (1997).

PI = (Uh/Ut)/(Ah/At), where; Uh is the number of wild dog observations in one habitat, Ut is the total number of wild observations in all habitats, Ah is the area for the habitat, and At is the total area.

1) [log10(PI+1)] then gives an index of preference for wild dog usage within each area of different lion density.

Duncan's (1983) method gives a preference index where 0.3 is parity, values above 0.3 show preference for that area, and below 0.3 demonstrates avoidance. Duncan's normalisation of the preference index removes the compression of avoidance values

relative to preference values that otherwise would occur, since avoidance is restricted to between values of 0 and 1.0 using only formula number 1) above.

Data from sightings reports submitted by safari guides and ZAWA Wildlife Police Officers was included where sufficient information was provided. This data was tested for bias before inclusion in this section, as there may be an increased likelihood of observing wild dogs in more open areas when not using telemetry methods and data may have been biased towards open habitats. The proportion of sightings in each habitat was tested against the proportion of the road network in each habitat. Although there was a significant difference between the proportion of sightings expected from each habitat (χ^2=172.6, df=3, P<0.0001), more sightings than expected were observed in thickets and less than expected in woodland, so no relative visibility bias towards open areas was apparent. No data were available on the relative road usage in each habitat so no adjustments to data were made. Wild dogs were frequently observed using roads for travelling and resting in all habitats, which may cancel out visibility limitations.

ii) Prey selection

Each prey species identified was presented as a proportion of the total observed hunts, kills and its total biomass contribution to the wild dogs' diet. Data for age and sex distribution of prey was incomplete so biomass was estimated for impala and kudu according to Mills and Gorman (1997), using weights of 40kg and 136kg respectively. Bushbuck weights were estimated visually from kills and an average of 50kg was used. Hunt data was then used to calculate the hunt effort spent on each prey species in each habitat and overall wild dog hunting success in each habitat. This was then compared to general wild dog habitat selection (PI) and prey density.

A hunt was defined as a chase in which dogs reached a run and where one adult or more pursued the prey. Pups and yearlings often participated in short "warm up" chases of a variety of prey including warthog and herds of fully grown buffalo and zebra. However, these chases were short, unsuccessful (often the prey did not even flee) and the adults did not participate so they were not considered a serious hunt. Consecutive individual hunts were difficult to observe due to limited visibility and accessibility in some habitats, so hunting success (kills/hunt) was determined from the

number of observed hunting periods, (morning, evening or night) and whether each period resulted in one or more kills.

GPS locations were taken for each wild dog hunt or kill observed, however prey was not always identified as carcass remains were often removed by spotted hyaenas before they could be identified. To calculate the proportion of prey species in the wild dogs' diet only observations where prey were identified were included, however to calculate hunting success in each habitat all hunt and kill data was used.

3.4 RESULTS

3.4.1 Habitat and Prey Species

3.4.1.1 Vegetation classification

The study area was classified into dominant habitats according to vegetation type. Table 4.1 below describes the four major valley floor habitats by growth form, height class, cover and dominant species, based on methods by Walker and Hopkins (1990). Vegetation species lists for each habitat are described in Appendix 2. Truncation distances from DISTANCE 4.1 (Thomas et al. 2003), analysis (section 3.3.1.2) were included with each habitat as a relative measure of vegetation density based on the visibility of prey.

The grassland habitat was characterised by isolated trees and shrubs, and a variety of grasses and forbs. This habitat also included sections of sodic soil relatively bare of growth, and other areas heavily dominated by tussocks and grasses more typical of savanna grasslands. In the groundcover structural layer grasses are listed as "mixed grass species" because vegetation surveys were carried out during the dry season and this made identification of many grass species difficult, since few were flowering. Groundcover was highly seasonal in all habitats except grasslands, with little groundcover remaining by the end of the dry season.

Table 3.1 Vegetation structure and composition for dominant habitats in the study area.

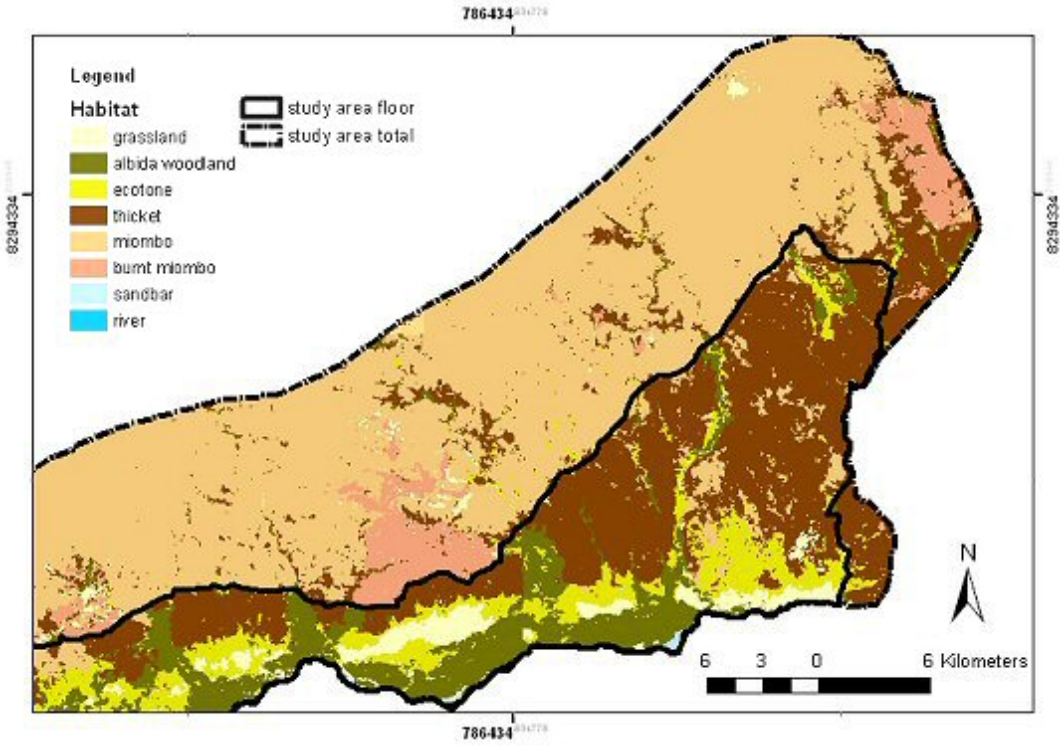
Habitat (visibility)	Growth Form	Dominant species	Average Height (m)	Cover class
Grassland (250m)	Tree	*Acacia nigrescens, Acacia tortilis, Hyphaenae petersiana, Combretum imberbe*	6-12	isolated plants
	Shrub	*Caparis tomentosa, Salvadora persica*	1-3	isolated clumps
	Ground cover	*Duospermum quadrangularis, Sphaeranthus flexuosus, Vernonia glabra, mixed grass spp.*	0.25-0.75	mid-dense
Albida Woodland (140m)	Tree	*Faidherbia albida*	12-20	sparse
	Shrub	*Senna singueana*	1-3	very sparse
	Ground cover	*Senna obtusifolia, Solanum panduriforme, Sphaeranthus flexuosus, mixed grass spp.*	0.5-1	mid-dense
Ecotone: grassland/woodland to thicket (80)	Tree	*Acacia tortilis, Combretum imberbe, Philenoptera violacea,*	6-12	very sparse
	Shrub	*Dichrostachys cinerea, Diospyros sinensis, Boscia mossambicensis,*	1-3	sparse
	Ground cover	*Crossandra spinescus, Duopsermum quadrangularis, Vernonia glabra, Ocimum canum and O. americanum,sphaeranthus flexuosis, mixed grass spp.*	0.5-1	dense
Thicket: shrubland (35)	Tree	*none*		
	Shrub	*Acacia ataxacantha, Boscia mossambicensis, Combretum elaeagnoides, Combretum adenogonium, Colophospermum mopane, Holmskioldia tettensis, Markhamia zanzibarica*	1-3	mid-dense
	Ground cover	*Crossandra spinescus, Dicoma anomela, Duospermum quadrangularis, mixed grass spp.*	0.25-0.75	sparse

The *albida* woodland habitat was an open woodland generally monodominant with *Faidherbia albida*, a sparse shrub layer of *Senna singueana* which was often associated with termite mounds, and a variety of forbs and grasses. Young *albida* forest with a closer canopy cover was included here, which otherwise had the same floral characteristics. The ecotone habitat was a heterogeneous transitional zone between grassland and thickets or open woodland and thickets, forming open

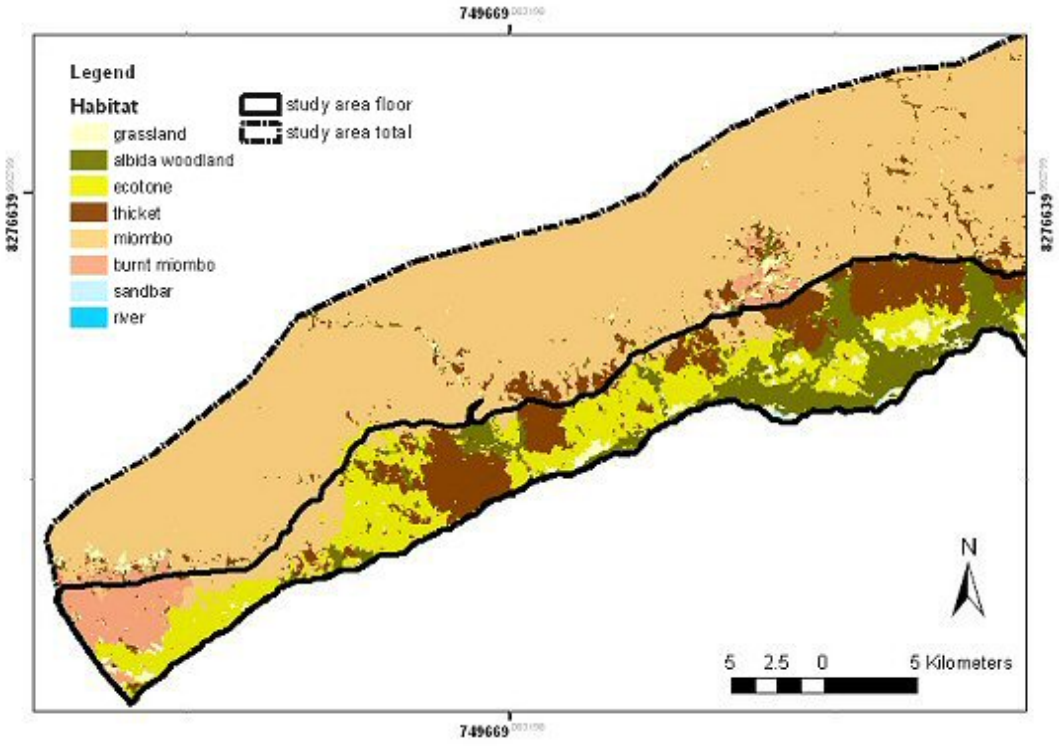
woodland at the tree canopy with an understory of open shrubland which was composed of a diverse array of species at different transect locations. The dense groundcover contained an equal prevalence of forbs to grasses. This habitat included areas of termitaria vegetation. Thicket habitats (shrublands) were composed of a variety of species, including some areas dominated by *Acacia ataxacantha* and *Combretum elaeagnoides* (jesse bush), and others by *mopane* scrub. Much of the *mopane* woodland in the valley floor had a thicket understory, so it was included in this habitat.

The study site also contained strips of riverine vegetation along watercourses and gullies, containing a variety of *Acacia* species and *Trichilia emetica*. However, these areas generally occurred in strips less than 100m wide and resembled the spectral properties and vegetation structure of either the *albida* woodland or ecotone, so they were classified within these general habitats for this study.

Surveys did not extend to the escarpment miombo habitat due to lack of road access. For the supervised vegetation classification this habitat was characterised from the spectral properties of the vegetation in the escarpment, which was clearly visible from the satellite image. Miombo is a specific type of African woodland, dominated by three tree genera; *Brachystegia, Julbernadia* and *Isoberlinia*, and covers much of the Zambian plateau (Jachmann 2000). It is generally typified by a closed canopy, but is still considered woodland since it supports an understory of grasses and herbs due to the low density of foliage at canopy level (Bingham 1995). Miombo undergoes regular seasonal burning, which is apparent on the vegetation map derived from the satellite image (Figure 3.1). It should be noted that the escarpment miombo habitat defined for the purpose of this study is more floristically diverse than represented here, particularly in the areas of escarpment where typical woodlands give way to riverine gulleys and steep valleys.



3.1a



3.1b

Figure 3.1a and b. Map of habitat distribution in the study area, developed from a supervised vegetation classification. Outlines show the overall study area and the river valley floor. The escarpment, dominated by miombo, is visible to the north of the valley floor.

Figure 3.1 shows the distribution of habitats in the study area. The alluvial terraces of the river valley floor area contain the greatest diversity of habitats, while the northern section of the study area is dominated by the escarpment miombo. Table 3.2 shows the proportion of the study area covered by each habitat. Thicket was the most dominant habitat type on the valley floor, but this was largely due to its prevalence in one north-eastern corner of the study area furthest from the Zambezi River (Figure 3.1a). This area was predominantly *mopane* scrub. *Albida* woodland and ecotone covered a large proportion of the remaining valley floor area on the alluvial terraces.

Table 3.2 Percentage of study area covered by each habitat. Values are given for both the entire study area, and for only the study area valley floor.

Habitat	Study Area		Valley Floor
	Km^2	% Cover	%Cover
Grassland	37.9	2.6	6.3
Albida woodland	118.9	8.2	19.7
Ecotone	131.0	9.0	21.7
Thicket	266.6	18.4	44.2
Miombo	893.7	61.7	8.1

3.4.1.2 Prey density

Impala density was found to vary between habitats. The highest densities of impala were found in the more open habitats of grassland and *albida* woodland (Table 3.3).

Table 3.3. Impala mean density and cluster size within each habitat. Sampling effort and truncation distances are included for each vegetation type. The percentage coefficient of variation is included (% CV). Superscript letters indicate significant differences between habitats (see text).

Vegetation Type	Sampling Effort (Km)	Truncation Distance (m)	Mean Density/Km2	% CV (Density)	Mean Cluster Size	% CV (Cluster size)
Grassland	13.5	250	174^{b}	36.9	17.4^{d}	28.16
Albida woodland	15.2	140	229^{a}	23.9	14.0^{c}	13.92
Ecotone	14.3	80	66	45.9	8.6	19.78
Thicket	14.5	35	$38^{a,b}$	52.2	$4.2^{c,d}$	32.45

Analysis of variance showed that there was a significant difference in impala density between habitats (d.f.=3, f=5.23, p=0.0022). Post-tests of multiple comparisons between vegetation types showed that the significant differences lay between the thicket and *albida* woodland (t=3.424, $p<0.05$, see superscript "*a*" in Table 3.3), and between thicket and grassland (t=2.857, $p<0.05$, see superscript "*b*" in Table 3.3).

Weighting impala density by the proportion of the valley floor covered by each habitat gave an average impala density of 95/km^2 (% C.V = 43.6), and an estimated abundance of 52,600 impala in the study area.

Impala herd sizes in each habitat were assessed as clusters in DISTANCE 4.1 (Thomas et al. 2003). Changes in herd size between vegetation types followed the same trend as impala density, with significant differences in ANOVA results (d.f.=3, f=3.93, p=0.011), found between thicket and *albida* woodland (t=2.790, p<0.05, see superscript "*c*" in Table 3.3) and thicket and grassland (t=3.133, p<0.05, see superscript "*d*" in Table 3.3).

The validity of herd size estimates was tested because vegetation density can affect counts as distance increases from each transect. There was no correlation between herd size and perpendicular distance from the transect in grassland (r=-0.12, p=0.07, n=117), *albida* woodland (r=-0.09, p=0.16, n=222), or ecotone vegetation (r=-0.07, p=0.28, n=46). In the thicket classification there was a moderate positive correlation (r=0.65, p=0.001, n=9) between herd size and increasing perpendicular distance. Larger group size may increase the probability of detection, particularly in thicker habitats where smaller groups may be obscured from view. However, thicker vegetation also increases the likelihood of failing to count all members of the group, and these effects are likely to counteract each other, as suggested by Dunham (1994).

Since there was no evidence of decreasing herd size with increasing distance from transects, the truncation distances used in DISTANCE 4.1 (Thomas et al. 2003) were taken as a reliable estimate of vegetation density, as measured by prey visibility. Plots of impala density and herd size against vegetation visibility showed positive relationships, as seen in Figures 3.2a and 3.2b, thus impala density and herd size declined with increasing vegetation density across habitats. Impala density was moderately positively correlated to vegetation visibility (r=0.72, p=0.27, n=4), although not significantly so. Herd size was strongly positively correlated to vegetation visibility (r=0.96, p=0.05, n=4).

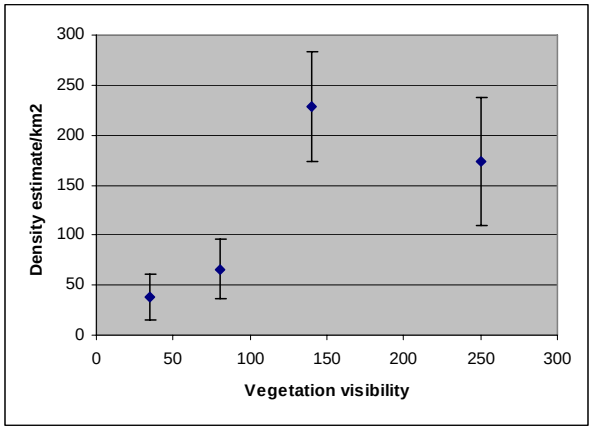


Figure 3.2a The relationship between impala density and vegetation visibility. Error bars represent (±)SE.

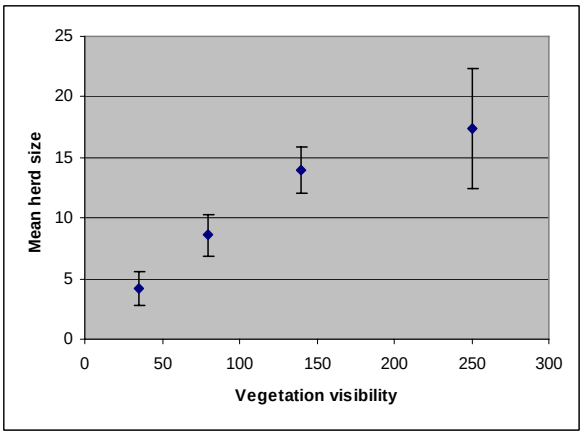


Figure 3.2b The relationship between impala mean herd size and vegetation visibility. Error bars represent (±)SE.

Impala counts in this study were timed to coincide with wild dog hunting periods, and were tested for temporal differences in impala density between these crepuscular periods, across habitats (Figure 3.3) Wilcoxon Matched-Pairs Signed-Ranks tests showed no differences between morning and afternoon in densities in any habitat; grassland (w+=10, w-=11, n=6, p<=1), *albida* woodland (w+=2, w-=8, n=4, p<=0.375), ecotone (w+=16, w-=5, n=6, p<=0.3125) or thicket (w+=1, w-=5, n=3, p<=0.5).

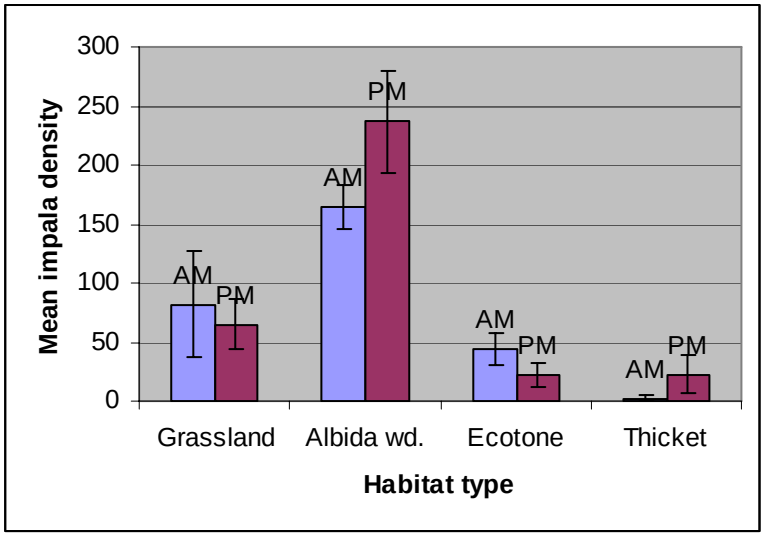


Figure 3.3 Comparison of morning (AM) and afternoon (PM) impala density estimates (impala/km^2) within different habitats. Error bars represent (±)SE.

3.4.2 Wild Dog Home Ranges and Habitat Utilisation

3.4.2.1 Home range analysis

Plots of the number of observations against home range area revealed a plateau in increasing home range area at approximately 40 observations, which accounted for 88.5% of estimated home range areas (Figure 3.4). Home range estimates for all pack years were then adjusted based on the number of observations using this non-linear regression curve.

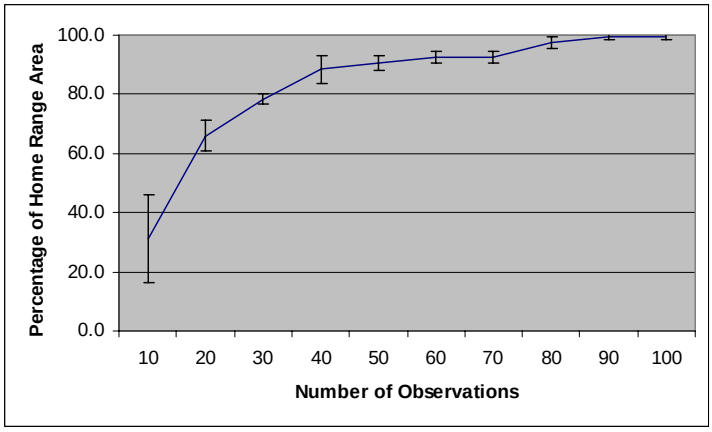


Figure 3.4 Graph showing the percentage area of home range generated from an increasing number of observations. Data were based on the mean values from two home ranges with n>100 observations.

Minimum convex polygon (MCP) estimates of wild dog multiyear ranges averaged 378km^2, ranging from 184km^2 to 665km^2 (Table 3.4). Annual home ranges were smaller on average, although not significantly so ($t=0.84$, $df=8$, $p=0.4$), with a mean size of 273km^2. The largest multiyear range occurred as a product of home range displacement as the pack shifted across the river valley floor, combined with large annual home ranges due to remote den locations in the escarpment (see Mushika pack 2000 and 2001 in Figure 3.5a)

Table 3.4 Home range sizes and overlap estimates for wild dog packs. Key: N= number of observations; 100% MCP = maximum home range estimate; Proportion of 50% Contour Area = proportion of 95% probability contour area covered by the 50% probability contour; 100% MCP overlap = percentage of total home range overlap; 50% Contour Overlap = percentage of overlap in 50% contour areas.

Pack Year	N	100% MCP (km^2)	Proportion of 50% Contour Area	100% MCP Overlap (%)	50% Contour Overlap (%)
Jeki99	50	255	0.18	n/a	0
Jeki00/01	36	141	0.20	71.5	19.9
GMA02*	112	74	0.08	0.2	0
GMA03*	103	442	0.23	32.8	8.6
Mush00*	58	459	0.17	5.7	0
Mush01	39	198	0.27	37.6	6.4
Simwen02/03*	37	345	0.13	42.1	29.3
Mean		**273.2**	**0.18**	**31.7**	**9.2**
SE		55.9	0.023	9.86	4.3

**indicates breeding pack year.*

Fifty percent probability contour core areas covered an average of 18% (SE=2.3%) of the 95% probability distribution (Table 3.4). In general, core areas received two to four times more use than would be expected from a random distribution of observations. In the case of the smallest 50% contour area, the GMA 2002 pack (Figure 3.6e), the core area received six times the expected random use. This was the result of a small home range with a heavily used den area which had many hunts occurring nearby to form the core.

Although spatial overlap in home ranges between packs reached up to 71.5% in any one year (Table 3.4 and see Figure 3.5), dogs were never observed directly encountering each other and are likely to have avoided each other temporally (Mills & Gorman 1997). Packs were observed within one kilometer of each other on only one occasion but typically were observed approximately 30km apart. Overlapping areas were shared with only one pack. Overlap in the 50% probability contour was significantly less (paired t-test, t=2.71, df=5, p=0.042) at only 9% (Table 3.4), further reducing the probability that packs would encounter each other in core use areas.

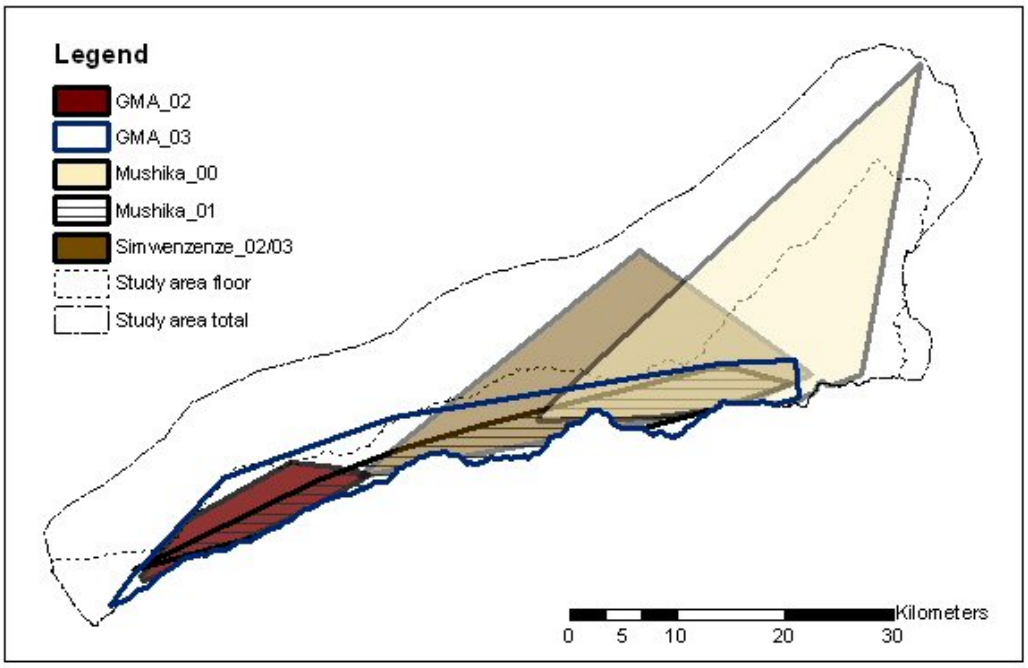


Figure 3.5a Map of 100% Minimum Convex Polygon home ranges for packs monitored by radio telemetry.

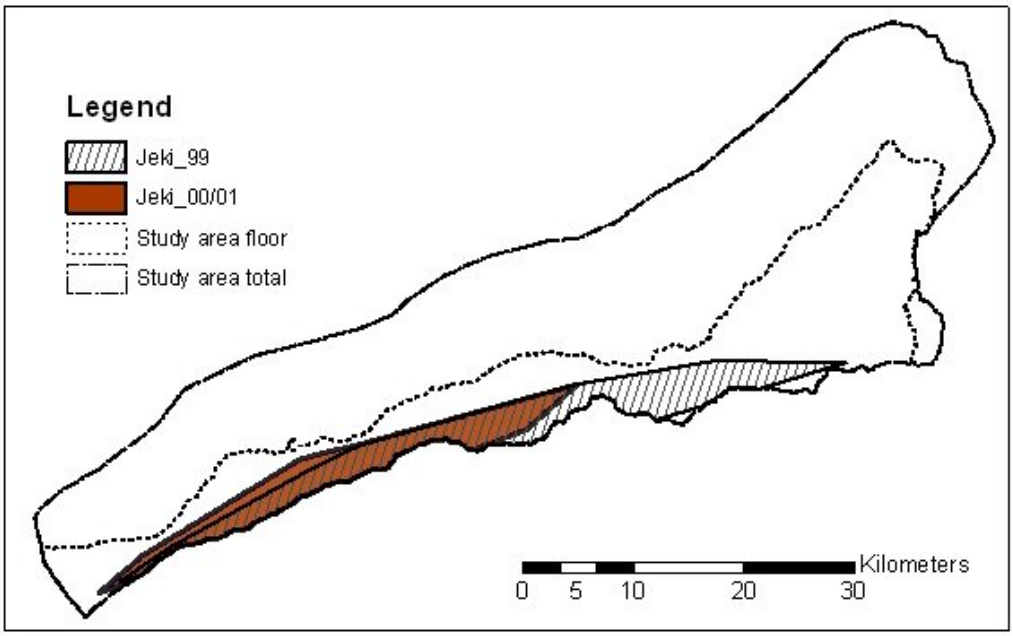
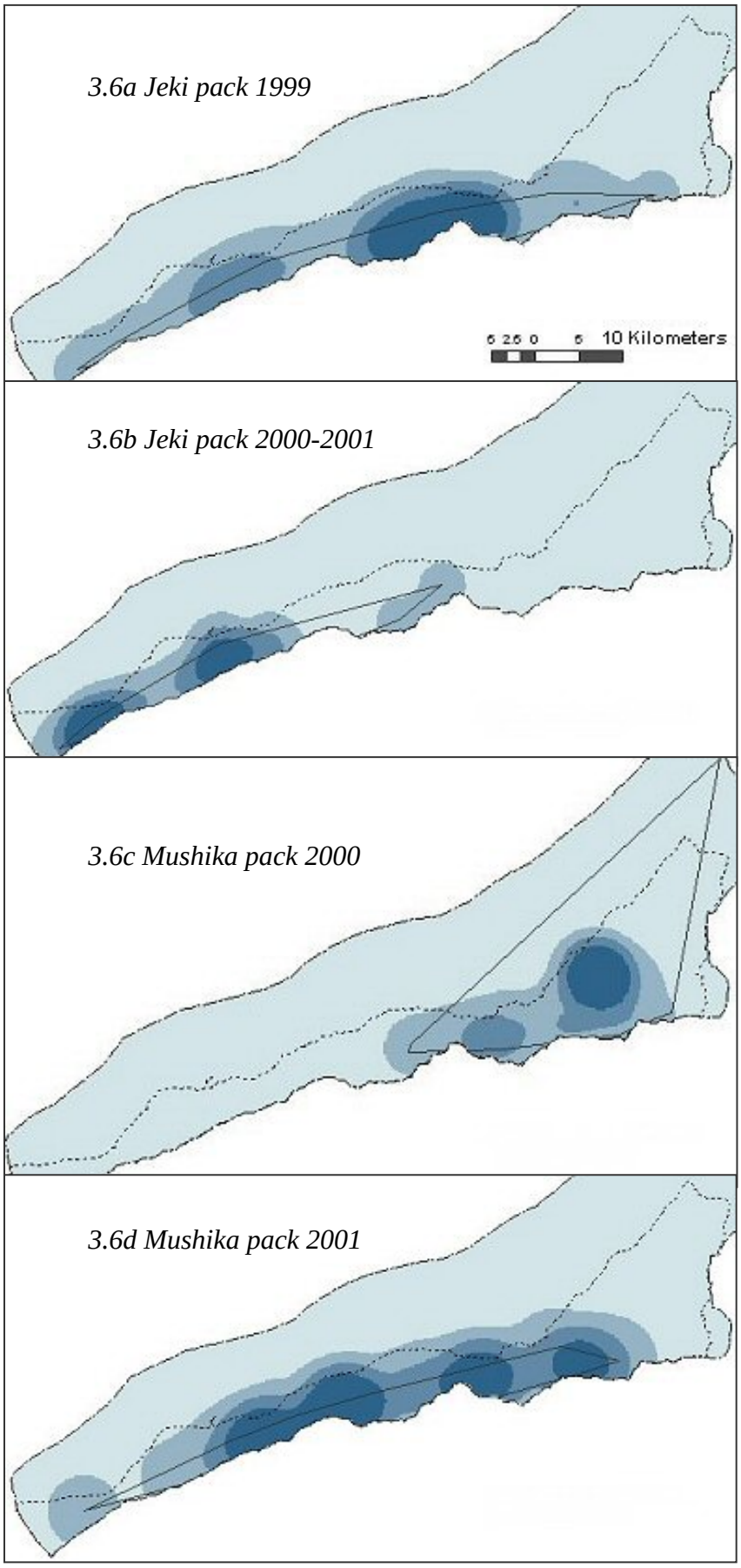


Figure 3.5b Map of 100% Minimum Convex Polygon home ranges for uncollared packs monitored by road tracking and sightings reports.

Home range data for the Jeki pack was limited to the valley floor since this pack was not radio-collared, so their home ranges did not extend into the escarpment. However, of the collared packs, only packs that were breeding were observed in the escarpment and the Jeki pack was a group of siblings (1999-2001) who did not breed. Therefore home range estimates for the Jeki pack are considered reasonably accurate. Thicket usage may be underestimated due to reduced visibility.

Figure 3.6 illustrates the 50%, 75% and 95% contour core areas of wild dog home ranges. Core areas in the escarpment were due to den locations, all other core areas were contained on the alluvial terraces of the valley floor. Packs which denned in the escarpment also maintained core hunting areas in the valley floor throughout the year (Figure 3.6c, e, f, and g).


3.6a Jeki pack 1999
3.6b Jeki pack 2000-2001
3.6c Mushika pack 2000
3.6d Mushika pack 2001
0 2.5 0 5 10 Kilometers
Legend
Study area floor
Study area total
100% MCP
Contour Value (%)
100
95
75
50

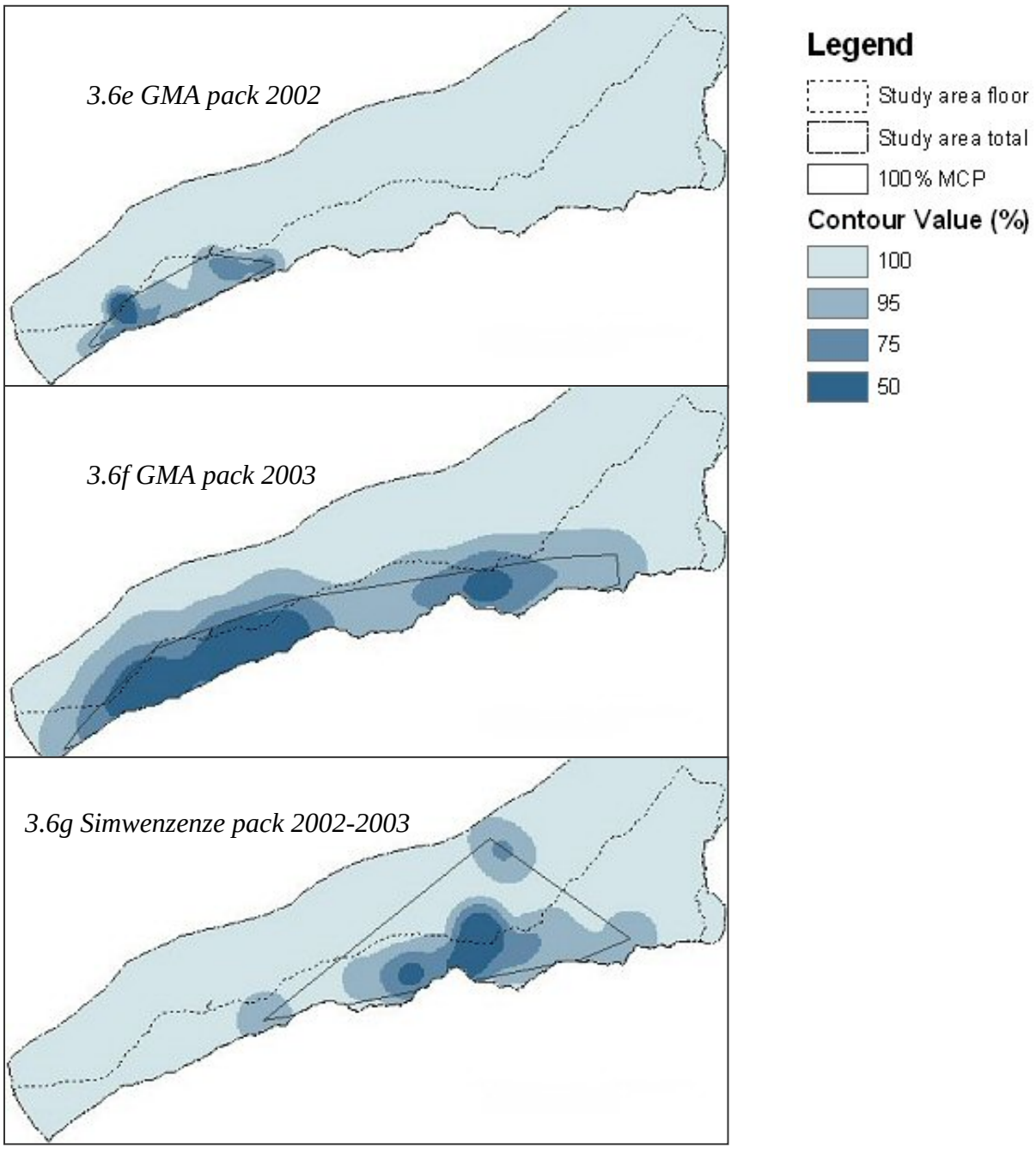


Figure 3.6 Utilisation distributions for annual wild dog home ranges, as density probability surface volume contours, showing 50%, 75% and 95% contour areas.

There was a significant positive correlation between annual home range size and the number of combined adults and yearlings (r= 0.81, df=6, p=0.028, see Figure 3.7). This relationship became non-significant if only adults were included in the analysis (r=0.36, df=5, p=0.47).

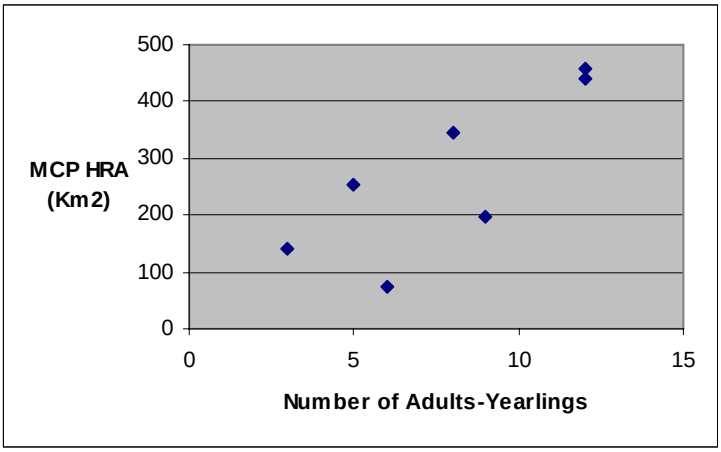


Figure 3.7 Graph of pack size against home range area estimates (100% MCP method).

There was no significant difference in the abundance of the dogs' main prey species, impala, in pack home ranges (ANOVA, df=11, f=0.27, p=0.77). Pack ranges all included similar sized areas of the two habitats containing the highest impala density, ranging from 24.6 km^2 to 28.8 km^2 of grassland (mean=26.2 km^2, ±SE=1.2), and 66.2km^2 to 97.1km^2 of *albida* woodland (mean=82.9km^2, ±SE=9.0). There was much greater variation in the size of low prey density habitats; thicket habitat sizes ranged from 17.2 km^2 to 173 km^2, and miombo from 1.2 km^2 to 220 km^2 (mean=79.8 km^2, ±SE= 47.6 and mean=83.5 km^2, ±SE=69.1, respectively). Despite larger areas of low density habitats in most breeding packs, there was no significant difference between breeding and non-breeding range sizes (t=0.978, df=2, p=0.38, data not shown).

Data from the wet season (December to March) was extremely limited, due to inaccessibility. All observations of wild dog packs involved locations on the valley floor, although only 5% of sightings utilised aerial tracking or telemetry (from n=22 sightings). There was a range of three to eight wet season sightings per year from the limited number of scouts and staff in the area. Wild dogs were never observed to enter or cross any part of the Zambezi river.

3.4.2.2 Habitat utilisation and prey selection

As might be expected from the high density of impala in the study area, the dominant prey species for the Lower Zambezi wild dogs was impala, with a value of over 80% whether assessed by hunting effort, number of kills or biomass in the wild dogs' diet (Table 3.5). Bushbuck formed the second largest component of the diet comprising 9.5% of kills, 80% of which occurred in the thickly vegetated habitats of the GMA area.

*Table 3.5 Wild dog prey selection within the study area, showing the proportion of hunting effort spent on each species and the proportion of successful kills and biomass in the diet. *n=165 hunt periods; #n=95 kills.*

Prey Species	Hunts (%)*	Kills (%)#	Biomass (%)
Impala	81.2	89.5	85.3
Bushbuck	12.1	9.5	11.3
Kudu	1.2	1	3.4
Warthog	2.4	0	0
Waterbuck	1.2	0	0
Buffalo	0.6	0	0

For general habitat selection including all activities, wild dogs showed the strongest preference for grassland habitat (Table 3.6), which comprised only 2.6% of the study area. A preference index below 0.3 demonstrates avoidance, while above 0.3 shows preference for that area. The dogs also had a strong preference for ecotone and *albida* woodland. Thicket was utilised roughly in proportion to its coverage in the study area, whilst miombo was strongly avoided.

Table 3.6 Wild dog habitat selection within the study area, expressed as an index of preference (PI).

Habitat	Wild Dog PI
Grassland	0.85
Albida Woodland	0.60
Ecotone	0.66
Thicket	0.32
Miombo	0.05

Figure 3.8 illustrates habitat utilisation per pack. The only pack to show a preference for thicket was the orphaned sibling Jeki pack (see section 2.4.1), which also preferred ecotone over grasslands and *albida* woodland. Overall hunting success was lowest in thicket areas (Table 3.8), which was also where most effort was spent hunting bushbuck (Table 3.7).

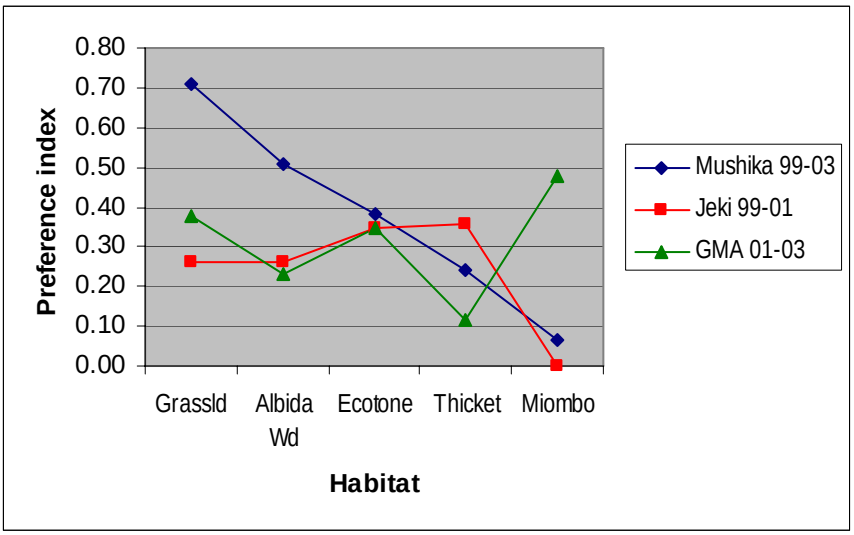


Figure 3.8 Habitat selection (PI) for individual packs.

The GMA pack preferred the escarpment miombo, then grassland and ecotone, but avoided thicket. The Mushika pack was based in the eastern end of the study area inside the National Park and had a strong preference for grassland and ecotone habitats, the two highest prey density habitats. This pack avoided both thicket and miombo. The Mushika and Simwenzenze packs were combined for this multiyear analysis because the Simwenzenze pack was formed from immigrant males and the Mushika females, and maintained the natal home range of the Mushika females.

Table 3.7 Percentage of hunting effort spent on each of the dominant prey species within each habitat type.

Prey Species	Grassld.	*Albida* Wd	Ecotone	Thicket
Impala	94.4	82.0	80.3	46.2
Bushbuck	0	8.0	13.6	53.8
Other	5.6	10.0	6.1	0

Table 3.8. Habitat selection during hunting by wild dogs, compared with hunting success (all prey species) and corresponding impala density in each habitat. Hunting success based on n=121 kills, n=237 hunts.

Habitat	Wild Dog Hunting PI	Hunting Success (%)	Impala Density (km2)
Grassland	0.55	55.8	174
Albida Woodland	0.35	57.4	229
Ecotone	0.39	52.8	66
Thicket	0.16	25.7	38

Hunting data collected from ground observations were restricted to the river valley floor habitats so miombo habitat was removed to assess wild dog hunting preferences and success in different habitats (Table 3.7 and 3.8). There was little difference in hunting success between grassland, *albida* woodland and ecotone habitats, which were all preferred by wild dogs, however hunting success was approximately halved in the thicket habitat (Table 3.8) which was strongly avoided. This corresponded to the lower prey density and visibility found in thicket habitat (Table 3.3). There was a strong positive correlation between wild dog hunting preferences and hunting success ($r=0.83$, $p=0.16$, $n=4$), although it was not significant, but the analysis had limited power due to low sample size. The wild dogs showed the strongest preference for grassland, which made up only 7% of the valley floor area. These areas had grasses less than 75cm high (Table 3.2), many of which died off to bare ground by the end of the dry season. There was therefore no restriction on visibility, combined with high prey density in this habitat. General habitat preferences follow the same trend as habitat selection for all activities (Table 3.6), except thicket was not actively avoided (PI =0.32) for general use, only hunting.

3.5 DISCUSSION

3.5.1 Habitat and Prey Species

The study site was naturally divided into an area of miombo escarpment habitat to the north, and the river valley floor which supported a diversity of habitats in the south (Figure 3.1). The vegetation mosaic formed by the horizontal alluvial terraces further divided by erosion gullies and rivers resulted in a large diversity of habitats condensed within a relatively small study area. Other wild dog study sites have generally been more homogeneous in comparison, thus the Lower Zambezi provided an ideal site to obtain more detailed data on wild dog spatial and temporal habitat selection.

Relative impala distribution over the study area habitats followed expectations based on published data, which found that impala prefer edge habitats between open and closed vegetation types (Leuthold 1970), and light woodland and grassland habitats (Estes 1991). Herd size and impala density may be interdependent and partially explain the finding that impala density and herd size increased with decreasing vegetation density (increasing visibility, see Figure 3.2). However the relationship between herd size and vegetation density also has a behavioural explanation; impala's preference for more open habitats would leave them more exposed to predation, therefore larger group sizes in open habitats carry the benefits of increased vigilance (Schenkel 1966) and a reduced probability of predation for each animal as group size increases. Impala were observed to congregate on the open grasslands and plains in the evening and disperse into other habitats to feed during the day, thus these open habitats were utilised even when food availability was limited by vegetation die-off during the dry season.

Impala densities for the study area were higher than would be expected, both on average and for the highest density recorded for an individual habitat (at 95/km^2 and 229/km^2 respectively). Average population densities at four sites spanning east and southern Africa ranged from 15/ km^2 to 80/ km^2 (Estes 1991; Jarman 1979; Leuthold 1970), although a high of 214/ km^2 was recorded in wooded savannah in Rwanda (Estes 1991). At such high densities the impala population could have adverse impacts on vegetation through overgrazing, and may also affect the species diversity of other antelope species since impala are mixed feeders and compete with more selective feeders for resources. Further study of the impacts of this species on Lower Zambezi

ecology is recommended. Impala densities in each habitat are likely to change seasonally since herbivores tend to disperse back towards the escarpment and ecotone when more water and forage is available during the wet season (Dunham 1994). However, wild dog and prey data were collected simultaneously (during the dry season) so the recorded impala densities remain relevant to the observed wild dog habitat selection.

The Lower Zambezi wild dogs had an abundance of prey. Impala formed the bulk of the wild dogs' diet which is in agreement with previous studies that found wild dogs preyed upon the most common medium sized prey (Fuller & Kat 1990; Mills & Gorman 1997; Woodroffe et al. 1997). Wild dogs were observed taking larger prey (kudu) only once; instead larger packs made multiple kills of impala in the one hunting session. This strong species preference may partially be a function of pack size, since smaller packs (<10) were observed to take more impala in the Selous (Creel & Creel 2002), while larger packs took more wildebeest and kudu. The mean pack size observed in the Lower Zambezi was 7.8 (range 3 to 12, Figure 3.7). However, in contrast to the Selous, larger prey were limited here; wildebeest are entirely absent from the area while kudu are restricted to localised areas of the Park. Therefore prey selection in this case is more likely to be simply a consequence of the relatively limited abundance of larger species.

Previous studies found that wild dog hunting success was independent of prey density (Creel & Creel 1998; Fuller & Kat 1993) and that wild dogs were found at their highest densities in low prey density areas, suggesting that competing predators were a more important determinant of range movements (Mills & Gorman 1997). In contrast to this, the wild dogs in the Lower Zambezi showed an active preference for high prey density habitats, particularly open grasslands. During hunting they avoided the lowest prey density habitat, thicket, where they had a markedly reduced hunting success (Table 3.8). The reduction of hunting success in thicket is likely to be related to the density of vegetation restricting movement and visibility. The heterogeneous nature of broadly categorised landscapes means that wild dogs can probably avoid thickets in many areas. In fact results suggest that packs who utilised thicket used it mostly for travelling and resting since thicket was avoided during hunting but used roughly in proportion to its coverage for overall usage. Utilisation of thicket may have

been underestimated due to limited access in some areas. However, thicket areas were relatively small and although wild dog kills were not always sighted it was still possible to detect the occurrence or absence of a kill. Telemetry signals indicated if the dogs became stationary to feed, and made it possible to track and observe the dogs leaving the thicket and thereby observe evidence of a recent kill including full bellies and blood on their muzzles.

3.5.2 Wild Dog Home Ranges and Habitat Utilisation

Minimum convex polygon (MCP) estimates of wild dog multiyear ranges (from 184km^2 to 665km^2, Table 3.4) were comparable to those observed in similarly wooded areas in Hwange National Park and Selous Game Reserve, at 423 km^2 and 379 km^2 respectively (Creel & Creel 2002; Woodroffe et al. 1997).There was substantial overlap in MCP home ranges at up to 72% (Table 3.4). Overlap was most likely due to the linear movement of the packs between the geographical boundaries of the river and escarpment, and the wild dogs' preference for grassland habitats in the valley floor.

Annual home ranges were small (mean =237km^2) and roughly equivalent to those observed in small fenced reserves such as Hluhluwe Umfolozi Game Reserve in South Africa (Andreka et al. 1999). The smallest home range recorded (74km^2) was for the GMA 2002 pack based in the Chiawa Game Management area where movements to the west were most likely inhibited by village settlements. No home ranges had a 95% probability contour which extended into village areas, several kilometres west of the core study area boundary (Figure 3.6). Packs rarely entered this area and always returned to the photographic safari area within a short period. However, no disappearances or mortalities were recorded during these westerly pack movements, nor reports of livestock losses from the villages. Wet season data was limited and inconclusive, but given the geography of the area and the data from dry season range movements, large seasonal wild dog range movements outside of the valley floor would be unexpected.

Contrary to previous studies, a significant positive relationship was found between pack size and home range size. Results from larger studies in Selous and Kruger found only a weak and non-significant positive correlation between adult/yearling

pack size and home range size using multiyear ranges, and no correlation using annual range data (Creel & Creel 2002; Mills & Gorman 1997). The authors suggested there was no evidence that larger packs defended larger territories and therefore resources, in fact Mills and Gorman (1997) found wild dogs were at their highest density in areas of lowest prey density. Despite the large variation in home range sizes in the Lower Zambezi, the lack of difference between both overall impala abundance, and the area of the highest density habitat in each range, provides no support for the resource partitioning hypothesis and concurs with previous studies.

In this study the correlation between pack size and home range size is likely to be a function of breeding behaviour. Those packs that bred retreated into the escarpment, while still utilising the valley floor to hunt, thereby increasing their home range size. Packs that bred successfully for more than one year had larger pack sizes. There was no significant difference between breeding and non-breeding range sizes largely because the smallest home range was for the first breeding year of the GMA pack of 5 adults. However this pack's home range increased dramatically the next year (442 km^2) when the pack was increased by eight yearlings (Table 3.4). With the exception of this single pack year, home ranges were generally smaller for packs that did not breed. Restricted access to the miombo escarpment habitat may have led to an underestimation of wild dog utilisation in this area, but the wild dogs were only ever located in the escarpment during breeding periods. During these periods the dogs remained in the low foothills of the escarpment and hunted down into the valley floor. The Zambian escarpment rises steeply from the valley floor and due to the slope and vegetation cover some areas may be low in density of preferred prey species (Estes 1991). It is therefore likely the steepest sections of escarpment form a natural deterrent to wild dog movements, except perhaps for emigrating groups who may travel over it to leave the area.

Wild dogs had a strong preference for all valley floor habitats except thickets, but despite impala density being highest in *albida* woodland this habitat was ranked 3rd in both general use and hunting preferences, favoured less than grassland and marginally less than ecotone (Tables 3.6 and 3.8). Ecotone was considerably lower in impala density than grassland and *albida* woodland but it was also preferred (Table 4.2). Ecotone habitat would have a higher abundance of bushbuck which occupies dense

vegetation (Waser 1975b), however densities of this species would be low compared to impala since bushbuck are solitary animals (Waser 1975a) and the lower visibility habitat would make hunting more difficult. Since the relationship between wild dog preferences and prey densities was not strictly linear, this suggests that wild dogs may have selected habitats to minimise interaction with competing predators, for instance when breeding.

The only pack that showed a preference for thickets, and also preferred ecotone over other habitats, was the orphaned sibling Jeki pack. This pack of 3-5 dogs would have been vulnerable to both interspecific and intraspecific competition, which may explain their active avoidance of higher prey density areas. The preference of the GMA pack for miombo was due to this packs' denning several times in the escarpment. The bulk of the *albida* woodland within their home range was in the National Park, which this pack utilised only in 2003. The GMA area is heavily dominated by ecotone and thicket, however this pack still showed a strong avoidance of thicket. The Mushika pack was based in the eastern end of the study area inside the National Park and had a strong preference for grassland and ecotone habitats, the two highest prey density habitats. This pack avoided both thicket and miombo.

Overall these results show that prey density was not a limiting factor for the Lower Zambezi wild dog population, and that in contrast to previous studies this population showed an active preference for high prey density habitats. Non-breeding home range sizes were small and limited to the river valley floor, but home range size increased during breeding periods to include remote areas of the Zambian escarpment. The effects of competing predators on the range movements of the Lower Zambezi wild dog population are further investigated in the following chapter.

CHAPTER 4: INTERPREDATOR COMPETITION

4.1 INTRODUCTION

Lions (*Panthera leo*) kill both adult wild dogs and pups, and spotted hyenas (*Crocuta crocuta*) often steal wild dog kills and can reduce the feeding success of dogs by harassment, which in turn reduces the dogs' ability to raise pups (Fanshawe & Fitzgibbon 1993; Fanshawe et al. 1991; Fuller & Kat 1990; Woodroffe & Ginsberg 1999a).

Creel and Creel (1996) compared densities of wild dogs with those of lions and spotted hyaenas across four ecosystems in east and southern Africa. They found strong negative correlations between wild dog and lion densities, and wild dog and spotted hyaena densities, and a positive correlation between lions and spotted hyaenas. Their data supported the theory that wild dog densities are limited by competition with these two carnivores. The significant correlation between lion and spotted hyaenas means that the effect of interpredator competition from these species on wild dog population success is difficult to separate. Diet overlap and subsequently increased competition was used to explain the negative correlation between spotted hyaena and wild dog density, however the competitive relationship between wild dogs and lions was less clear, and the existence of a causal link between the two, rather than a correlation, was not established. Direct predation by lions on wild dogs is a common occurrence in some areas and may explain wild dogs avoiding areas of high lion density (Mills & Biggs 1993; Woodroffe et al. 1997). Wild dogs have also been shown to avoid areas with high lion densities even when these habitats have the highest densities of wild dog prey (Mills & Gorman 1997).

4.1.1 Interpredator Competition from Spotted Hyaenas (*Crocuta crocuta*)

Wild dogs are generally found at considerably lower densities than spotted hyaenas and lions (Creel & Creel 1996). Hyaenas have been found to have a greater impact on wild dog feeding rates in areas where the hyaenas are more common and visibility is good (Creel & Creel 1996). Kruuk (1972) found that hyaenas fed at 60% of wild kills and were present at 74% in the high visibility area of the Serengeti and Ngorogoro

areas. In contrast, in more wooded areas such as the Selous and Kruger National Park, hyaenas rarely fed at wild dog kills and if present were usually in lower numbers than dogs (Creel & Creel 1996; Mills & Biggs 1993).

By measuring the daily energy expenditure of six wild dogs, Gorman et al. (1998) found the energy costs to wild dogs while hunting to be high, up to 25 times the basal metabolic rate. Therefore a small loss in food due to kleptoparasitism by spotted hyaenas may have a substantial impact on the amount of time the dogs need to spend hunting to achieve energy balance. Measurements were based on a pack which was hunting very intensively due to a high ratio of adults to dependent young, and it was estimated that if the dogs were to lose 25% of their food they would have to hunt for up to 12 hrs a day to maintain energy balance, instead of the observed average of 3.5hrs a day (Gorman et al. 1998).

Successful kleptoparasitism of wild dog kills by spotted hyaenas is mainly dependent on the numbers of hyaenas present. Although hyaenas may be present at a large percentage of wild dog kills, up to 92% (Fanshawe et al. 1991), the hyaenas often only feed after the dogs have eaten their fill (Fanshawe et al. 1991; Fuller & Kat 1990). This level of competition would not be expected to have a significant effect on the feeding success of the dogs. A study in Kenya found there were rarely more than four hyaenas observed at a wild dog kill; dogs often chased single hyaenas and no hyaenas fed before the dogs abandoned the kill of their own volition (Fuller & Kat 1990). Similarly wild dogs were frequently observed chasing and attacking hyaenas in the Selous (Creel & Creel 1996).

In contrast, Carbone et al. (1997) modelled data from a Serengeti study and found that more than four hyaenas at a kill considerably reduced the wild dogs' access time to that kill, and individual "gut fill" time for a dog often exceeded access time in this situation. An earlier study in the Serengeti (Fanshawe & Fitzgibbon 1993) of an extensively studied single wild dog pack found that the wild dogs' time at the kill was longer where there was a higher ratio of dogs to hyaenas, and that therefore larger packs of dogs would be more successful in areas of high hyaena density through improved defence of kills (Fanshawe & Fitzgibbon 1993). However, Carbone et al. (1997) suggest that the advantages of a large pack with an increased ability to defend

a kill rarely compensates for the reduction in feeding due to intrapack "scramble competition" for food. Therefore at least three important variables are involved determining the effects of kleptoparasitism: the number of wild dogs, the number of hyaenas, and the prey mass. Intermediate pack sizes of three to ten adults (Carbone et al. 1997) may be most effective in achieving a balance between the defence of kills and meeting nutritional demands for each individual dog.

Direct predation by hyaenas on wild dogs is uncommon and usually opportunistic. In the Serengeti two litters of pups were left unattended at a time of food scarcity and were killed by hyaenas (Malcolm & Marten 1982), while in the Selous two pups were abandoned due to deterioration from anthrax infection and were subsequently killed by hyaenas (Creel et al. 1995). Since pups cannot be collared and remain underground for several weeks, or at inaccessible den sites, it is often difficult to determine causes of mortality. Hyaenas may play an important role as a disease reservoir for wild dogs since they are found at higher densities than wild dogs, and often interact with them (Creel & Creel 1996).

4.1.2 Interpredator Competition from Lions (*Panthera leo*)

The negative effect of lions on wild dog populations has been widely cited (Creel & Creel 1998; Creel & Creel 1996; Mills & Gorman 1997; Vucetich & Creel 1999; Woodroffe & Ginsberg 1999a; Woodroffe et al. 1997; Woodroffe et al. 2004b). In a comparison of wild dog populations in five different countries, predation by lions was the single most important cause of natural mortality in adults, accounting for 12% of adult mortality (Woodroffe & Ginsberg 1999a). These results were an average of values across sites, but in fact high lion predation was only found in two of the five study areas. Another study confirmed the important effects of lions in one of these two sites, Kruger National Park, where lions caused 39% of pup mortality and 43% of adult deaths (Mills & Gorman 1997). It is important to note that these results are site-specific and in some populations, including south-western Zimbabwe and parts of Zambia, direct predation of adult wild dogs by lions has been negligible (Creel & Creel 2002; Woodroffe et al. 1997; Woodroffe et al. 2004b).

In addition to high densities of lions being correlated with low densities of wild dogs (Creel & Creel 1996; Mills & Gorman 1997), in Kruger National Park wild dogs were

also found at their lowest densities where prey was most abundant (Mills & Gorman 1997). Behavioural avoidance of lions could conceivably force dogs out of areas of high prey density and into high risk areas outside of National Parks and game reserves. A case study in the Ngorogoro Crater in Tanzania showed that wild dogs were observed in the area in the 1960s after a crash in the lion population. The lion population recovered, the dogs disappeared, and they have remained absent while the lion population increased five fold then stabilised (Creel & Creel 1996). No causal effects were established.

Reports across study sites suggest kleptoparasitism of wild dog kills by lions is rare compared to that seen by hyaenas (Creel & Creel 2002). In a study in Kenya wild dogs never lost a kill to hyaenas, however Fuller and Kat (1990) observed a single lioness appropriate a juvenile wildebeest killed by wild dogs; the lioness was then joined by another resulting in the dogs abandoning the kill. The lack of observed kleptoparasitism by lions may be at least partly a result of successful avoidance behaviour by wild dogs.

Some studies recommend that in-situ conservation and reintroduction programs should be focused in areas where wild dog population viability, or recovery, will not be compromised by the impact of a dense lion population (Mills & Gorman 1997; Vucetich & Creel 1999). However, since results to date are not consistent across study sites, this suggestion emphasises the need for further collection of site-specific information on the impact of lions and spotted hyaenas to ensure effective management.

4.2 OBJECTIVES

This objective of this section of the study was to determine the role of interpredator competition on wild dog home range movements and population dynamics. Specifically, aims were to:

1. Estimate the density of competing predators lion and spotted hyaena in the study area.

2 . Assess the effects of interpredator competition on;

i) kleptoparasitism of wild dog kills,

ii) wild dog movements and habitat utilisation.

4.3 METHODS

Annual surveys of lion and spotted hyaena were carried out to establish species density distributions. Density data were then compared to wild dog habitat utilisation.

4.3.1 Lion Surveys

Two methods were combined to assess lion densities in the study area:

i) Annual survey forms were distributed to all safari operators in the study area, who collected data on recognisable individuals and prides in their safari area. Data consisted of opportunistic sightings; lions were regularly tracked by safari guides as they provided a major tourist attraction, and sightings were therefore frequent (approximately once per week). Most individuals and groups were recorded by name and identifying features. A copy of the survey form is contained in Appendix 3.

ii) Photographic and sketch records were collected by AWDC throughout the season. On average, 208 field days per year were spent covering the study area to track the wild dog packs, and all lions encountered during this period were recorded. Individuals were identified using permanent scars, age, body size, mane colour and size for males, whisker-spot patterns, and any other distinguishing features such as missing tail tips and ear notches. This data was then cross-checked against sightings provided by the safari operators to compile a final annual count. Population numbers changed throughout the year; only adults and cubs surviving in September of each year were included in the annual density estimates.

Lion surveys were carried out between April to November each year, from 2001 to 2003. Surveys were restricted to the existing road network and valley floor of the study area. Annual home ranges for each pride or individual were estimated from sighting location data. Data for some animals were insufficient to develop reliable range estimates using minimum convex polygon methods, so home ranges were digitized over satellite images of the study area in ArcMap (ArcGIS 8.1), using biologically meaningful boundaries.

Eastern and western home range boundaries were based on sighting information and often followed natural landscape features, such as ridges and large tributary rivers, and also dry river beds. Although these features do not present barriers to lion

movements, they either corresponded with the limits of pride or group movements, or in some cases lions were seen to patrol and scent mark using the feature as a territory boundary. The Zambezi River was used as the southern boundary and the Zambian escarpment provided a boundary to the north.

Since no lions were collared, sightings data may have been biased towards open areas which would underestimate lion ranges. There was insufficient survey data to test this. Homes ranges were therefore extended to include all habitats up to the base of the escarpment, based on data from Mana Pools showing that lions frequently utilised thickets, jesse bush and *mopane* habitats on the Zimbabwe side of the Zambezi valley floor (N. Monks, unpublished data). Lions may occur in the escarpment area, however they generally reach highest density in areas of high prey density (Creel & Creel 1997; Spong 2002; Stander 1991, 1993). The research in Mana Pools showed only 3% of prey density occurred in the escarpment, so lion densities would be expected to be correspondingly low in that habitat. Occasionally, lions were observed to cross the river for short stays on small islands or to emigrate, however wild dogs were never observed entering or crossing the river so only mainland areas within the study area boundaries were included to assess interspecific competition.

Annual lion density in each home range was calculated. Overlap occurred in all lion home ranges, up to 100% in some cases where male coalitions overlapped more than one female pride. Once digitized, overlapping ranges created a map of intersection polygons which were partially used by several groups. The proportion of each home range used by each individual or group was used to estimate density in each polygon area. For example, if the home range of a group of four lions overlapped another range by 25%, three lions were used to calculate density in the exclusive part of their range, and one lion was added to density calculations in the 30% overlap area.

Using ArcMap (ArcGIS 8.1) “joins and relates” functions and annual data for both species, the number of wild dog GPS activity points occurring in each lion density polygon was calculated to investigate the effects of lion density on wild dog habitat use.

Wild dog activity was broken into three categories, 1) all annual GPS fixes combined 2) the four month breeding season of each pack, and 3) the non-breeding season when

home ranges increased. A four month breeding period was arbitrarily chosen based on the ten to twelve week observed denning period, plus an additional month when pups were small and often cached during hunts, thus they still restricted pack movements and made the pack more vulnerable to predation. Although pups had trouble keeping up with the pack for some months longer, the packs returned to normal home ranges soon after denning.

Lion densities obtained from mapping information from the annual lion surveys were ranked into four lion density categories of equal interval; Low 0-0.045, Low-moderate=0.046-0.090, Moderate-high=0.091-0.135, and High=0.136-0.180. The category ranking was based on lion density figures from other regions of Africa with stable lion populations, which ranged from .086 in a low density open plain habitat in Serengeti to 0.2 in Ngorongoro Crater in Tanzania, and averaged 0.127 adults/km (n=5 populations; Creel and Creel 2002). Wild dog habitat selection for ranked areas of differing lion density was analysed using Duncan's (1983) index of preference (PI) as above (3.3.2.2), comparing the number of wild dog observations found in each lion density area. The far eastern lion home range section (polygon10, see section 4.4.1) was deleted from analysis as data coverage from lion surveys was poor in this area.

Using the vegetation map composed in ArcGIS (see section 3.4.1.1) and the digitised lion density polygons, the habitat composition of the four ranked lion density category areas was calculated. The relationship between lion density and habitat composition was tested using Spearman Rank correlations.

4.3.2 Spotted Hyaena Surveys

Hyaena density was determined using highly amplified playbacks of noises found to attract spotted hyaenas (*Crocuta crocuta*), adapted from three methodologies: Mills (1985), Creel and Creel (1996), and Monks (personal communication). Where methodologies differed that used by Monks was chosen so that data would be comparable with that for Mana Pools National Park. Sounds played included noises of a bleating wildebeest calf, spotted hyaenas mobbing lions, an inter-clan hyaena fight, hyaenas squabbling on a kill (provided by M.G.L. Mills), and noises of a squealing pig (provided by N. Monks). The tracks played were varied for each survey.

Four surveys were carried out between May and November, one in 2000, two in 2002 (five months apart), and one in 2003, nine months after the previous survey. No permits were available for a 2001 survey. For the 2000 and 2002 surveys, two RCF 45.7cm, 8 ohm horn speakers wired in series and pointed in opposite directions were connected to a Goldstar TCC-320 High Power Hi-Fi car stereo and a 12V PW-100 Sharp Stereo Power Amplifier. New equipment was used for the 2003 survey consisting of two 40cm Max Trumpet Speakers (40 watts, 8 ohm, Model no. TC-1640) connected to a Sony ESPmax CD Walkman (D-E226CK) and a 12V Max Power Amplifier (Model No. SSB-60).

The sounds were played continuously for 5-minutes at a time. A five-minute pause followed, the speakers were rotated 90 degrees, and the sounds were played for another 5 minutes. The tapes were played 4 times and 50-minutes were spent at each station. Approaching hyaenas were observed by two to eight people in two to three vehicles using high intensity spotlights and binoculars. The majority of hyaenas stayed at the station until the end of the playbacks, and some arrived up to 10 minutes after playbacks were completed. The maximum number of hyaenas simultaneously in view was recorded. Hyaenas less than one year old generally remain at the den, so counts were for animals over one year of age (Mills et al. 2001). All other carnivores that appeared were noted. Hyaenas that were heard whooping but not seen at the time of the count at each station were counted. These hyaenas are thought to be itinerants who may not be members of the local clan and therefore may not be confident enough to confront the intruders (the taped animals) directly and therefore do not come in sight of the calling station , as proposed by Mills (1985).

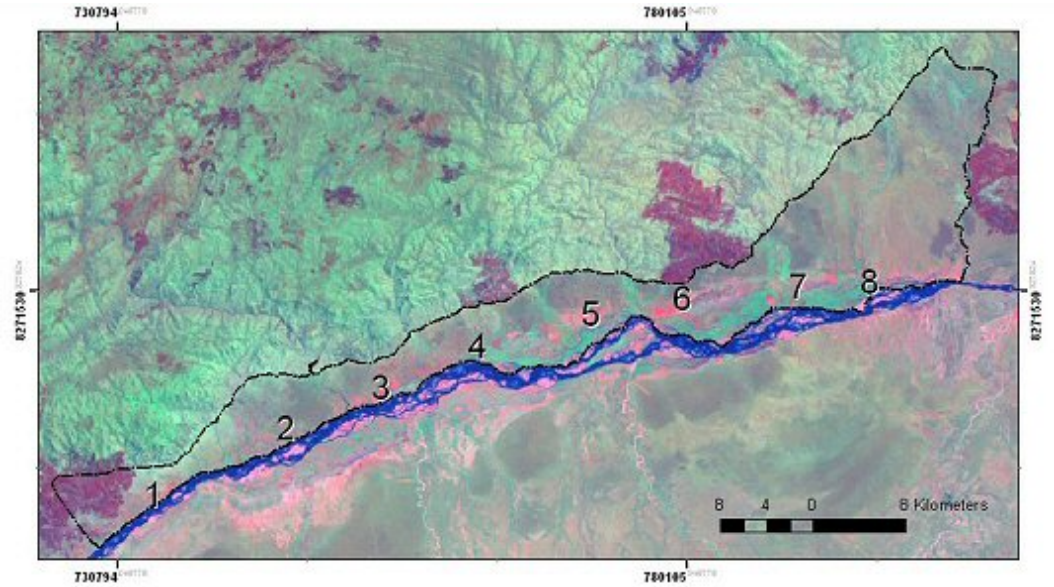


Figure 4.1 Satellite image of the Lower Zambezi, illustrating hyaena calling station locations (numbered 1 to 8). The valley floor area falling within the study site is outlined in black.

An area of approximately 360km^2 of the Zambian valley floor was sampled, along a transect of 72km of road running southwest to northeast, over eight stations (Figure 4.1). The narrow width of the valley floor meant a single transect sampled the majority of the study area, although the far north east corner of the study area was inaccessible since surveys were restricted by road access. The sample area included the Eastern Chiawa Game Management Area (GMA), from Kayila property to the National Park boundary, then 50km into the Lower Zambezi National Park safari area, to the Mushika river. The first survey was completed during the pilot project in 2000 and was only carried out over the six stations within the National Park (290km^2, over a 50km transect).

The tapes were played at night, beginning 30 minutes after sunset, over two consecutive nights. Three independent experiments were carried out in which hyaenas were located in one vehicle, and a second vehicle carried out playbacks to test the maximum response distance. During these experiments, the longest distance from the calling station in which hyaenas were observed to respond was 3km. Although experiments in this study were limited, this distance is identical to a study which used the same equipment in Zimbabwe (N. Monks personal communication) and comparable to a more comprehensive study by Mills et al. (2001) who found all respondents were within 3.2km in a similar variety of habitats (n=17 experiments).

Survey stations were chosen for good visibility and spaced an average of 9.4 km apart to minimise the chance of double counting.

4.3.3 Data Analysis

Spotted hyaena density was calculated from the mean number of respondents at each site and a response radius of 3km established from in-situ experiments. Calling stations were often less than 3km from the Zambezi River, which was not utilised by the wild dogs, so the area for each individual calling station was further adjusted by removing the area of the river, using ArcMap (ArcGis, 8.1). The area for each calling station ranged between 18.1 km^2 to 28.3 km^2 using a 3km radius. Densities were not adjusted by a probability of failure to respond to playbacks in this study (see Mills et al 2001) because they were intended for comparison with density estimates of spotted hyaenas from other studies, which had not been adjusted for non-response.

A repeated measures ANOVA was used to test for overall differences in hyaena density at each site using the four temporal surveys as dependent observations. An unpaired Students T-test compared the density of hyaenas at sites where lions were present against sites where lions were absent, and Pearson's product moment correlations were used to investigate the relationship between the density of hyaenas and the number of lions present at call-in sites.

Annual hyaena density estimates at each station were used to test for a relationship between spotted hyaena density and wild dog activity. For year 2002 the mean of the two hyaena surveys was used. Using ArcMap (ArcGIS 8.1), the number of wild dog observations (points) falling with the 3km buffer of each calling station (polygon) was obtained. Ordinary least-squares regression was used to find the model of best fit. Linear relationships were described using Pearson's product moment correlations.

The number of direct encounters observed between wild dogs and both lions and spotted hyaenas was recorded throughout the entire study, to assess the effect of kleptoparasitism on wild dogs.

4.3.3.1 Comparison across study sites.

Finally, overall densities of the three large carnivore species, wild dog, lion and spotted hyaena were compared to published data from other study sites across Africa, to assess the relative state of the Lower Zambezi populations. Least-squares regression was used to investigate correlations between densities of wild dogs, lions and spotted hyaenas across study sites. Creel and Creel (1996) previously used residual plots to determine that an exponential model of the form $y=e^{a+bx}$ maximised r^2 for comparing wild dog density against lion density, and wild dog against spotted hyaena densities. For comparison, the same exponential model was used here in a re-analysis of a modified data set, again comparing wild dog density to lion and spotted hyaena density. The model also gave good data fit in this case. A linear model ($y=a+bx$) was used to test the correlation between spotted hyaena and lion densities.

4.4 RESULTS

4.4.1 Lions

4.4.1.1 Density

Lion population density in the lower Zambezi ranged between 0.06 to 0.086 adults per km^2 (Table 4.1). Although overall lion density was moderate, the observed population was small (<50 adults) and declined over three years of study. The population had an unusual mean adult sex ratio of approximately 1:1 males to females (mean=0.97:1, ±SE=0.08). Low cub survival rates were observed, with 0% survivorship from the seven new cubs recorded in 2001 and a single new cub observed in 2002 which by the time of the survey in September was the only survivor from a minimum of three litters recorded during the previous months. There were 5 transient males, observed as singles or pairs, which moved through the area during 2001.

Table 4.1 Lion density estimates (adults/km^2) and population structure in the study area, from 3 annual surveys.

Year	Males	Females	Cubs	Total Adults	Density
2001	18	16	7	34	0.086
2002	13	14	1	27	0.068
2003	11	13	9	24	0.060

Maps of lion densities from the annual surveys show a central core of the highest lion densities, from the Chongwe River area on the western border of the National Park (Range number 2, Figure 4.2a-c), and east into the Park. A list of annual lion density figures for each area is included in Appendix 3.

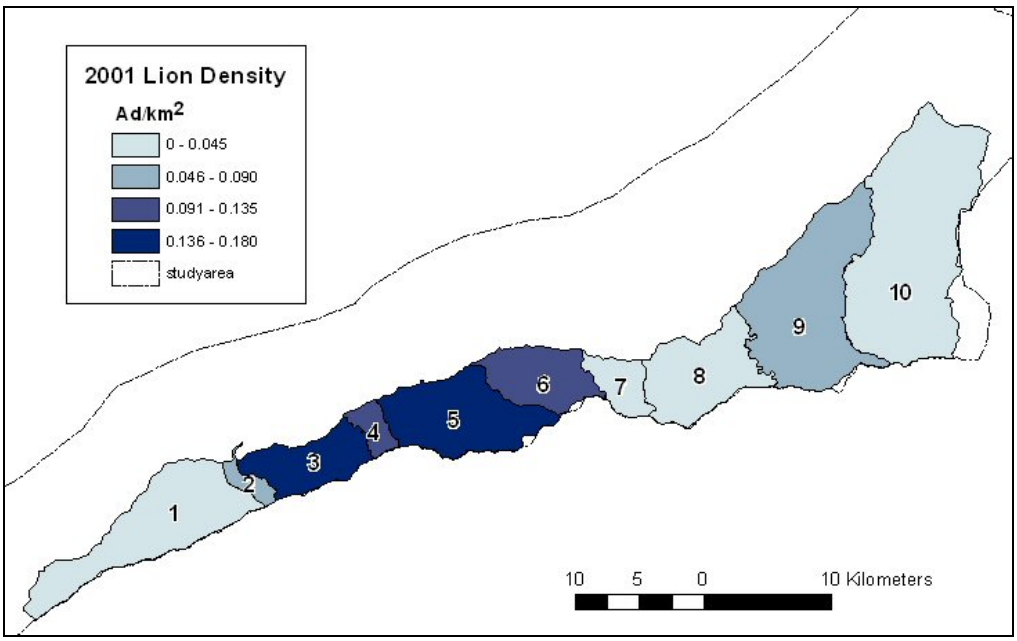


Figure 4.2a

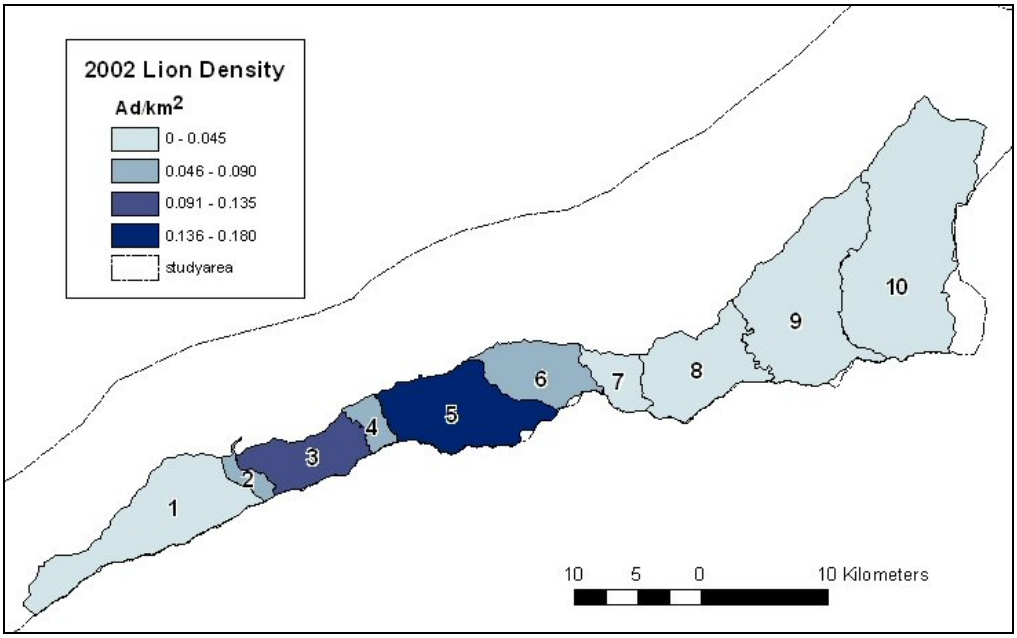


Figure 4.2b

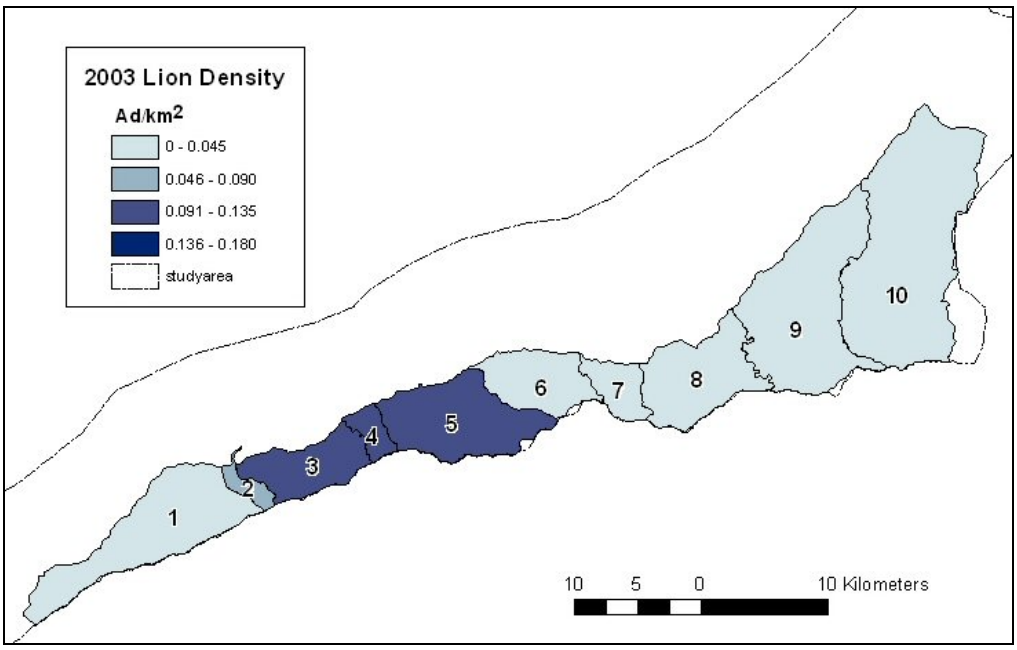


Figure 4.2c

Figure 4.2 Map of annual lion densities in the valley floor of the study area. Numbered areas of lion density were formed from lion territories and overlap estimates.

4.4.1.2 Competition

Analysis of wild dog habitat use in ranked areas of differing lion density showed temporal avoidance of high lion density areas (Table 4.2). During the breeding period, wild dogs showed preference (PI>0.3) for the lowest lion density areas over all three years, with active avoidance of low-moderate through to high lion density areas. There was a significant negative correlation between the wild dog preference index during breeding periods and areas of increasing lion density ($r=-0.60$, $p=0.038$, $n=12$). During the wild dog non-breeding period habitat selection was more varied, with a preference for all levels of lion density occurring at some stage, and no significant correlation between the wild dog preferences and lion density ($r=-0.17$, $p=0.59$, $n=12$). Notably, moderate-high to high lion densities were actually preferred above other areas in years 2001 and 2002 outside of the breeding period. As would be expected from combining these results, annual data show no clear trend of preference or avoidance over the lion density gradient ($r=0.08$, $p=0.80$. $n=12$).

Table 4.2 Index of preference for wild dog use of areas ranked by lion density during wild dog breeding and non-breeding periods, and for annual wild dog data combined. Lion areas are ranked by increasing lion density (adults/km^2): Low= 0-0.045, Low-moderate=0.046-0.090, Moderate-high=0.091-0.135, High=0.136-0.180, n= the total number of dog observations, from 6 pack years.

Year	Lion Density	Wild Dog Index of Preference		
		Breeding	Non-breeding	Annual
2001 *n=62*	Low	0.48	0.32	0.38
	Low-moderate	0.13	0.13	0.13
	Moderate-high	0.00	0.15	0.11
	High	0.21	0.46	0.39
2002 *n=134*	Low	0.38	0.21	0.32
	Low-moderate	0.07	0.45	0.25
	Moderate-high	0.22	0.73	0.49
	High	0.12	0.00	0.08
2003 *n=112*	Low	0.35	0.27	0.30
	Low-moderate	0.00	0.65	0.48
	Moderate-high	0.17	0.35	0.28
	High	0	0	0

The ranked categories of lion density are intended as a relative measure for the study area, however they are based on figures from other areas where stable lion population densities ranged from 0.065 to 0.14 adults/km^2 for freely dispersing populations, up to the highest density recorded in the geographically isolated Ngorongoro Crater, of 0.24 adults/km^2 (see Table 4.5 for details).

Direct encounters between lions and wild dogs were rare. Lions were present at 2.0% of wild dog sightings (n=440), and 2 kills (n=122) both of which were lost to the lions. When lions were encountered the dogs actively moved out of the area, in one case moving 30km in two days. On two occasions a dog pack was seen to interact with lions; once where 8 adult dogs encountered a pair of adult male lions, and another where 6 adult dogs with 10 pups encountered a lone male. In both instances the adult dogs harassed the lions from a safe distance, while the lions occasionally charged them. There was no direct contact or injury during either encounter.

Table 4.3 Percentage habitat composition for areas of ranked lion density.

Habitat	Lion Density			
	Very Low	Low	Medium	High
Grassland	8	5	9	7
Albida Wd.	14	21	18	31
Ecotone	29	13	24	38
Thicket	38	58	44	18
Miombo	12	4	5	7

Lion survey data was insufficient to establish lion habitat selection within each range, however analysis of habitat composition in areas of differing lion density (Table 4.3) showed a strong correlation between lion density and the proportion of *albida* woodland in lion ranges (Spearman Rank test; r=0.85, df=3, p<0.3). There was a weak positive correlation between lion density and increasing proportions of ecotone habitat (r=0.4, df 3, p<0.75), and a weak negative correlation for thicket (r=-0.4, df=3, p<0.75). All correlations were not significant but levels of significance were limited by the small sample size and the Spearman Rank Correlation test.

In addition to containing the highest proportion of *albida* woodland habitat, the highest lion density areas also had the lowest proportion of thicket areas (Table 4.3). Grasslands contained high impala density, however they made up only a small proportion of all lion home ranges due to their relative scarcity (mean=7.3%, ±SE=0.85). No data was collected on lion prey species, however lions were observed preying on impala as well as larger species including buffalo and zebra. *Albida* woodlands are likely to contain a high density of these larger prey; *Faidherbia albida* pods form an important part of the diet for both browsers and grazers, including buffalo (Dunham 1994; Palgrave 1997), and many areas of this habitat support a seasonal understorey of grasses.

4.4.2 Spotted Hyaenas

4.4.2.1 Density

Spotted hyaena density in the Lower Zambezi averaged 0.34 adults/km^2 (see Table 4.4 for annual data). A repeated measures ANOVA using the four temporal surveys found no significant differences in hyaena density between any of the eight calling stations (d.f=3, f=2.02, p=0.14). A table of hyaena densities at each calling station is included in Appendix 3. Each calling station contained a variety of habitats, from

albida woodland nearest the river in the south to thickets by the escarpment in the north. Data was therefore insufficient to assess hyaena habitat selection.

Table 4.4 Spotted hyaena population density (adults/km^2) in the study area, for three years. 2002 figures are based on the mean of two surveys.

YEAR	Mean Density	SE
2000	0.35	0.11
2002	0.34	0.08
2003	0.18	0.05

Lions responded to the call-ins at a minimum of two sites and maximum of four sites at each of the four surveys. The number of respondents ranged from 1 to 8 lions at any one site. There was no correlation between the number of lions present and the number of hyaenas observed responding to the call-in (r=-0.16, n=30 calling stations, p=0.38). An unpaired Students t-test also showed no significant effect of the presence or absence of lions on the density of hyenas observed at each site (df=28, t=1.48, p=0.15). Although the pride of eight lions which responded was observed to chase 2 hyaenas from the site, its presence did not prevent the hyaenas from initially responding.

4.4.2.2 Competition

Correlations between the annual number of wild dog observations within the hyaena calling station areas (radius 3km) and hyaena density showed weak to moderate positive correlations, but none were significant. In 2000 the correlation was weakest with an r-value of 0.46 (p=0.35, n=29 wild dog observations), for 2002 the r-value was 0.47 (p=0.25, n=46) and for 2003 the r-value was 0.56 (p=0.14, n=52). The strength of the correlation increased with sample size. Therefore there was no evidence of wild dog avoidance of hyaenas in the valley floor based on density figures, however the scale of temporal and spatial data for hyaenas was very limited.

Direct encounters between wild dogs and spotted hyaenas were more numerous than for lions, but rarely affected wild dog feeding success. Spotted hyaenas were observed at 8.6% of wild dog sightings (from n=440 sightings), ranging in number from 1 to 5 (mean=1.18, ±SE= 0.12). Of 122 observed wild dog kills, hyaenas were present at 17.2%. Of these the wild dogs lost their kill to hyaenas on only four occasions (3.2%). Three of these occasions involved the same pack of three to four adults and five to six

yearlings (GMA pack 2003, see Appendix 1). In all other cases hyaenas remained at a safe distance or were successfully fended off by the dogs, and claimed the carcass remains once the dogs had finished feeding and abandoned the kill.

Wild dogs did not appear to be antagonised by hyaenas when food or young pups were not present. One pack of 8 adult and yearling wild dogs lying in grassland habitat in the late evening allowed 2 hyaenas to approach and come into physical contact 3 times. A single hyaena came into contact with the same adult female wild dog each time, who initially stood to face the hyaena. On the third occasion the hyaena approached when the wild dog pack had settled to sleep and the last contact sniff prompted only a raised head from the wild dog, while the rest of pack ignored the hyaena's presence.

4.4.3 Comparison Across Study Sites

Lion and spotted hyaena densities were compared to wild dog data across study sites to assess interpredator competition. Lower Zambezi lion and hyaena density estimates both fell within the range of values observed in other study areas, with lion density comparatively low, and spotted hyaena density in the mid range of observed values (Table 4.5)

Table 4.5 Predator population densities in study sites across sub-Saharan Africa.

Study Area	Wild Dog	Spotted Hyaena	Lion
Lower Zambezi, Zambia	0.018	0.34	0.071
Selous, Tanzania	0.04[a]	0.32[a]	0.11[a]
Hwange, Zimbabwe	0.015[a]	0.17[b]	0.035[b]
Moremi, Botswana	0.04[c]		
Kruger, RSA	0.02[d]	0.45[d]	0.065[d]
Serengeti, TZ (1967-79)	0.015[a]	0.17[a]	0.079-0.094[a]
Serengeti, TZ (1985-91)	0.0067[a]	0.82[a]	0.14[a]
Ngorongoro	0[a]	1.43[a]	0.16-0.24[a]
Aitong, Kenya	0.036[e]	0.3[e]	

Data from: a) Creel and Creel, 1996; b)Woodroffe et al. 1997; c) McNutt, 1995; d) Mills and Biggs, 1993; e) Fuller and Kat, 1990

Lower Zambezi figures were taken from the mean of all survey years for wild dogs and lions, however 2003 data was omitted from spotted hyaena estimates due to suspected low response rates (see section 4.5.2). Figures from other study sites were slightly updated from those that appeared in Creel and Creel's (1996) comparable

analysis. The lion density figure for Kruger National Park was taken from figures published in Mills and Biggs (1993), where previously they were from Pienaar (1969) and personal communication, and corresponding wild dog figures were used from Mills and Biggs (1993) for spatial and temporal consistency. Lion density figures were included from Hwange National Park which were absent from Creel and Creel's (1996) figures. Correlations between lion, hyaena and wild dog densities were compared across study sites using the updated figures. Comparisons of wild dog density against competing predators only included sites were wild dogs were present.

Least-squares regression found no relationship between lion and wild dog densities across five study sites (r=0.02, t=-0.54, p=0.61, n=6, Figure 4.3b). Since wild dogs were thought to have declined due to disease outbreaks in the Serengeti area, figures from this site were then removed and there was a moderate positive relationship between lion and wild dog densities, although this was not significant (r=0.73, t=1.84, p=0.16,n=5).

There was a moderate negative relationship between wild dog and spotted hyaena density, but again this relationship was not significant (r^2=-0.56, t=-1.52, p=0.19, n=7). There was a significant, positive relationship between lion and spotted hyaena densities (r=0.91, t= 4.78, p=0.005, n=7).

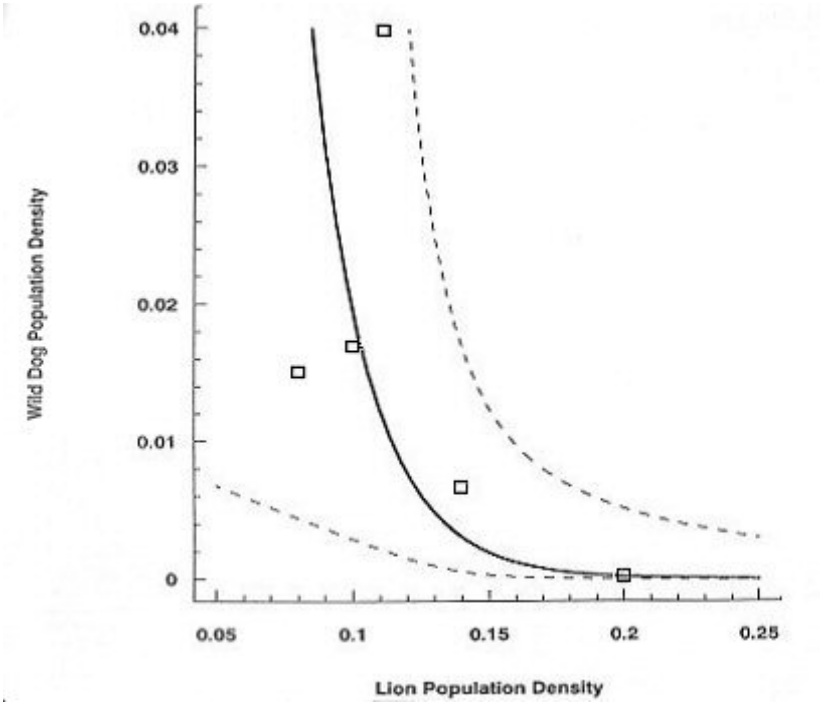


Figure 4.3a Previous analysis of the relationship between lion and wild dog densities across study sites, figure taken from Creel and Creel (1996). A negative exponential model was fitted (see text).

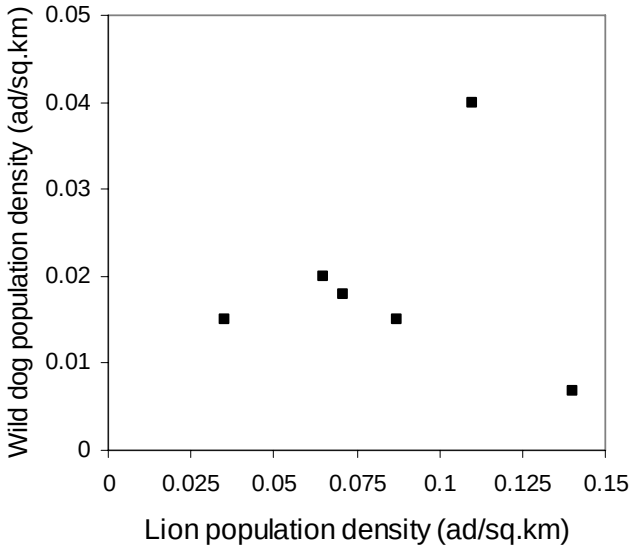


Figure 4.3b Current analysis of the relationship between lion and wild dog densities across study sites, data from Table 4.5 above. No relationship was found.

The results above prompted re-analysis of Creel and Creels (1996) published data (Figure 4.3a), using their exponential model, to investigate the significant negative relationship that was previously found between lion and wild dog densities across study sites. Where a range of densities for one study site were given in Creel and Creel (1996), the mean was used in analysis here (Table 4.5). Results matched Creel

and Creel (1996) with a strong negative relationship found between lion density and wild dog density across sites (r=-0.91, t=3.72, p=0.03), however standard residuals and leverage for one point were high and therefore were poorly fitted to the equation and also had a large effect upon the curve (Table 4.6). The Ngorongoro population data gave a leverage of 0.85 (> 4/n=0.8) and a standardised residual of -2.88 (> ±2.0). Thus one area with no wild dogs present had a significant effect on the predictive relationship.

Table 4.6 Ordinary least-squares regression details, from input data and exponential model as per Creel and Creel (1996).

Study site	Density (adults/km2)		Regression analysis		
	Lion	Wild dog	Leverage	Residuals	Std.Residuals
Selous	0.110	0.040	0.237	-3.194	-0.592
Kruger	0.100	0.017	0.293	-4.017	-0.773
Ngorongoro	0.200	0.000	0.849	-6.908	-2.883
Serengeti (1967-79)	0.087	0.015	0.401	-4.135	-0.865
Serengeti (1985-91)	0.140	0.007	0.220	-4.828	-0.885

4.5 DISCUSSION

4.5.1 Interpredator Competition from Lions

Lion population density in the lower Zambezi was comparable to lion density in Kruger National Park at 0.065 adults/ km^2, but estimates were lower than those in the Selous Game Reserve at 0.11 adults/ km^2, and recent estimates in the Serengeti of 0.14 adults/ km^2 (Table 4.5). The observed population was small (<50 adults) and declined over the three years of study. The mean adult sex ratio (1 male: 1 female) was unusual since lion populations generally have a higher proportion of females; surveys of the large and stable lion population in the Selous recorded 64% females (Creel & Creel 1997), an estimate similar to the lion population in the Serengeti which had 67% females (Packer & Ruttan 1988).

The high proportion of males was accompanied by low cub survivorship. The dominant male of the central study area died in 2001 and this may account for the high infant mortality observed in the two prides he associated with, the two largest in the study area. His death coincided with a high number of male coalitions and single males moving through the area in 2001, outnumbering the females (Table 4.1). Five of these males were not seen again from 2002 onwards. Infanticide is common in lion populations during male takeovers (Whitman et al. 2004). The immigration of new male groups into the area suggests that dispersal mechanisms in the lion population were not as compromised as those in wild dogs. Lions were often observed crossing to islands in the Zambezi River, and several were identified after crossing to the directly opposite Mana Pools National Park.

Lion density surveys were restricted to the valley floor, so results do not represent density estimates for the entire National Park. However, lions have been shown to reach highest density in areas of high prey density (Creel & Creel 1997; Spong 2002; Stander 1991) and in the study area prey was concentrated on the alluvial terraces of the river valley where vegetation is diverse and water abundant. Research in the Zambezi River valley in Mana Pools, directly across the river from the Lower Zambezi, showed only 3% of prey density occurred in the escarpment and lion densities were correspondingly low in that habitat (N. Monks unpublished data). Therefore, figures presented here are likely to be an over-estimate for lion density throughout the National Park. Lion density was positively correlated with the

proportion of *albida* woodland within each lion range, however grassland areas were relatively small and scattered and therefore any habitat preferences for this area would be probably be difficult to detect using this method. Grasslands generally adjoined *albida* woodlands and lions were frequently observed in both habitats.

In striking contrast to other studies which have shown wild dogs avoid high lion and prey density areas (Creel & Creel 2002; Creel & Creel 1996; Mills & Gorman 1997), wild dogs in the Lower Zambezi avoided high lion density areas only during the breeding season and demonstrated preference for these areas during other times of the year. Lower Zambezi lion densities were positively correlated with the proportion of the highest prey density habitat within each range. Mills and Gorman (1997) demonstrated that wild dogs in Kruger National Park avoided high impala density areas due to high lion density, even though impala was by far their favoured prey (81.0% of biomass). However, in that study lions were a major cause of adult mortality (43%), which was not the case in the Lower Zambezi (see Table 2.1).

Broken hill country was actively preferred by the wild dogs in Kruger National Park (Mills & Gorman 1997), and miombo woodland itself is certainly not unsuitable for wild dogs since this habitat dominates much of Zambia and Tanzania and is one of the major habitats in the Selous Game Reserve, which contains a large and stable population of wild dogs (Creel et al. 2004). In this study the miombo areas would be considerably lower in prey than the river floor, if only due to more limited water availability. Although the escarpment appeared to be a geographical barrier to wild dog movements in this study, there were large areas of low lion density available to the east and west of the high lion density core area (Figure 4.2) which results suggest were under-utilised. Interpredator competition was cited as the most likely cause of wild dogs' avoidance of high prey density areas in Kruger, through resource competition and the threat of direct predation by lions. The seasonal variation in habitat selection by the Lower Zambezi wild dogs implies a threat of intraguild predation since high prey and lion density areas were avoided during breeding, but it also implies a lack of interpredator competition for resources since these areas were heavily utilised at other times of the year.

These results have important management implications for wild dog populations, by suggesting that wild dogs may successfully compete with other large predators where there are sufficient refuge areas available for breeding. The wild dog habitat selection observed here may be specific to the geography, prey densities and habitat composition in the Lower Zambezi. However, they may also be applicable to other areas in central Africa, including eastern Zambia and portions of the Rift Valley system. The South Luangwa National Park in Zambia is part of the southern most section of the Great Rift Valley, with the Luangwa River forming its eastern boundary. The Luangwa River valley extends south to meet the Zambezi River in the eastern end of the Lower Zambezi National Park. Reports of wild dog sightings from the South Luangwa National Park suggest seasonal wild dog movements also occur there. The dogs are observed in the valley floor safari area only during periods either side of the breeding season, and quite probably retreat into the escarpment to den. The South Luangwa National Park covers an area of over 9000km^2 and is surrounded by adjoining GMAs (Jachmann 2000). This area could potentially support a large and viable population of wild dogs.

Further investigation of the temporal use of refuge areas could be applicable for the management of smaller wild dog populations, particularly in fenced reserves containing populations of other large predators. Instead of focussing wild dog conservation in areas with low overall lion densities or managing interpredator competition, areas containing a combination of poorer prey density habitats and high prey density habitats may provide sufficient refuge for wild dogs, depending on habitat type.

4.5.2 Interpredator Competition from Spotted Hyaenas

Spotted hyaena densities in the Lower Zambezi (see Table 4.4), fitted within the range of hyaena densities observed in other study sites across sub-Saharan Africa (0.17 to 0.82 adults/km^2). Estimates were similar to those in the wooded habitats of the Selous Game Reserve and Kruger National Park (Table 4.5).

Hyaena response rates appeared to drop by the 3rd survey (Table 4.4), even though the audio tracks used for calling were varied and no more than two surveys per year were carried out (three of the four were at least eight months apart), as recommended by

Mills et al. (2001). Non-response was not determined experimentally, however results for 2003 were probably not representative of total hyaena densities; increases in both the number and frequency of hyaenas sighted in comparison to previous years were reported by safari guides, including up to 43 hyaenas observed on one kill in the area of one calling station. However, only 35 hyaenas responded in total over 8 sites in 2003. 2003 figures were therefore dropped from the population estimate (used in Table 4.5.). Assuming equal probabilities of non-response at each site, figures still give a relative indication of hyaena density per area and were included in analyses of annual data. Although the pride of eight lions which responded at one hyaena playback site was observed to chase two hyaenas from the site, its presence did not prevent the hyaenas from initially responding. These results agree with Mills et al. (2001) who found no effect of lions on hyaena response.

Spotted hyaena densities were not adjusted for non-response so they are a conservative estimate. Future surveys should include more experiments to measure response rates in different habitats to utilise the non-response model proposed by Mills et al. (2001), together with a reward system to avoid habituation and increased non-response to playbacks.

Spotted hyaenas had minimal effect at wild dog kills, stealing carcasses at only 4% of them. These figures are very similar to those from the Selous study, where spotted hyaenas were present at 18% of wild dog kills and ate at only 2% (Creel & Creel 1996). However, a substantial reduction in wild dog feeding rates due to hyaenas was found in the open plains habitat in the Serengeti where hyaenas ate at over 70% of wild dog kills (Kruuk 1972). Previous research suggested that more wooded habitats reduce the impact of interpredator competition by reducing the probability of kill detection (Creel & Creel 1996; Mills & Biggs 1993), and because hyaena density and clan structures differ in these environments and fewer hyaenas generally arrive at wild dog kills. Findings in the Lower Zambezi further strengthen this argument.

Despite the lack of competition at kills, hyaenas appear to have to affected wild dog pup survivorship in the last year of this study, and wild dog habitat selection during breeding periods (see section 2.4.1.2). Predator competition is likely to have caused the long distance den moves observed in the wild dog population, where two packs

shifted over 20km over a two week period (section 2.4.1.2). It was difficult to accurately identify causes of pup mortality due to restricted den access and visibility, so predators may have had a greater effect than detected. Predation is often cited as the main cause of pup mortality in other studies (Woodroffe et al. 1997; Woodroffe et al. 2004b).

There was no significant correlation between wild dog and spotted hyaena densities (section 4.4.2.2), however information on range and habitat utilisation for hyaenas was limited here in comparison to that for wild dogs and lions. Other studies have found lion and hyaena densities are positively correlated (Creel & Creel 1996), so the effect of interpredator competition from these two species is difficult to separate.

4.5.3 Comparison Across Study Sites

Analysis of updated figures on wild dog, lion and spotted hyaena densities showed no significant correlation between wild dog and lion densities, in contrast to previous research by Creel and Creel (1996) who detected a significant negative relationship based on data from four ecosystems. Data analysed in this study used similar figures but added two study sites (Lower Zambezi and Hwange). The main difference in findings was due to the omission of the Ngorongoro data which was not included in this analysis since no wild dogs were present at this site (Figure 4.3). If this data is removed from the data used in Creel and Creel's (1996) study the relationship between wild dog and lion densities no longer exists ($r=0.047$, $t=-0.07$, $p=0.52$). The cause of the wild dog's disappearance from the Ngorongoro is not known, therefore it was considered dubious to include data from this site for lack of a causal link and since it becomes the key point in suggesting a predictive relationship between lion and wild dog densities.

The negative correlation between wild dog and lion densities found by Creel and Creel (1996) was later used as a basis for modelling wild dog extinction probabilities (Vucetich & Creel 1999), which then found that wild dogs were extremely sensitive to competition with lions and subsequently recommended management of interspecific competition in wild dog conservation strategies. Since the correlation did not hold due to a difference of one study site and with new data added, caution should be exercised

in extrapolating those findings into evidence of a sound ecological relationship and a basis for long-term species management.

Creel and Creel (1996) acknowledged that the inclusion of the more recent Serengeti estimates was questionable, and re-ran the correlation without data from this site but found the relationship continued to be strongly negative. Although lion populations were increasing at the time that wild dogs declined to near local extinction in the Serengeti area, there were also viral disease outbreaks which have been cited as one likely cause of the population decline (Alexander & Appel 1994; Ginsberg et al. 1995a; Woodroffe 2001). High densities of sympatric large carnivores may have been a contributing factor through interpredator competition or disease transmission (Creel et al. 2004; Vucetich & Creel 1999), but the population was also small and susceptible to local extinction from stochastic events, its extinction predicted in 1979 (Ginsberg et al. 1995a). In any case, if the figures from the Serengeti site were removed here there was in fact a moderate positive correlation between lion and wild dog densities, although this was not significant (section 4.4.3). The deletion of data points is subjective, however it serves to illustrate that the previous findings of a negative correlation between lion and wild dog densities was effectively based on two sites where wild dogs declined due to unknown causes.

The Selous wild dog population is estimated to be one of the largest remaining in Africa, with the highest recorded density of dogs, although there is a comparable population in northern Botswana. Lion density in the Selous was judged low compared to other populations and this was proposed as an important factor in the wild dogs' success there (Creel & Creel 2002). In fact the lion density figure published for Selous was almost double that of Kruger National Park, and several times higher than that of Hwange National Park (Table 4.5). Wild dog densities were correspondingly low in Kruger and Hwange compared to the Selous.

There seems to be no consistent relationship across study sites between lion densities and their observed effect on wild dog populations. Lions were identified to be a major cause of wild dog mortality in Kruger NP which had low lion density (Mills & Gorman 1997), while lion density in the Lower Zambezi was higher than Kruger and yet lions were not a major cause of wild dog mortality, and avoidance of high lion and

prey density areas was observed only during the breeding season. More data from a larger number of sites would be required to establish if any type of consistent relationship exists between these two species.

A simpler explanation may be that the relationship changes with variations in other related ecological factors. There is substantial evidence that wild dogs do avoid high lion and prey density habitats in some protected areas (Creel & Creel 2002; Creel & Creel 1996; Mills & Gorman 1997), and this may also be correlated with avoidance of spotted hyaenas since lion and hyaena densities are positively correlated. Although not significant in this analysis, the negative relationship between wild dog and hyaena density was more evident than that for lions. This corresponds with evidence of hyaenas causing a decrease in wild dog feeding rates, which could have important impacts on wild dog energy balance (Carbone et al. 1997; Fanshawe & Fitzgibbon 1993; Gorman et al. 1998; Kruuk 1972), and the more limited evidence of direct predation (Woodroffe et al. 2004; this study), particularly on wild dog pups. Increased resource competition from hyaenas is likely given their larger diet overlap with wild dogs (Mills & Gorman 1997) compared to diet overlap between wild dogs and lions.

Findings from the Lower Zambezi wild dog population suggest that competition theory, where increased resources lead to decreased competition, may explain the seasonal avoidance by wild dogs of high predator and prey density habitats. With sufficient cover from vegetation and a high density prey base, interpredator competition over resources would be reduced in high prey density habitats, while the threat of direct predation may have induced wild dog avoidance of these areas during breeding periods when packs are more vulnerable. Mills (1995) observed a parallel rise in both lion and wild dog densities during a time of drought in Kruger National Park, which further supports competition theory. This conflicts with an alternate hypothesis published by Creel (2001); that higher prey density actually increases interpredator resource competition for wild dogs due to the increased value of a carcass over live prey, combined with the wild dogs' hunting success and vulnerability to kleptoparasitism by larger predators such as lions. This hypothesis was based on data from two study sites and the previously recorded negative relationship between wild dog densities and lion densities across study sites. More

detailed information on prey densities and related hunting success and kleptoparasitism across study sites is needed to further clarify this supposition.

The evidence suggests that management of interpredator competition from both lion and spotted hyaenas should be assessed on a site by site basis, along with other interacting ecological factors, including the nature and density of habitat and other possible threats to wild dogs. This is an important consideration given the economic value of all large carnivores to ecotourism in Africa, which is intrinsically linked to the conservation value of protected areas. Areas that support a high diversity of large carnivores are generally more appealing to tourists, and wild dog management should not be unnecessarily focussed towards areas containing low levels of competing predators.

CHAPTER 5: GENETIC ANALYSIS

5.1 INTRODUCTION

5.1.1 The Role of Genetics in Wildlife Conservation

Population declines may be caused by a range of environmental and ecological factors, including overexploitation, pollution, the impacts of introduced species, as well as by stochastic events of a demographic, environmental or genetic nature (Brook et al. 2002). Loss of habitat and increasingly fragmented landscapes contribute to species decline by interfering with natural dispersal mechanisms and population dynamics, particularly of highly mobile large mammal species. Habitat fragmentation can interrupt natural dispersal patterns, alter philopatry and mate selection, and effect juvenile survival (Bjørnstad et al. 1998; Boudjemadi et al. 1999). Reintroduction and artificial augmentation of populations of endangered species may therefore play an increasingly important role in conservation management, to compensate for compromised gene flow and lack of population recolonisations in fragmented landscapes.

Reintroduction presently has a limited role in African wild dog conservation; current recommendations suggest the priority is to maintain extant populations *in situ* (Woodroffe & Ginsberg 1999; Woodroffe et al. 2004). Early reintroduction programs of captive wild dogs had limited success largely because the dogs were naïve to competing predators or had underdeveloped hunting skills (Scheepers & Venzke 1995). A range of reintroductions using a combination of wild caught and captive dogs have since been more successful (Woodroffe & Ginsberg 1999; Woodroffe et al. 1997). More recently, reintroduction and translocation are being used in South Africa to develop and manage a metapopulation of wild dogs by utilising a network of small fenced reserves (Moerhrenschlager & Somers 2004; Woodroffe & Ginsberg 1999; Woodroffe et al. 2004). This strategy is management intensive, therefore preserving larger protected areas that sustain viable populations has received first priority for wild dog conservation strategies for areas outside of South Africa (Woodroffe et al. 2004). Nevertheless, while perhaps not required for larger free-ranging populations, reintroduction or augmentation may have a more important role to play in managing the remaining small, free-ranging populations where natural recruitment is compromised. Ideally, any reintroduction program should utilise animals that are

genetically unrelated to avoid inbreeding, while still maintaining the genetic integrity of the population and its capacity to respond to selection pressures. Therefore the collection of information on the genetic diversity of populations is an important component of any conservation project.

Genetic factors also contribute to population viability by interacting with other pressures. For example anthropogenic threats and habitat fragmentation can lead to population decline, resulting in inbreeding. Inbreeding further reduces survival and fecundity, and the continued interaction of these factors can carry a population into an "extinction vortex" (Gilpin & Soulé 1986). However, in very small populations, which are particularly vulnerable to local extinction through stochastic events (Ginsberg et al. 1995), more immediate factors are likely to be more deterministic of population survival, including environmental variables, natural catastrophic events, and demographic stochasticity (Harcourt et al. 2001; Hedrick & Miller 1992).

Genetic information has become increasingly important in setting evolutionarily significant units (ESUs) for management purposes, a concept proposed to define the minimum unit used in conservation and avoid debates over definitions of species (Ryder 1986). The definition of ESUs has changed over time and continues to be debated (Crandall et al. 2000; Fraser & Bernatchez 2001; Kelt & Brown 2000; Moritz 1994, 1999; Ryder 1986). From earlier concepts based on strictly phylogeographical genetic data, there is now a strong argument for inclusion of more ecological data and a focus on adaptively significant genetic variability (Crandall et al. 2000). The strictly phylogenetic approach to species management may be particularly limited in small populations of endangered species, where the indication of differentiation, or lack of it, may simply be the result of small sample size (Fraser & Bernatchez 2001).

Two components were previously used to define ESUs; "reproductive and historical isolation, and adaptive distinctiveness" (Crandall et al. 2000). Limitations of this definition included: 1) that ESUs are less likely to be found in highly mobile species with a high level of gene flow, ie many large mammal species, including wild dogs, and 2) that many genetic techniques do not necessarily survey loci that are adaptively important (Fraser & Bernatchez 2001; Hedrick & Miller 1992). More recent definitions incorporated the "ecological exchangeability" of genes rather than

maintaining emphasis on the existence of "distinctiveness". Ecological adaptations include morphology, demographic characteristics, and life-history traits which should be heritable (Crandall et al. 2000). Fraser and Bernatchez (2001) emphasised that focusing on ecological exchangeability ignored the fact that genetic distinctiveness may represent an important evolutionary step towards speciation, and suggested a more flexible approach combining various aspects of previous definitions of ESUs, depending on each specific situation. They termed this approach "adaptive evolutionary conservation". These approaches give more scope for managing adaptive differences rather than just gene flow, and also differentiate historic from recent gene-flow.

Although distinctive genetic divergence may still be used to determine ESUs, from a practical management viewpoint populations within ESUs are often further broken down into Management Units (MU), to determine appropriate policies for translocations and maintaining population differentiation (Moritz 1994). Manel et al. (2003) give a current definition of the MU as "populations with significant divergence of allele frequencies at nuclear or mitochondrial loci regardless of the phylogenetic distinctness of the alleles, (i.e. demographically distinct populations that should be managed to ensure the viability of the larger evolutionary significant unit, subspecies, or regional populations)."

5.1.2 Genetic Effects on Populations

Given sufficient generation time, genetic effects can have important implications for the persistence of any population. These effects include loss of genetic diversity, inbreeding depression, outbreeding depression, and mutational accumulation (Frankham et al. 2002).

Genetic drift is the loss of alleles by chance, and this process occurs more rapidly in small or declining populations. Rare alleles are the most sensitive to genetic drift and are lost easily (Frankham et al. 2002). Further loss of alleles will eventually lead to reduced heterozygosity (Amos & Balmford 2001). Genetic variability is lost slowly, since loss is dependent on the number of generations the population has spent at its reduced size. The long-term effects of the loss of genetic variability on populations are still debated to some extent. As discussed in a review by Hedrick and Kalinowski

(2000), when populations contract and genetic variation is reduced, deleterious alleles may be "purged", leading to only short-term effects. However, Amos and Balmford, (2001) show that evidence for this is limited; inbreeding depression is reduced by purging to limited degrees and only in some populations. Uncertainty about the effectiveness of purging is reiterated in Brook et al. (2002), particularly in small populations where inbreeding generally continues. Regardless of this phenomenon, loss of genetic variability reduces the capacity of a population to respond to selection.

On a more contemporary time scale, when large amounts of genetic diversity have been lost individuals may be forced to breed with genetically similar conspecifics, leading to inbreeding depression from a lack of heterozygosity (Brook et al. 2002; Hedrick & Miller 1992). This is often symptomatic in populations which have declined dramatically via other causes, but the genetic effects of inbreeding then become causal and further contribute to decline. Inbreeding depression is caused by mating with genetically similar individuals, and is a function of effective population size and generation time (Amos & Balmford 2001; Brook et al. 2002). Effective population size is the size of an ideal population that would lose genetic diversity at the same rate as the actual population; for example this takes into account the population structure, sex ratio, and generational overlap rather than the absolute population size (Frankham et al. 2002). Most deleterious alleles are recessive and only expressed in the homozygous state, therefore their expression increases as effective population size becomes reduced. Brook et al. (2002) carried out a study on a range of taxa, including 20 threatened species, and used population viability analysis (PVA) to model the effect of inbreeding on extinction risk. Inbreeding, at the level of 3.14 lethal equivalents per diploid genome, was found to increase extinction risk by 25-30% in population sizes ranging from 50 to 1000 individuals. However, the effect was dependent on time; all populations were modelled right through to extinction which required a minimum 60 years for the mammals studied.

Although the effects of inbreeding were controversial at first, there is now an abundance of studies on the various effects of inbreeding depression (Hedrick & Kalinowski 2000). Early studies by Ralls et al. (1988) and Ballou and Ralls (1982) provided evidence of inbreeding costs on juvenile survival and fecundity in a variety mammal species. Effects on juvenile weight in captive wolves were detected by

Laikre and Ryman (1991). Hedricki et al. (1992) found inbreeding effects on male mating success as well as fecundity, and suggested previous estimates of inbreeding effects were probably underestimated since many were carried out on captive populations where there were no environmental stresses, predators or competitors. Amos and Balmford (2001) reviewed evidence of inbreeding depression effects at the population level and suggested that where environmental stresses (stochastic events) may lead to population crashes, inbred animals were more likely to die. Brook et al. (2002) emphasised the effects of inbreeding on all stages of the lifecycle, which should be taken into account in all PVA, rather than the earlier focus on inbreeding effects on juvenile survival.

Inbreeding and loss of genetic variation is likely to be less of a problem in populations of highly mobile species, where more gene flow is maintained. Active avoidance of inbreeding has been detected in a variety of species, ranging from skinks (Stow & Sunnucks 2004) to African wild dogs (Girman et al. 1997; McNutt 1996). Population structure is also an important consideration in the detection of loss of genetic variation, particularly in group-living species, since co-ancestry can result in high levels of relatedness and lower genetic variation (Spong et al. 2002).

Small populations exhibiting inbreeding effects may require introduction of unrelated individuals. However, outbreeding depression is an important consideration when developing reintroduction and translocation policies (Pitra et al. 2002), even though there is limited data on its significance in populations. Outbreeding depression is a reduction in fitness resulting from crosses between distantly related individuals, which can be a problem in some endangered species (Hedrick & Miller 1992). This effect is generally less of a problem than inbreeding depression, and requires high levels of variation between populations to come into effect. However, there is a strong argument for preserving local diversity and a caution against introducing alleles that do not coincide with local adaptations, for example seasonal breeding variations, the introduction of more deleterious alleles, and the introduction of diseases that local populations may not be able to adapt to (Amos & Balmford 2001). Any management program should consider all available evidence of previous gene flow patterns and aim to mimic realistic genetic exchange.

The last genetic effect which may affect population viability is mutational accumulation; chance mutations which result in deleterious alleles that accumulate over time (Frankham et al. 2002). The build up of deleterious alleles in small populations requires several generations, especially in sexually reproducing species, although there is some evidence there is a greater effect in smaller populations of <100 (Amos & Balmford 2001). This accumulation may take 100-200 years in outbreeding populations, and is more of a concern in asexually reproducing populations (Amos & Balmford 2001; Frankham et al. 2002). Inbreeding is more of a concern since it increases the chances of expression of deleterious alleles through altered gene frequencies.

5.1.3 Relevance to African Wild Dog Conservation

African wild dogs have a short generation time (approximately 2 years) and occur at low densities. As a consequence they have the potential to lose genetic variability relatively quickly compared to some large mammal species. However, they also demonstrate behavioural avoidance of inbreeding through their dispersal methods, where full-sibling mating is actively avoided by dispersal of single-sex sibling groups. Long-distance dispersal also increases gene flow. Nevertheless, habitat fragmentation and restricted dispersal may impact on wild dog outbreeding behaviour in some populations.

There have been several previous studies of wild dog genetic diversity in various African populations, spanning a geographic range from Kenya to the Republic of South Africa (Girman et al. 2001; Girman et al. 1997; Girman et al. 1993). Early studies of mtDNA genetic variability and morphology in wild dog populations detected two clades in eastern and southern Africa, which were originally thought to be sufficiently distinct to be classified as separate subspecies (Girman et al. 1993). More recent sampling of a larger number of populations found mtDNA haplotypes were not geographically restricted but covered a more recent and extensive admixture zone, which included populations in Botswana, Zimbabwe, and south-eastern Tanzania (Girman et al. 2001, and see Figure 5.2; Methods for sample locations). The phylogenetic relationship of the mtDNA control region haplotypes and their frequency in eastern and southern regions can be seen in Figure 5.1. Zambia lies in the middle of the admixture zone but no data from this country were available for

Girman et al.'s (2001) analysis.

Girman et al.'s (2001) study used mtDNA DNA control region sequences and 11 microsatellite loci to assess seven populations of wild dogs. The level of genetic diversity in free-ranging populations was comparable to that found in other large carnivore populations. All the sampled populations were relatively large and stable in comparison to the Lower Zambezi population. Girman et al. (2001) did find relatively reduced levels of genetic diversity in captive wild dog populations, and there is a possibility that smaller and more isolated populations in the wild may show similar reductions.

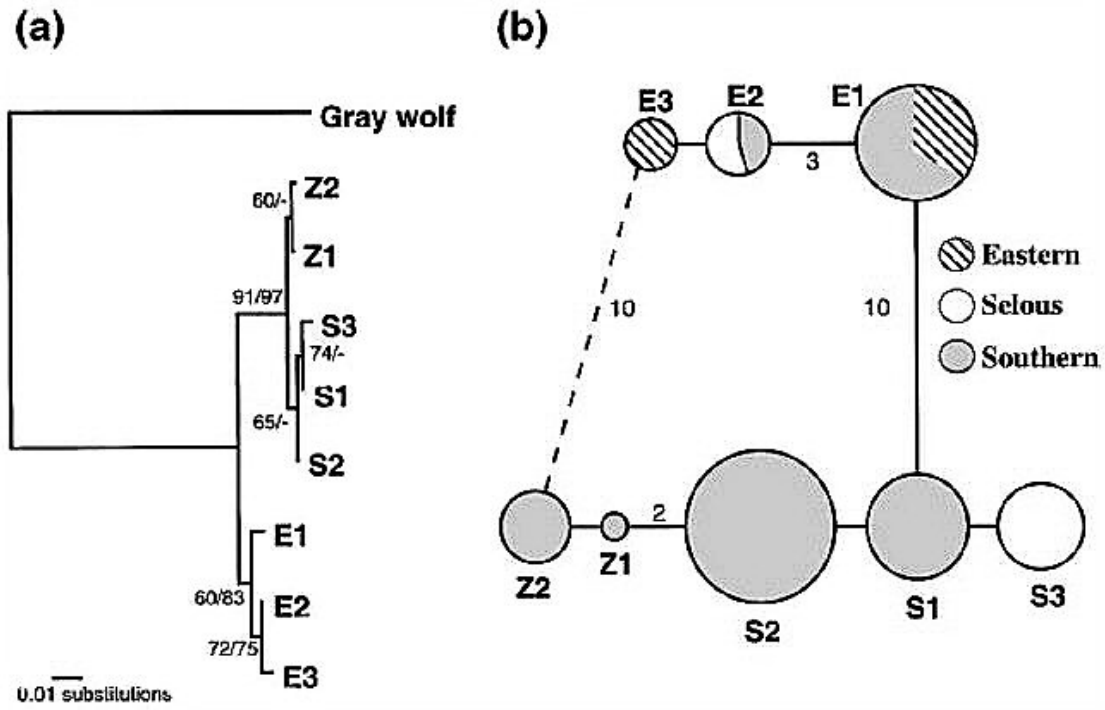


Figure 5.1 Phylogenetic relationships of wild dog control region mtDNA haplotypes as described by Girman et al. (2001). Figure (a) is a distance neighbour joining tree (Tamura and Nei, 1993, gamma correction, α=0.5) showing bootstrap support at nodes for neighbour joining (numerator) and maximum parsimony (denominator) trees from 1000 replicates. Figure (b) is a minimum spanning network with the proportional sizes of the nodes indicating the frequency of haplotypes in the entire sample. The frequency of each haplotype in eastern (Masai Mara and Serengeti), Selous, and southern (all others) is indicated by shading. The number of substitutions differentiating haplotypes is shown (where different from 1), and an alternative link between eastern and southern genotypes is shown by a dashed line.

Girman et al.'s (2001) analysis found that populations were generally differentiated from each other with regard to mtDNA haplotype frequency and microsatellite allele frequency. The exceptions to this were the Masai Mara and Serengeti populations which were geographically contiguous, and Namibia which was restricted by a small sample size of only six dogs. All populations had at least one unique allele, with a maximum of three unique alleles found in the Selous. Allele frequencies differed between southern and east African populations, but were shared among populations in each region. Zambia lies in the middle of these two regions, between the east African Masai Mara, Serengeti and Selous populations, and the well sampled southern populations of Kruger, Hwange and Okavango.

Conservation management should mimic gene flow between contiguous populations (Crandall et al. 2000) and increase connectivity, therefore there is a need to identify the most closely related populations to Zambian wild dogs. The present study aims to assess the first samples from Zambian wild dogs and compare their genetic diversity to other African wild dog populations. Information on historical and contemporary population structure is investigated here to provide insights into the phylogenetic history and population dynamics of the Zambian population. This information is vital for developing sound conservation strategies.

Girman et al. (2001) suggested that climate change during the Pleistocene period and its effects on rainforest expansion and the Rift Valley may have been sufficient to cause the divergence of east and southern African wild dog clades. Information from the Zambian populations may provide further insights into this theory since Zambia lies south of the Rift Valley and north of previously sampled southern African populations. Hewitt (2000) provided a review of evidence for climate change during recent quaternary ice ages and its effects on speciation and genetic population structure, for a variety of species. In tropical zones, forests moved lower in mountainous areas, and tropical mountains provided a stable, moist habitat which conserved older species as well as generating new ones. Wieczorek et al. (2000) also discuss shifts in vegetation, rainforest expansion and contraction during the Pleistocene age. The Rift Valley ecosystem has been proposed as contributing a barrier to gene flow in a variety African animals, including mammals, birds and

amphibians (Freitag & Robinson 1993; Pitra et al. 2002). Therefore, the forests of Rift Valley mountains could conceivably have been a barrier to wild dog gene flow during these ice age fluctuations.

Two other hypotheses are proposed by Girman et al. (2001) to explain the divergence of the two wild dog clades; firstly, the distribution of miombo forests which interrupted the distribution of other species, including canids. However, miombo is a preferred habitat for wild dogs in some areas so miombo forest alone is an unlikely barrier to gene flow. Secondly, the derivation of current wild dog populations from refugia in western or central Africa was proposed, rather than divergence *in situ* in eastern and southern populations. This last hypothesis assumes no geographical barriers to wild dog dispersal.

A single west African wild dog museum skin was sampled in the previous study, and found to have a distinct mtDNA haplotype unique to this population (Girman et al. 2001). Overall, mtDNA and microsatellite results to date suggest that populations in west, eastern and southern Africa must all be conserved to preserve the current levels of differentiation and genetic diversity in the species, and more information is required from western and central African populations to shed more light on the phylogenetic history of the species.

5.1.4 Genetic Techniques

The combination of maternally inherited mtDNA DNA and highly variable nuclear microsatellite markers has been widely used to assess species' phylogenetic history, population structure and genetic diversity, and to infer demographic characteristics such as dispersal behaviour.

Mitochondrial DNA (mtDNA) control region sequence has a high mutation rate, no recombination, and traces only the maternal line (Frankham et al. 2002). An early study of mtDNA in 100 species of animals including fish, birds and mammals, showed the utility of using this form of DNA for a wide variety of phylogenetic studies (Kocher et al. 1989). Consequently, mtDNA DNA was quickly adopted to establish measures of genetic distinctness, for use in the management of wild and captive populations (Ashley et al. 1990; Avise & Nelson 1989; Hedrick & Miller

1992). Apart from the above mentioned studies of African wild dogs, mtDNA has been used to determine the phylogenetic structure of populations in several other highly mobile canid species (Lehman et al. 1991; Pilgrim et al. 1998; Randi et al. 2000; Roach et al. 2001; Vila et al. 2003; Vila et al. 1999; Wayne et al. 1992).

Microsatellites are particularly useful for population studies, due to their high mutation rate and associated level of diversity per locus, their location throughout the genome, and their co-dominant mode of inheritance (Frankham et al. 2002). Microsatellites have been widely used to gain insights into population and social structure in carnivores (Girman et al. 1997; Kim et al. 2001; Roach et al. 2001; Spong et al. 2002). Patterns of dispersal can be inferred from parentage assignment methods, and have been found to increase estimates of dispersal rate and scale compared to field observations (Telfer et al. 2003; Zenger et al. 2003).

The current study utilised non-invasive sampling methods through the collection of faecal samples for genetic analysis using both mtDNA and microsatellites, to enable comparison of Zambian wild dog populations with those previously analysed by Girman et al. (2001).

5.2 OBJECTIVES

Genetic samples from the Lower Zambezi were analysed using mtDNA control region sequences and 11 polymorphic microsatellite loci to determine:

1) The levels of genetic diversity in the population

2) The Zambian population's place in the phylogenetic history of wild dogs

3) Current population structure, and levels of differentiation from previously studied free-ranging wild dog populations.

This information contributed to an assessment of the status of the Lower Zambezi population, and identified which, if any, wild dog populations may be a suitable source of stock for translocations into Zambia to augment declining populations.

5.3 METHODS

5.3.1 Sample Collection

Thirty eight samples were obtained from three Zambian wild dog populations; 30 wild dogs were sampled from the Lower Zambezi population (*n*=3 tissue, *n*=4 blood, *n*=23 faecal samples, see section 2.3.1.1 for collection methods). Additionally, 1 faecal sample was obtained from Kafue National Park, and 7 faecal samples from South Luangwa National Park. Blood and tissue samples were taken from individuals immobilized for radio-collaring or snare removal. Faecal samples were collected opportunistically; in the Lower Zambezi only individuals of known identity were sampled, while samples from South Luangwa and Kafue National Parks were randomly collected by safari guides from unidentified wild dogs. Additionally for this study, 41 African wild dog DNA samples were obtained from the University of Pretoria in RSA, representing populations from the Transvaal in RSA (21 samples), Namibia (9 samples) and Botswana (11 samples).

Fresh faecal samples obtained in the field were either refrigerated and extracted within three days, or frozen and stored at -4˚C for up to four months before extraction. Samples from three dogs had been collected during the pilot study prior to incorporation of the genetic aspect of the project and these samples were stored in 70% ethanol for a two year period (two of these samples yielded sufficient mtDNA for analysis).

5.3.2 DNA Extraction

DNA was extracted from faecal samples using a QIAamp DNA Stool Mini Kit (QIAGEN; available from www1.qiagen.com). The supplied protocols were observed with the following alterations:

A larger amount of stool was extracted due to low product from initial extractions. Three scrapes (approximately 220mg each) were taken from the outside of each faecal in an effort to target wild dog epithelial cells rather than DNA from prey. The scrapes were pooled and mixed with 4.8mL of ASL buffer and homogenized, and 2mL of this mixture was then aliquoted for each of two replicate DNA extractions. Replicates were spun down then 1.4mL of the supernatant was removed and used for step 4 of the QIAamp DNA Stool Mini Kit protocol, which was then followed for the remainder of the extraction process.

Blood samples

A QIAamp DNA Stool Mini Kit was used for extraction of DNA blood samples, with the following modifications: 3 X 200µL of blood was taken from each sample, and each aliquot added to 1.6mL of ASL then spun down for 2 minutes at high speed (14000rpm on Eppendorf MiniSpin Plus Personal Micro Centrifuge, MBCO No. 90412-0207). The supernatent was then used as per Step 8 of the QIAamp DNA Stool Mini Kit protocol, which was then followed. This procedure omitted the addition of the inhibitex tablet for removal of PCR inhibitors from stool samples.

Tissue samples

The ear-notch tissue samples were sent to the University of Pretoria and extracted by standard salting out protocols using treatment with Sodium Dodocyl Sulphate and proteinase K, and subsequent phenol/chloroform extraction (Sambrook et al. 1989).

5.3.3 Amplification and Sequencing

5.3.3.1 Mitochondrial control region

Lower Zambezi population maternal lines were confirmed from field observations. Two to three samples from each generation of each maternal line were sequenced to confirm maternity and haplotype (*n*=24 samples in total). A total of six maternal lines were sequenced from Zambia: four from the Lower Zambezi, one from Kafue National Park and two from South Luangwa National Park populations. Maternal lines from the three geographical regions represented by the samples from the University of Pretoria were sequenced and added to the mtDNA analysis.

Amplification

Canid-specific primers were designed to overlap the 381-bp sequence of control region I of the mitochondrial genome sequenced in African wild dogs by Girman et al. (2001; Genbank Accession number: AF335724-32). Due to the presence of prey DNA in the faecal samples used in this study, the general vertebrate primers used by Girman et al. (2001) were not suitable for this study. Therefore canid-specific primers were designed which overlapped the sequence used by Girman et al. (2001) and covered all the variable sites. The new primers began at position 93 of Girman et al.'s (2001) sequence (the first variable site occurred at 171-bp) and overlapped at the 3'-end by a further 22-bp. Primer sequences were forward primer 5'-

ACTATTCCCTGATCTCCCCC-3' and reverse primer 5' - CCTGAAGTAAGAACCAGATGCC-3'. The forward primer was labeled with an M13(-29) tail, the reverse with an M13(-38) tail, according to the tailed primer methods developed by Oetting et al. (1995).

The mtDNA fragments were amplified using Polymerase Chain Reaction (PCR) in a 20µL reaction volume containing 2.5mM $MgCl_2$, 50mM KCl, 10mM Tris-HCl (pH 8.4), 5mM dNTP mix, 1 unit of *taq* polymerase, 20 pmoles of each primer and ~ 10ng template genomic DNA. PCR amplifications were carried out on three 96 well PCR machines; PTC-100, PTC-200 and PTC-200 Gradient Cycler, (MJ Research Inc); the Gradient Cycler was primarily used for optimizing PCR reactions.

Faecal derived samples were run with initial denaturation at 95˚C for 5 minutes, then 40 amplification cycles of 95˚C denaturation for 30s, 55˚C annealing temperature for 30s, and extension at 72˚C for 30s. A final extension at 72˚C was carried out for 5 minutes after the last cycle. Blood and tissue samples were run with the same thermal cycling profile with the modification of an annealing temperature of 60˚C, run for 35 amplification cycles.

Faecal derived samples which resulted in weak amplification product were then run for 45 amplification cycles at an annealing temperature of 50˚C. If amplified PCR product remained faint when run on a 2% [w/w] agarose gel, subsequent sequence was often weak and difficult to read. In this case the illuminated bands of PCR product were cut out from the agarose gel, dissolved in 30µL 1X TBE buffer [90 mM Tris-borate, 2 mM EDTA] at room temperature (Sambrook et al. 1989), and used to seed a second booster PCR of 45 cycles at 50˚C annealing temperature.

Agarose gel electorphoresis

Prior to sequencing PCR products were visualized on a 2% [w/w] agarose gel (Progen). The gel was prepared by melting 1g of agarose into 50mL of 1X TBE buffer [90 mM Tris-borate, 2 mM EDTA] (Sambrook et al. 1989). After cooling to approximately 50˚C 0.5µg/mL of ethidium bromide was added. The gel was then poured into a casting plate to a depth of ~ 4-7mm and a comb inserted. After cooling and comb removal, 5µL of PCR product DNA and 2µL of agarose gel loading buffer

(15% Ficoll Type 400 [Pharmacia], 0.25% bromophenol blue and 0.25% xylene cyanol) were mixed then added to the gel in an electrophoresis tank containing 1X TBE buffer, then run for 20mins at ~90-100 volts to separate bands. Size standards were run every 10 wells to determine DNA concentration and size. Bands were visualized on an Ultra.Lum UV trans-illuminator, and recorded using either a DS -34 Polaroid camera, 2 megapixel Kodak camera or ImageMaster VDS version 2.0 (Pharmacia Biotech).

Sequencing

PCR product was cleaned up, from PCR reagents, double stranded DNA and salts solution, using either a JetQuick PCR Purification Spin Kit (Genomed) according to the supplied protocol, or by using a 5:1µL ratio of PCR product to ExoSapIT enzyme (Amersham Biosciences) incubated for 45 min at 37°C, then 15 min at 80°C for enzyme inactivation.

I) In-house sequencing

A SequiTherm Excel II DNA sequencing kit – LC (Epicentre Technologies) was used for the sequencing reaction, with IRD- labelled primers. These primers were complimentary to the M13 tails on the canid-specific primers used for initial PCR. DdNTPs (2µL) were plated out in separate wells and 4µL of bulk mix was added to each. Bulk mix for each sample contained 1X buffer, 1pmol each of IRD700 primer and IRD800 primer, 8-9µL of cleaned up PCR product, 4U of SequiTherm Excel II Polymerase, and water added to make up a total of 17µL. The sequencing reaction was run with an initial denaturation at 95˚C for 5 minutes, then 35 amplification cycles of 95˚C denaturation for 30s, 60˚C annealing temperature for 15s, and extension at 72˚C for 60s. A final step of 72˚C for 60s was run after the last cycle. This reaction was based on methods by Sanger et al. (1977).

Samples were mixed with loading buffer, denatured at 95˚C, and loaded onto a 0.25mm thick, 41cm 4% nondenaturing polyacrlylamide gel containing; 3.1mL stock Acrylamide (Acrylamide PAGE 40% aqueous solution, Amersham Pharmacia Biotech), 6.2mL of 5x TBE [90 mM Tris-borate, 2 mM EDTA](Sambrook et al. 1989), 13.1g of urea (BDH AnalaR, Merck), 210µL of 10% Ammonium Persulfate (APS) (Amresco), 28µL of TEMED (Progen), and water. The sequence was then

visualised using a LI-COR 4200 automated sequencer according to manufacturer's instructions.

Sequences were visualized using the software programs Base ImagIR Image Manipulation (v4.00), Base ImagIR Image Analysis (v4.10), and SCF File Creation (v4.10) (LI-COR). Sequences were edited by eye via chromatograms using the program Sequencher (Gene Codes Corporation, www.genecodes.com). The sequences were then aligned using the program GeneDoc (Nicholas & Nicholas 1997).

II) Commercial DNA Sequencing

Samples sent out for sequencing were pre-prepared by mixing 8-10µL of purified PCR product with 3.2pmole of primers made up to 12µL mix with Milli-Q water, as per instructions from Westmead DNA (www.westmead-dna.org.au). These were then sequenced on an Applied Biosystems ABI PRISM 3100 Genetic Analyser.

5.3.3.2 Microsatellite alleles

All DNA samples collected in Zambia (n=38) were analysed for 11 microsatellite loci known to be polymorphic in African wild dogs and identical to those used by Girman et al. (2001) in their analysis of other African wild dog populations.

PCR amplification of faecal samples was carried out on the same machines used for mtDNA PCR (above), in a 20uL volume reaction containing: 5mM $MgCl_2$, 50mM KCl, 10mM Tris-HCl (pH 8.4), 5mM dNTP mix, 1 unit of HotStarTaq DNA polymerase (QIAGEN), 20 pmoles of each primer and approximately 40ng DNA (proportion of target DNA unknown). Microsatellite primers were labeled with M13-tails and PCR reactions contained complimentary IRD700 dye labels for electrophoresis.

Faecal samples were run with hot-start denaturation at 95˚C for 15 minutes, then 45 amplification cycles of 95˚C denaturation for 30s, 48˚C annealing temperature for 30s, and extension at 72˚C for 30s. A final extension at 72˚C was carried out for 5 minutes after the last cycle.

Blood and tissue samples were run with the same protocols as above except PCR reactions contained 1 unit of commercial *taq* polymerase and 2.5mM $MgCl_{2,}$ and 10ng target DNA. These samples were run for 40 amplification cycles with an initial denaturation at 95˚C for 5 minutes, and an annealing temperature of 55˚C.

Primers that failed to effectively amplify faecal samples were further optimized in PCR reactions. After extensive optomisation trials, Primer L155 was run with the addition of DMSO solution (Dimethyl Sulfoxide, 1%), and L173 and L677 were run with the addition of Tween-20/NP40 (0.1%). Primers L366 and L423 were amplified with *taq* polymerase.

Polycacrylamide gel electorphoresis

Microsatellites were size separated following electrophoresis on a polyacrylamide gel using the LI-COR 4200 automated sequencer as for mtDNA above, using a 25cm, 6% nondenaturing polyacrlylamide gel. Gel images were manipulated using Base ImagIR (v4.0) and final scoring of alleles was carried out using the program Gene ImagIR™ software (v 4.05, Scanalytics), and by eye. An M13 control sequencing reaction was run at intervals across each gel as an absolute size marker, which allowed scoring and sizing of microsatellite data for analysis.

The principles of a multiple PCR approach (Piggot & Taylor 2003a; Taberlet et al. 1996) were followed for faecal samples, in addition to individual optimization of each primer. Heterozygous alleles were run until scored at least twice, and faint samples and homozygous alleles were run until scored consistently a minimum of three times. Results were checked against field records of observed parentage. Problematic samples were run in PCR up to 7 times. If, after multiple PCR, allelic dropout was suspected from visual comparison of band intensity with true homozygotes, and comparison with family pedigree, the sample was dropped from final analysis.

5.3.4 Statistical Analysis

Zambian African wild dog mtDNA d-loop control region sequences and microsatellite loci data were compared to those generated by Girman et al. (2001). Girman et al. (2001) collected 228 samples from seven free ranging African wild dog populations in eastern and southern Africa (Figure 5.2): Masai Mara National Park in Kenya; Serengeti National Park in Tanzania; Selous Game Reserve in Tanzania; Hwange National Park in Zimbabwe; Moremi Wildlife Reserve in Botswana; north-west Namibia; and Kruger National Park in the Republic of South Africa (RSA).

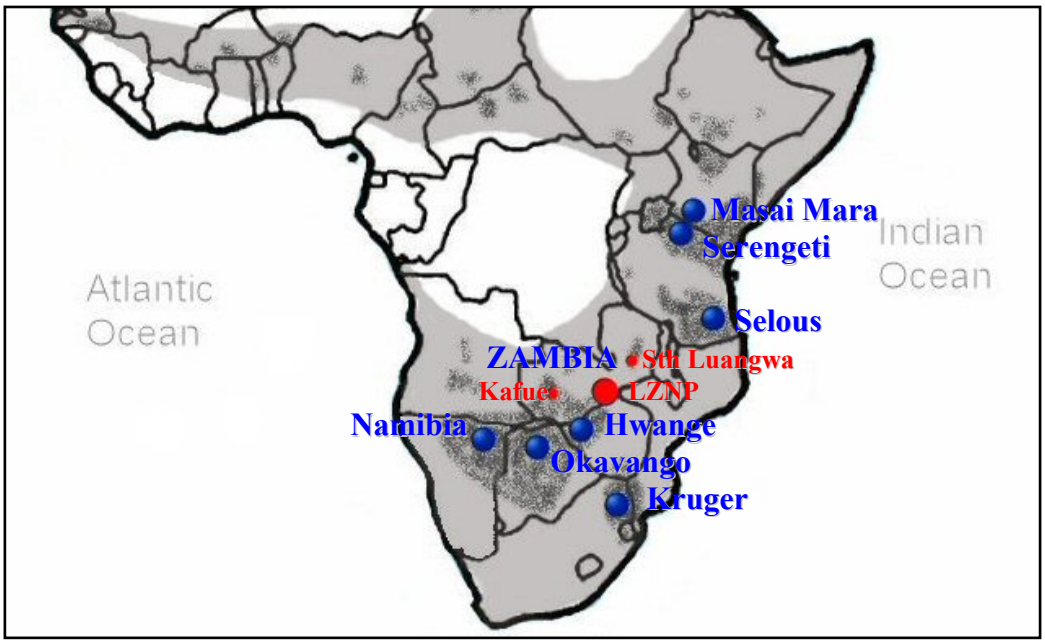


Figure 5.2 Map of geographic distribution of sampled free-ranging African wild dog populations. Zambian populations are shown in red, and populations previously sampled by Girman et al. (2001) in blue. The smaller red symbols represent the small sample sizes from Kafue and South Luangwa.

Statistical analysis incorporated a variety of methods to derive diversity indices, phylogenetic history, and population genetic structure. Methods were chosen to allow direct comparison with data from Girman et al. (2001), with additional up-to-date statistical analysis techniques carried out to resolve the data further.

I) Mitochondrial DNA analysis was used to determine: phylogeny and sequence divergence; population structure; and genetic diversity.

II) Microsatellite loci data were used in both genic and genotypic analysis, and to assess genetic diversity and test for evidence of population bottlenecks.

5.3.4.1 Analysis of mitochondrial data

Analysis was carried out on the 403-bp sequence formed by combining the sequence used by Girman et al. (2001) with that of the canid-specific primers. The first 93-bp of Girman et al.'s (2001) sequence, although free of variable sites, was included so results would be comparable to those of the previous study.

For mtDNA analysis the samples consisted of; samples from Zambian populations from this study which amplified successfully (n=33), those supplied by the University of Pretoria (n=41), and the results from Girman et al. (2001) for samples throughout eastern and southern Africa (n=228). Samples were pooled to assess geographical distribution and gene flow, and to compare Zambian population genetic diversity to the populations in other geographic regions.

Tests for mtDNA genetic diversity were carried out by examining haplotypic diversity (h) and nucleotide diversity (π) within populations. Haplotypic diversity is a measure of the number and frequency of haplotypes present in a population, while nucleotide diversity measures the degree of polymorphism between haplotypes within a population. Nucleotide divergence (d_A) was calculated to measure diversity between populations, using the software program REAP (McElroy et al. 1991). The program MODELTEST 3.06 (Posada & Crandall 1998) was used in this analysis to test for the best-fit model for sequence substitution and gamma distribution of rate heterogeneity for all sequences. All genetic distance methods were then calculated using the best-fit model, incorporating Tamura and Nei (1993) substitution model using a gamma distribution and invariant sites (TrN + I). The model provided the estimated parameters of an equal gamma rate and the proportion of variable positions = 0.8804. The Tamura-Nei method outputs a corrected percentage of nucleotides for which two haplotypes are different. This correction allows for different transversion and transition rates, and also distinguishes between different transition rates between purines and between pyrimidines.

Historical divergence was determined by measuring sequence divergence, calculated in MEGA version 2.1 (Kumar et al. 2001). Both Maximum parsimony (MP) and Neighbour Joining (NJ) distance methods were used for phylogenetic reconstruction of populations using mtDNA haplotypes, in the program PAUP 4.0b8 (Swafford

2000). Genetic distance was used for the NJ analysis, while the MP methods utilised a heuristic search with gaps identified as a 5th state.

Analysis of molecular variance (AMOVA, (Excoffier et al. 1992)) was calculated within the program ARLEQUIN version 2.0 (Schneider et al. 1997) to test for mtDNA genetic differentiation and patterns of geographical structuring. This method estimates the proportion of variation within and between populations based on the frequency distribution of haplotypes and pairwise distances (Φ_{ST}). AMOVA was used to test biologically meaningful divisions of populations into various groups, including populations separated by Rift Valley. Differentiation between populations was analysed by an exact test of population differentiation using 10000 Markov chain steps (Raymond & Rousset 1995) in ARLEQUIN 2.0. Mismatch distribution analysis (Schneider & Excoffier 1999) was calculated in ARLEQUIN 2.0 to test for evidence for rapid historical population expansion.

A Neighbour Joining (NJ) tree showing the hierarchical structure of haplotypic diversity in wild dog populations was calculated according to methods by Holsinger and Mason-Gamer (1996) using Nucleodiv software, version 1.7. This method provides a bias correction to Nei's (1982) nucleotide diversity statistics and groups populations based on the average time to coalescence for pairs of haplotypes. It does not require any pre-specified hierarchical structure. Statistical support for each node was estimated by random resampling 10,000 times, to provide a null distribution for sample comparison.

5.3.4.2 Analysis of microsatellite data

Zambian population microsatellite results were pooled with that used in Girman et al. (2001), the raw genotypic data from that study was kindly supplied by Derek Girman and Carles Vila (n=203 individuals). Only data from free-ranging populations were included in this analysis.

Genetic diversity and population characteristics were analysed with a variety of methods. Using FSTAT version 2.9.3.2 (Goudet 2002), the mean number of alleles per locus was calculated, in addition to allelic richness per locus and per population since this measure is independent of sample size and allows comparison of different

sample sizes. FSTAT (v2.9.3.2, Goudet, 2002) was also used to calculate observed heterozygosity ($H_{O)}$, and expected heterozyogisty (H_E) based on Hardy-Weinberg assumptions (Saitou & Nei 1987). H_E was presented for previous wild dog population data in Girman et al. (2001) since H_E is strongly correlated with H_O but is a more unbiased index (Nei & Roychoudoury 1974), so it was also used here for direct comparison.

Genotypic linkage disequilibrium is the non random association of genotypes occurring at different loci. This was calculated using the log-likelihood ratio G-statistic (FSTAT v2.9.3.2, Goudet 2002) where only individuals typed at both loci are analysed and where the P-value is estimated as the proportion of statistics from randomised data sets that are larger or equal to that observed. This method weights each sample by its content. Exact tests for deviation from Hardy-Weinberg equilibrium were carried out across loci using the Markov chain method (1000 iterations) in the program GENEPOP version 3.4 (Raymond & Rousset 1995). The number of unique alleles in each population was also identified.

Null alleles (alleles with an absence of gene product) were tested for using MICRO-CHECKER version 2.2.3 (van Oosterhout et al. 2003) by evaluating the significance of heterozygote deficiency after Bonferroni adjustment. Null alleles and allelic dropout were also checked against pedigree data.

The program BOTTLENECK (Piry et al. 1999) was used to test for recent reductions in effective population size based on allele frequency data. Within this program, the Wilcoxon's heterozygosity excess test (Piry et al. 1999) was used together with the allele frequency mode shift analysis (Luikart & Cornuet 1998). Assumptions were based on the two-phased model (TPM) of mutation-drift equilibrium, which is considered best-suited to microsatellite data (Piry et al. 1999).

Genic differentiation between geographical populations was first assessed in FSTAT (v2.9.3.2, Goudet 2002) by calculating the inbreeding coefficient F_{IS}, which measures the probability that two alleles in an individual are identical by descent, a positive F_{IS} indicating a deficiency of heterozygotes. A multi-locus Hardy-Weinberg global test for heterozygote deficiency, based on the Markov chain method, was used to test for

overall heterozygote deficit or excess in populations. For multiple comparisons a Bonferroni adjustment was made. Pairwise comparisons of populations were then evaluated using θ_{ST} [an unbiased F_{ST} estimator, (Weir & Cockerham 1984)] and significance was estimated from 10,000 randomisations carried out in FSTAT. Genetic differentiation between geographical populations was calculated using the AMOVA application (Excoffier et al. 1992) within the program ARLEQUIN version 2.0 (Schneider et al. 1997). An NJ topology was then built using Nei's (1978) unbiased distance method in the program MICROSAT (Minch et al. 2004). To give statistical support to NJ tree topology, 1000 bootstrapped distance matrixes were run in MICROSAT then a consensus tree was built using PHYLIP version 3.6 (Felsenstein 2004).

Genotypic methods were used to further resolve fine-scale population structure and differentiation. An assignment test was used to test for the likelihood of finding an allele in each population. To avoid zero values a frequency value of 0.01 was assigned to alleles missing in one population. Based on a Bayesian model and using criterion by Rannala and Mountain (1997) and a simulation algorithm by Cornuet et al. (1999), the program GENECLASS version 2.0 (Piry et al. 2004) was used to assign individuals to each population. This model and parameters were chosen since first generation migrants would not be expected between most populations. The assignment is based on the percentage of individuals not excluded from assignment to each population, given a probability of 0.05 or greater.

In addition to the assignment test a model based clustering program, STRUCTURE version 1.0 (Pritchard et al. 2000) was used here to infer population structure from individual genotypic data. This method uses posterior probabilistic assignment to infer (k) number of populations, by the user incrementing the number of populations for each run to obtain a significant value ($P>0.95$) for k. The program was run using an admixture model which assumes populations may have mixed ancestry, and a frequency model which assumes allele frequencies may be similar in different populations, as expected from migration or shared ancestry. The model was run with a burn-in length of 10,000.

Lastly, an NJ tree was built based on the proportion of shared alleles between individuals. A distance matrix using $Dps_{ij}=1-(ps_{ij})$ (where ps=proportion of shared alleles between individuals *i* and *j*) was generated in MICROSAT (Minch et al. 2004) and the NJ tree was built using MEGA version 2.1 (Kumar et al. 2001).

5.4 RESULTS

5.4.1 Mitochondrial DNA Analysis

5.4.1.1 Genetic diversity

Eight mtDNA haplotypes were identified, each 403-bp in length. These haplotypes contained seventeen variable sites, twelve of which were parsimony informative. The extension of 22-bp of sequence created by the new canid-specific primers designed here contained one additional variable site and was therefore included in sequence analysis. No new haplotypes were found in the Zambian wild dog population samples. From the 79 wild and captive samples sequenced in this study, all matched two of the mtDNA haplotypes found by Girman et al. (2001); listed as S2 and Z1. Both haplotypes were found within the wild Lower Zambezi population, while captive dogs originating from the Transvaal and Namibia were all of haplotype S2, and those with Botswana origins were Z1. S2 was the most common haplotype found in southern African wild dog populations, while the other haplotype found in Zambia, Z1, was shared only with the two nearest neighbouring populations to the south, Hwange in Zimbabwe and the Okavango in Botswana. The haplotypes found in the captive dogs matched those found by Girman et al. (2001) in dogs from the same geographic regions (Table 5.1).

The bulk of the Zambian data is from the Lower Zambezi region, however one sample from Kafue National Park and two from the South Luangwa National Park (5 faecal samples failed to amplify) were included in analysis. The Masai Mara and Serengeti populations were pooled for analysis as per Girman et al. (2001) who found no significant genetic difference in a pairwise comparison (Φ_{ST}) between them.

Table 5.1 Mitochondrial DNA haplotypes from eight geographic regions. Data is composed from samples sequenced in this study combined with data from free ranging wild dog populations published in Girman et al. (2001).

Population location	Haplotype							
	S1	S2	S3	Z1	Z2	E1	E2	E3
Masai/Serengeti						18		9
Selous			24				7	
Zambia		14		19				
Hwange		12		1	13	1	1	
Namibia		15						
Okavango		6		12	3	29	3	
Kruger	37	57						
Transvaal		21						

The best-fit model resulting from MODELTEST (version 3.06, Posada and Crandall, 1998) was the Tamura and Nei (1993) model of sequence evolution, which matched the model assumed by Girman et al. (2001), with the modification of an equal gamma rate. Both NJ and MP phylogenetic methods gave topologies and bootstrap values corresponding to those described by Girman et al. (2001), and shown here in Figure 5.1 (see Chapter 5 Introduction). Inclusion or exclusion of the grey wolf sequence as an outlier had no effect on branch topology, so the sequence was not included in further analysis.

Sequence divergence for all the mtDNA haplotypes ranged from 0.27% to 5.1%, with a mean of 2.7% (SE±0.35%). Sequence divergence was high between eastern (E1,E2,E3) and southern (S1,S2,S3,Z1,Z2) haplotypes with a mean divergence of 4.4%, while within group mean sequence divergence was 0.75% and 0.74% for eastern and southern groups respectively.

Haplotypic diversity (h) within populations ranged between 35% and 63%, and nucleotide diversity (π) within populations ranged between 0% and 1.61% (Table 5.2). Nucleotide divergence (d_A) between populations ranged between 0% (where Transvaal and Namibia shared a single haplotype) to 3.97%. High nucleotide divergence (>3.0%) was found between the Mara/Serengeti populations and all of the southern African populations including Zambia, with the exception of the Okavango (0.64%) which shared two haplotypes with the Mara/Serengeti (see Appendix 4 for table of (d_A) values).

Table 5.2 Mitochondrial DNA and microsatellite genetic diversity in African wild dog populations. Number of samples for mtDNA and microsatellite analysis is shown by "N". Haplotypic diversity (h) and nucleotide diversity (π) are shown for mtDNA data. Mean expected heterozygosity (H_e), allelic richness (Al) and average sample size analysed for each locus (n/Locus) are shown for microsatellite data, for 11 loci tested.

Population location	*mtDNA diversity*			*Microsatellite diversity*			
	N	% *h* (±SE)	% π	H_E (±SE)	N	Al	n/Locus
Masai/Serengeti	27	45.3 (4.43)	0.53	0.621 (0.032)	28	3.229 (0.771)	26.6
Selous	31	35.5 (5.88)	1.61	0.667 (0.049)	22	3.598 (1.159)	17.8
Zambia	33	49.4 (1.68)	0.57	0.579 (0.034)	19	2.788 (0.648)	14.6
Hwange	28	60.8 (3.62)	0.92	0.654 (0.031)	22	3.346 (0.811)	21
Namibia	15	0	0	0.615 (0.053)	6	3.223 (0.819)	5.8
Okavango	53	63.6 (3.94)	2.3	0.605 (0.037)	31	3.095 (0.863)	29.7
Kruger	94	47.8 (1.66)	0.13	0.556 (0.044)	94	2.933 (0.755)	93.8
Transvaal	21	0	0	n/a	0	n/a	n/a

Mismatch analysis gave significant results (SSD p-value <0.019) for all populations excluding the Okavango, thereby giving no evidence of recent historical population expansion in these areas. The value for Okavango was not significant (SSD p-value=0.057), and this population, along with nearby Hwange, had a large number of haplotypes (5) compared to other samples populations (1 to 2). Admixture events and population sub-structure can affect the shape of the mismatch distribution to an as-yet unknown extent. Given the large dispersal capabilities and relatedness in pack structure in wild dogs, there is little evidence of population expansion to be concluded from these results.

5.4.1.2 Population differentiation

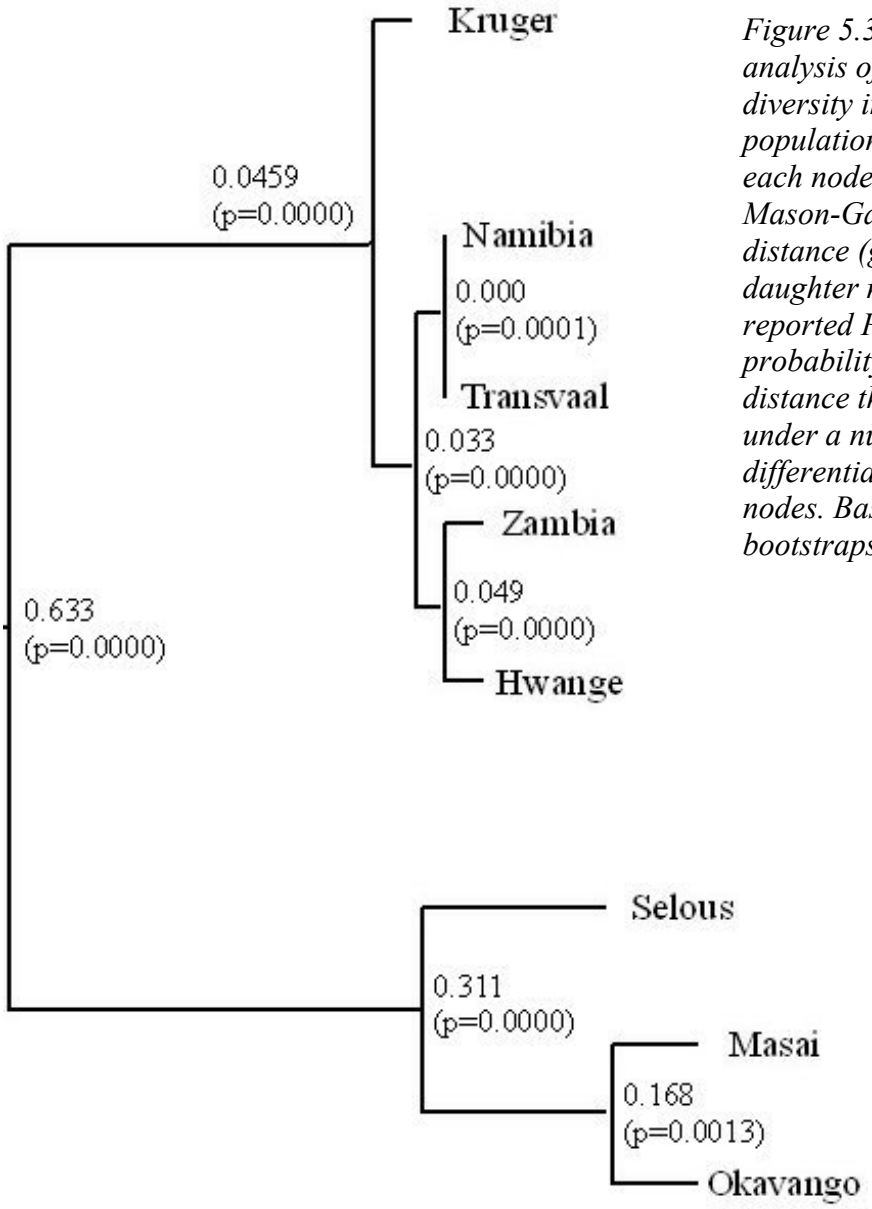


Figure 5.3. Hierarchical analysis of mtDNA haplotype diversity in African wild dog populations. The numbers at each node are Holsinger and Mason-Gamer's (1996) genetic distance (g_{ij}) between the two daughter nodes, and the reported P-value is the probability of obtaining a distance that size or greater under a null hypothesis of no differentiation between the nodes. Based on 10,000 bootstraps.

Hierarchical analysis of population structure revealed several population groupings (Figure 5.3). The Masai Mara/Serengeti and Okavango populations are closely grouped since they both have the most common haplotype as E1, and the Selous is grouped nearby. The remaining southern African populations are grouped together, with Zambia grouped closest to Hwange, then Transvaal and Namibia (who shared the same single haplotype), and Kruger is more distantly grouped with these populations. All distances between nodes were highly statistically significant with $p<0.0015$.

An extensive comparison of population groupings based on the proportion of mtDNA genetic variation within groups (Φ_{CT}) is provided in Girman et al. (2001), with the exclusion of Zambia. That study found roughly equivalent support for a variety of groupings, including grouping the Masai/Serengeti and Okavango together, then all the other populations, or grouping Masai/Serengeti and Okavango plus separating the Selous and/or Hwange from the southern group of populations. Equal support was found for considering all populations independently. Further AMOVA was carried out here to test for any likely geographical differentiation between populations, based on mtDNA, by including Zambia with its nearest neighbouring populations. The strongest support (57.4% variation accounted for among groups) was found in grouping the Masai Mara/Serengeti population separately and Zambia together with all the remaining populations including the Selous (Table 5.3).

Table 5.3 AMOVA analysis of geographic groupings of wild dog populations. Groups are separated by parenthesis under the population grouping. P-value is shown in parenthesis alongside percentage of variation.

Population Grouping	**Percentage Variation**		
	Within populations	**Among populations within groups**	**Among groups**
[Masai/Serengeti, Selous] [Zambia, Okavango, Hwange] [Namibia, Kruger, Transvaal]	31.53 (0.000)	61.62 (0.000)	6.85 (0.262)
[Masai/Serengeti] [Selous, Zambia, Okavango, Hwange, Namibia, Kruger, Transvaal]	18.08 (0.000)	24.53 (0.000)	57.39 (0.000)
[Masai/Serengeti, Selous] [Zambia, Okavango, Hwange, Namibia, Kruger, Transvaal]	29.03 (0.000)	49.24 (0.000)	21.73 (0.036)
[Masai/Serengeti] [Selous, Zambia, Okavango, Hwange, Namibia] [Kruger, Transvaal]	29.41 (0.000)	44.76 (0.000)	25.84 (0.152)
[Masai/Serengeti, Selous, Zambia, Okavango, Hwange, Namibia] [Kruger, Transvaal]	33.51(0.000)	65.75 (0.000)	0.75 (0.290)

Exact tests of population differentiation based on mtDNA revealed all populations were significantly differentiated from each other ($p<0.001$), except for Namibia and Transvaal. Both these populations had small sample sizes and contained only one haplotype. Pairwise population comparisons (Φ_{ST}) showed Zambia was most differentiated from the Masai-Serengeti population (Table 5.5). Groupings here differ slightly from Girman et al. (2001) whose inclusion of museum skins found three haplotypes in Transvaal (S1, S2, and Z1).

5.4.2 Microsatellite Analysis

5.4.2.1 Genetic diversity

Levels of expected heterozygosity (H_E) per population ranged from 0.556 (Kruger) to 0.667 (Selous), see Table 5.2. The amount of allelic richness in the Lower Zambezi population was the lowest recorded over all populations (2.78; see Appendix 4 for table of allelic richness per locus). All populations except the Lower Zambezi contained at least one unique allele; two were found in Kruger and Masai-Serengeti, and three in the Selous. A table of microsatellite genotypic data for all Zambian dogs sampled is contained in Appendix 4.

No null alleles were detected amongst any of the loci examined. Significant genotypic linkage disequilibrium (after Bonferroni adjustment; $p<0.00091$) was detected across all samples in seven pairs of loci: between 263 and 366, 155 and 453, 155 and 671, 173 and 250, 173 and 453, 173 and 677, 250 and 671.

The number of complete multi-locus genotypes per population was low for three populations; Namibia, Selous and Lower Zambezi all had 5 or fewer individuals with all loci complete, and these three populations also had the lowest number of average samples per locus (n/Locus, Table 5.2). No locus had significant deviation from Hardy-Weinberg equilibrium (HWE) across populations.

Table 5.4 F_{IS} values per population and per locus. Significance at the 5% level is indicated by shading: yellow indicates heterozygote deficit, and blue indicates heterozygote excess.

Locus	Masai/Ser	Selous	Lower Zambezi	Hwange	Namibia	Okavango	Kruger
155	-0.166	0.078	0.509	-0.204	0.063	0.081	-0.045
173	-0.083	0.382	0.275	0.192	-0.25	-0.025	0.086
250	0.028	0.111	0.14	-0.197	-0.143	-0.145	0.114
263	0.067	-0.027	0.349	-0.141	0.318	-0.119	-0.167
366	-0.235	0.162	0.182	-0.235	0.143	0.04	-0.036
423	0.017	-0.076	-0.06	0.108	-0.19	-0.174	-0.095
442	0.036	-0.024	-0.36	0.372	0	0.03	-0.086
453	-0.235	0.154	-0.168	-0.069	-0.22	0.065	0.066
606	-0.071	-0.25	-0.103	-0.317	-0.081	0.255	-0.039
671	0.099	0.254	0.262	0.097	-0.176	-0.116	-0.129
677	0.095	-0.075	0.122	-0.027	0.063	0.03	0.062
Mean	**-0.038**	**0.076**	**0.125**	**-0.047**	**-0.049**	**-0.022**	**-0.029**

F_{IS} values for the Lower Zambezi population were high compared to the other populations, averaging 0.125 compared to other averages in the range of -0.047 to 0.076 (Table 5.4). Table 5.4 presents F_{IS} results per locus per population. The multi-locus Hardy-Weinberg global test for heterozygote deficiency gave a significant value for the Lower Zambezi population, after Bonferroni adjustment (p=0.0018, ±SE 0.0003, H_1=heterozygote deficiency). All other populations had a p-value in the range 0.34 to 0.98; no other population had a significant overall heterozygote excess or deficit at the population level. Analysis per locus found 4 loci in the Lower Zambezi population with significant heterozygote deficiency, L155, L173, L263, L671, and one in Kruger (L250). Significant heterozygosity excess was found in loci in three other populations: L263, L423, and L671 in Kruger; L250 in Hwange; and L155 and L453 in the Masai-Serengeti.

Evidence of a recent reduction in effective population size was detected in the Lower Zambezi population, and to a lesser extent in the Selous population. Mode shift was present in both populations, and the Wilcoxon's heterozygosity excess test gave significant results of p=0.00073 for the Lower Zambezi and p=0.011 for Selous.

5.4.2.2 Population differentiation

Microsatellite pairwise comparison values (θ_{ST}) showed Zambia was most highly differentiated from the Masai-Serengeti and Kruger populations (Table 5.5). Pairwise population comparisons were not significant between Selous and Namibia, Lower Zambezi and Namibia, and Lower Zambezi and Selous. Analysis was limited to 6 samples for Namibia, therefore the lack of significance for this population was most likely due to small sample size. An θ_{ST} with a value of one indicates a population is completely differentiated (unique), while an θ_{ST} of zero would indicate no difference. Populations were therefore differentiated but θ_{ST} values were low.

*Table 5.5 Population differentiation. Pairwise Φ_{ST} estimates between populations for mtDNA (below diagonal), and pairwise θ_{ST} estimates for microsatellite markers (above diagonal). For microsatellite θ_{ST}, significance level is indicated by superscript: *p<0.05 ,**0.05>p<0.01,#0.01>p<0.001, N=not significant. All mtDNA Φ_{ST} values were significant at p=0.0000.*

Population	Masai/S.	Selous	Zambia	Hwange	Namibia	Okavango	Kruger
Masai/Serengeti		0.0887*	0.1776#	0.0883#	0.1401**	0.1233#	0.1215#
Selous	0.6702		0.1531N	0.0841**	0.0949N	0.1305**	0.1272#
Zambia	0.8832	0.4916		0.1481**	0.1486N	0.1286#	0.2176#
Hwange	0.7845	0.3526	0.1125		0.0598**	0.0614#	0.1141#
Namibia	0.8992	0.4052	0.4499	0.2883		0.0408**	0.0849#
Okavango	0.1529	0.4547	0.6866	0.5590	0.6487		0.0968#
Kruger	0.9284	0.5129	0.5518	0.4802	0.2486	0.7752	

Microsatellite AMOVA results from Girman et al. (2001) again gave the highest support for grouping all populations separately, although support was also shown for separating the Masai Mara/Serengeti population from the remaining southern African populations. An additional ten AMOVA analyses were run here including Zambia in various population groupings, however no grouping accounted for more than 5% of variation among groups, and all had over 89% of variation accounted for within populations (p=0.000), strongly supporting differentiation of all populations (data not shown). This result is further supported below by assignment tests and model based clustering results.

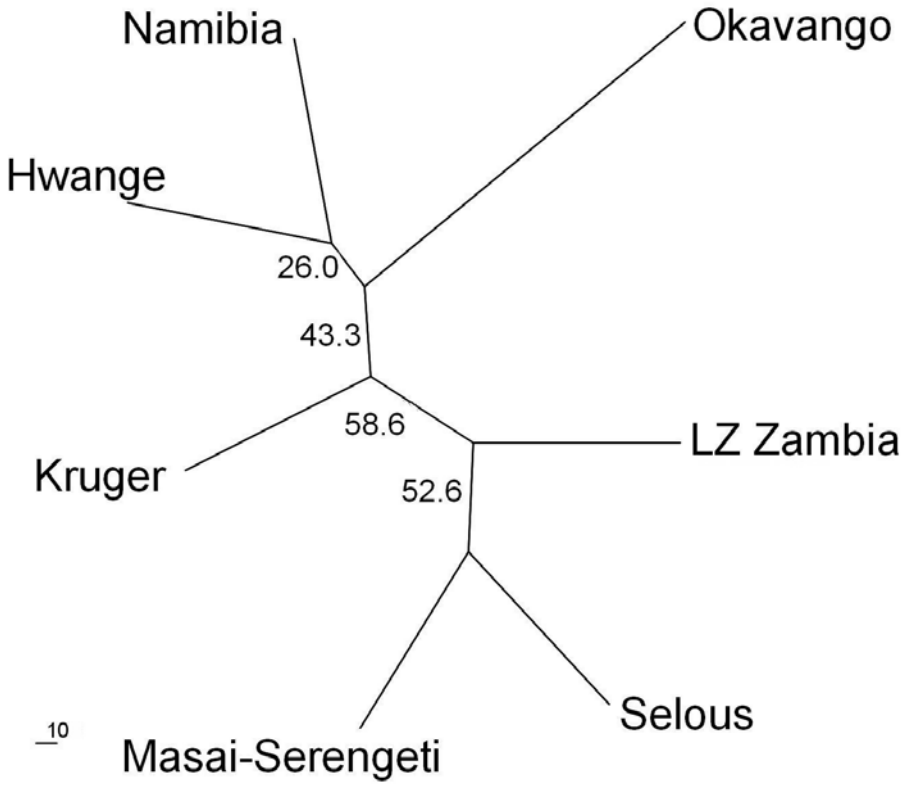


Figure 5.4 Neighbour Joining tree showing the relationships between African wild dog populations based on Nei's (1978) standard genetic distance from microsatellite data. The percentage of bootstrap support (1000) is shown at each node.

Differentiated populations were confirmed in an unrooted NJ tree based on Nei's (1978) standard genetic distance (Figure 5.4). Grouping of populations by microsatellite data more closely resembled the geographic distribution of populations than mtDNA results. Notably, the Zambian samples were placed on a branch in between the eastern African populations (Masai-Serengeti and Selous) and remaining southern African populations. Central southern neighbouring populations Hwange, Botswana and Namibia were grouped together, with Kruger differentiated with less than 50% support from bootstrapping. The Selous and Masai-Serengeti populations were very closely grouped. The branching topology results concur with pairwise θ_{ST} values, which showed the highest population differentiation between Zambia and Kruger, then between Zambia and the Masai Mara-Serengeti and Selous populations. Bootstrap values and branch lengths confirm population differentiation was low.

Table 5.6. Percentage of individuals not excluded from assignment to each population (probability 0.05 or greater). Columns contain source populations of individuals, rows show percentage of individuals assigned to each population.

Population	Masai/Seren	Selous	Zambia	Hwange	Namibia	Okavango	Kruger
Masai/Serengeti	92.9	0	5.3	4.5	0	6.5	12.8
Selous	3.6	77.3	0	0	0	0	0
Zambia	0	0	73.7	0	0	0	0
Hwange	7.1	4.5	10.5	86.4	16.7	41.9	11.7
Namibia	0	4.5	5.3	0	83.3	16.1	3.2
Okavango	3.6	0	10.5	9.1	33.3	93.5	11.7
Kruger	10.7	0	0	0	0	12.9	87.2

On average 84% (SE±2.8) of individuals were assigned to their correct population of origin (Table 5.6). Since this test was based on exclusion, individuals could be assigned to more than one population. The three largest cross-assignments (16% to 42%) were between geographically neighbouring populations Namibia, Okavango and Hwange. For the Lower Zambezi, the majority of cross-assignment was to the nearby Hwange and Okavango populations.

The model-base clustering analysis run in STRUCTURE version 1.0 (Pritchard et al.., 2000) gave a highly significant probability of assignment of individuals into 7 clusters, indicating a best-fit population structure which matched the number of populations sampled in the study ($p=1$, where k source populations is the null hypothesis). All other values of assignment to k populations were improbable ($p<0.0001$).

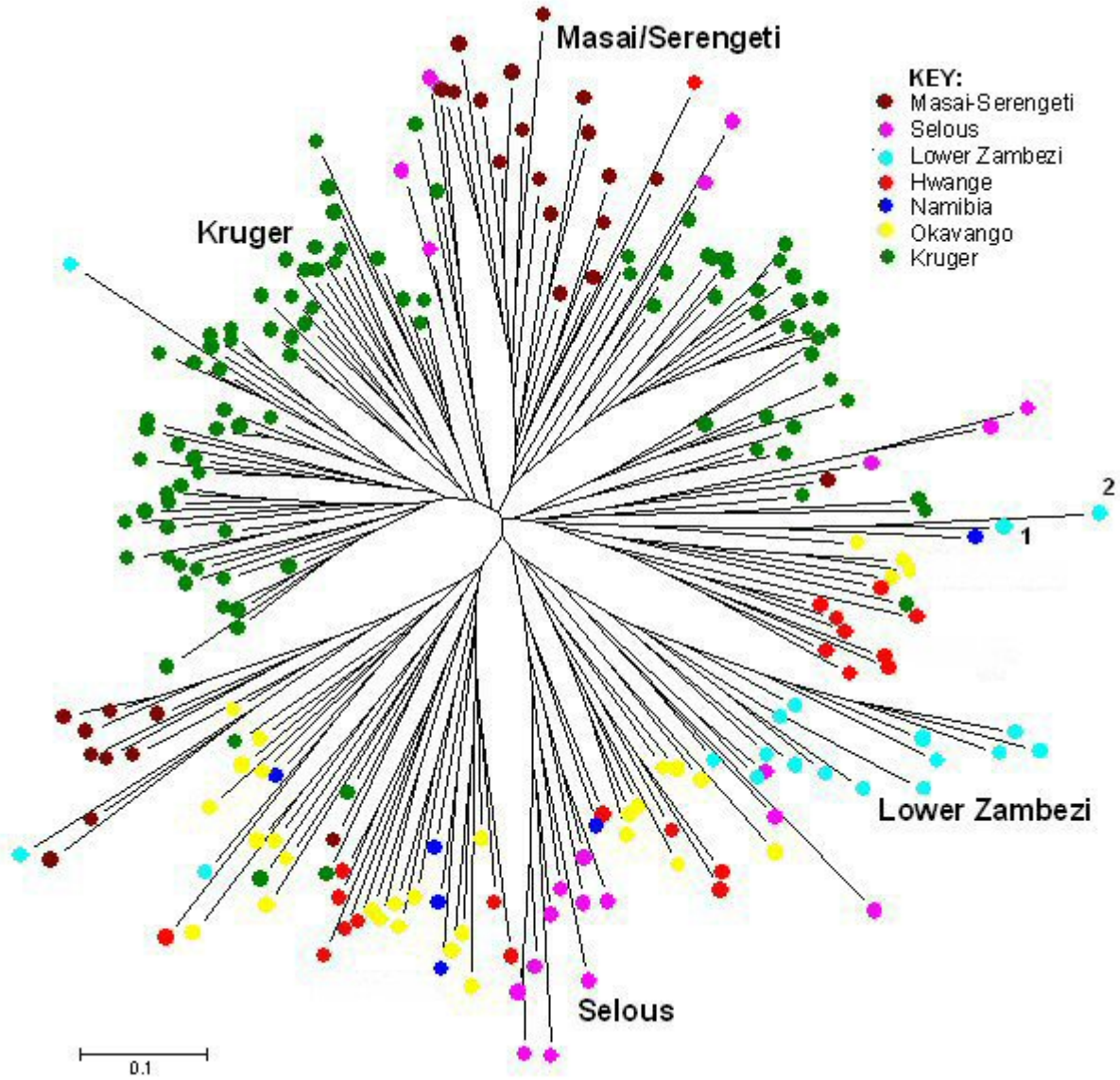


Figure 5.5 Neighbour joining tree of individual African wild dogs grouped by proportion of shared alleles. Samples from South Luangwa and Kafue in Zambia are labelled "1" and "2" respectively.

Grouping individuals by the proportion of shared alleles resulted in five main clusters with varying levels of admixture of individuals from different populations. Within these clusters individuals were generally grouped with others from the same population of origin. Individuals from the Lower Zambezi population were grouped together with the exception of 3 animals. Individuals from Kafue and South Luangwa were grouped separately from the Lower Zambezi individuals. Areas of tightly grouped individuals and short branch lengths indicate either low diversity or closely related individuals, for example in the Kruger population.

5.4.3 Amplification of Faecal Samples

For faecal samples, microsatellite primers had a mean of 49.7% (±SE 6.25) success rate in amplification, discounting the samples which were excluded because allelic dropout was suspected from gels or pedigree data. There were some sample specific effects; 5 animals gave samples which amplified from 5 or less of the 11 primers. Amplification of faecal samples proved more difficult for some individual primers. Primers L423, L453, L155, and L366 gave the poorest results of between only 19% and 33% success, despite extensive optimisation.

Since pedigree records on maternity were available from field data, only two to three dogs from each known maternal line were amplified for mtDNA, and tissue and blood samples were used where possible. Of the 33 faecal samples screened, 58% yielded mtDNA and were sequenced. All samples sequenced from one or more generations of each maternal line in the Lower Zambezi population confirmed field observations on maternity.

5.5 DISCUSSION

5.5.1 Amplification of Faecal Samples

The current study utilised non-invasive sampling methods through the collection of faecal samples for genetic analysis using both mtDNA and microsatellites, to enable comparison of Zambian wild dog populations with those previously analysed by Girman et al. (2001). The use of non-invasive samples has been found to limit the reliability of results in some studies, particularly for microsatellites. Creel et al. (2003) looked at error rates from non-invasive sampling (hair and faeces) and found errors in marker assignment despite multiple PCRs. Typical problems from faecal samples include contaminants which inhibit PCR, false alleles from contaminating DNA sources and allelic dropout. These can be particularly problematic when using results to estimate population sizes rather than for relatedness or population structure analysis. A multiple tubes approach, aimed at increasing replication, was developed by Taberlet et al. (1996) to help overcome these problems, and several studies have since optimised methods and documented the reliability of faeces for microsatellite typing (Ernest et al. 2000; Frantzen et al. 1998; Kohn et al. 1995; Kohn et al. 1997; Piggot & Taylor 2003a; Piggot & Taylor 2003b). Pilgrim, Boyd & Forbes (1998) found canid specific primers amplified poorer quality DNA samples better than universal primers, which was taken into account in methodology in this study.

The faecal derived DNA samples amplified well for mtDNA control region sequences, but as would be expected microsatellite loci amplification had a lower success rate. In this study the use of field pedigree data reduced error rates and sampling was sufficient to obtain results from all generations of all known packs in the study area.

Extensive optimisation of polymerase chain reaction conditions improved success in amplifying most microsatellite loci, but sample and primer specific effects were important limitations. Non-invasive sampling methods greatly increase sampling opportunities for such a highly mobile species, therefore further screening of alternative canid specific primers specifically for use in faecal analysis is recommended.

5.5.2 Genetic Variability

Previous analysis of genetic diversity in African wild dogs spanning a wide geographic region detected similar levels of genetic diversity indices among large free-ranging populations (Girman et al. 2001). The Lower Zambezi population, in contrast, showed evidence of decreased heterozygosity, and bottleneck analysis showed evidence of a recent decline in effective population size. Habitat fragmentation, demographic population decline and the subsequent lack of dispersal success observed in the field all support the detection of small population genetic effects in this population. Captive populations also displayed decreased levels of genetic diversity in the previous study (Girman et al. 2001).

Mitochondrial DNA analysis did not detect any unique haplotypes in the population. The Lower Zambezi population contained the most common haplotype found in southern African populations (S2), and one additional haplotype that it shared with Hwange and Okavango (Z1). Haplotypic diversity was comparable to the other populations studied, the majority of which contained one to two haplotypes. Nucleotide diversity fell within the range of the other populations. The highest nucleotide diversity was detected in the Okavango, Selous and Hwange, which all contained haplotypes from both southern and eastern Africa.

Microsatellite analysis showed low expected heterozygosity (H_E) and allelic richness in the Lower Zambezi population. F_{IS} analysis showed a significant deficiency of heterozygotes in the Lower Zambezi population, which was not present at a population level in any other population. The detection of significant heterozygote excess or deficiency found in some loci in other populations may be an artefact of substructure in sampling. Evidence of a recent reduction in effective population size was also detected in the Selous population. The BOTTLENECK (Piry et al. 1999) program is sensitive to population sub-structuring, and this population sample had a low number of complete multi-locus genotypes which may have affected analysis. More extensive sampling and genetic analysis of the population is recommended to further investigate evidence of a past bottleneck in Selous.

The Lower Zambezi microsatellite results were based on a high proportion of faecal samples (63%), and allelic dropout from these samples could potentially result in detection of a false heterozygote deficiency in this population. However, gels were checked against field pedigree data for correct allele typing and allelic dropout, and all individuals with suspected dropout were deleted from the sample prior to analysis. The highest recorded excess homozygosity in the population was at locus L155, and in this case all the faecal samples gave heterozygous results. This locus had poor amplification results and was based on a sample size of only ten individuals, nevertheless, the other three loci with a significant deficiency of heterozygotes were based on 16-18 samples, and all loci had representatives of at least 4 pack lineages.

Given the level of relatedness in African wild dog packs and their breeding structure where only the alpha pair breed, often for several generations, loss of genetic diversity in small populations might be expected to occur quickly particularly if outbreeding behaviour is compromised. Although individual sample size for the Lower Zambezi was reasonable, the number of packs detected in the population was low and therefore only a few individuals are likely to have contributed alleles to the population.

5.5.3 Population Differentiation

Results generally fit Girman et al.'s (2001) model of two historical wild dog clades and recent admixture through migration. Mitochondrial DNA and microsatellite data confirmed differentiation of the Lower Zambezi population, concurring with differentiation of all other populations studied in Girman et al. (2001). Zambia was grouped with the southern African mtDNA clade, sharing a haplotype unique to neighbouring populations to the south, Hwange and Okavango, and AMOVA results further supported this grouping. Genic analysis of microsatellite data also supported clustering in line with geographic separation, with θ_{ST} pairwise comparisons placing Zambia furthest from the populations to the extreme north and south of the sampled region. Girman et al. (2001) also found a significant negative correlation between microsatellite pairwise comparisons of the number of migrants per generation (*Nm*) and the geographical distance between localities for the 7 populations studied. Although populations were differentiated, none were unique, supporting evidence of historical and recent gene flow between populations.

The NJ tree based on genetic distance (Figure 5.4) supported θ_{ST} population pairwise comparison results, with low to moderate bootstrap support on all nodes separating the populations (all <60%). This analysis differentiated the Zambian population from the other populations more than would be expected from geographic location. This is likely to be a factor of the low genetic diversity and recent bottleneck in the Lower Zambezi population, which would hasten the process of population genetic drift (Frankham et al. 2002). More samples from larger Zambian wild dog populations over an increased geographic area would be required to clarify the relationship between Zambian populations in general to those in other areas of Africa. Although no other free ranging populations in this analysis contained significant levels of heterozygote deficiency, the Kruger national park population had comparably low nucleotide diversity, H_E, and allelic richness (Table 5.2). Girman et al. (2001) provided further evidence to suggest the current Kruger NP population may have expanded from a smaller founder population and thus have reduced levels of genetic diversity. This may account for the strength of Kruger's distinction from the Lower Zambezi and the other southern African populations. Mismatch analysis failed to detect evidence of a recent population expansion in Kruger, however this analysis is likely to have been limited by the small number of haplotypes (Schneider & Excoffier 1999).

Genotypic analysis using the assignment test, model-based clustering, and proportion of shared allele analysis all differentiated the wild dog populations, and supported a stepwise model of admixture to the nearest neighbouring populations. The weak to moderate level of differentiation concurred with results from genic analysis. The neighbour joining tree of individual African wild dogs grouped by proportion of shared alleles (Figure 5.5) showed only 5 major clusters, suggesting considerable admixture amongst populations, particularly Okavango, Hwange, and Selous. The NJ tree analysis was not bootstrapped so results should be interpreted with caution. However, the admixtured individuals originated from populations which also showed high levels of cross-assignment in GENECLASS [v.2.0 (Piry et al. 2004)] analysis, therefore Figure 5.5 approximates a pictorial representation of the assignment test results.

It is important to note the effect of sampling regimes here. The tight clustering and short branch lengths observed in some sections of the NJ tree are likely to be the

result of substructuring in sampling; in their original analysis of data from populations outside of Zambia, Girman et al. (2001) found clustering matched pack affiliations in the Kruger and Masai Mara/Serengeti samples. There was a lack of pedigree information available on genotypic data for most populations here, however, of the 94 Kruger samples, 59 samples are likely to have belonged to only 6 maternal lineages (M.G.L. Mills, personal communication). Of the 31 Botswana samples typed for microsatellites, there were 6 known maternal lines which accounted for 20 samples, although this could possibly be reduced to 4 lineages since several may be linked (J.W. McNutt, personal communication). The 33 Zambian samples were based on 6 maternal lineages represented in both mtDNA and microsatellite analysis, including one each from South Luangwa and Kafue.

Genotypic linkage disequilibrium was detected in this study between several pairs of loci. Previously, Girman et al. (2001) found that over-sampling of related individuals due to wild dog pack structure accounted for a large proportion of apparent linkage disequilibrium. When analysis in that study was restricted to the alpha male and female from each pack, linkage disequilibrium fell from 60% to 15% in the Kruger population. Based on pack size and structure in African wild dogs it is logistically difficult to collect a large number of samples from unrelated individuals, in fact in the case of small populations it may not be possible. However, long term studies should aim to collect samples from as many different lineages as possible to avoid over-sampling of related individuals from frequently observed packs, and subsequent effects on the assessment of genetic diversity (Hansen et al. 1997; Spong et al. 2002). Analysis could then be restricted to one or two representatives of each lineage.

Results here present further support for Girman et al.'s (2001) hypothesis of the Rift Valley and associated climate and vegetation changes as an historical barrier to gene flow. The Zambian samples were consistently highly differentiated from the Masai Mara-Serengeti population and the Rift Valley lies between the two. The data also provides support for the second hypothesis of expansion through migration from southern African refuges and subsequent differentiation in east Africa. The presence of shared haplotypes between Hwange, Okavango and the Selous support an admixture model and wider sampling of the Zambian region may provide more information on levels of gene flow between these populations. Specifically, more

sampling of the larger South Luangwa NP population to the north east in Zambia may provide further information on levels of genetic differentiation and phylogeny between Zambian populations and the Selous, which is the nearest east African population but lies to the other side of the western Rift Valley lakes and mountains.

One caution for the use of genetic markers is that panmictic populations are often more suited for testing population-genetic hypotheses, since sampling large numbers at all locations is less important whereas for differentiated populations a representation of all populations is a priority (Lehman & Wayne 1991). Small samples sizes can lead to inferring distinction between populations, when sampling a large range of populations may detect a more continuous pattern of gene flow. As mentioned above this occurred with previous wild dog studies which originally recommended the classification of two different subspecies (Girman et al. 1993), but more extensive sampling found recent mixing of the two clades (Girman et al. 2001).

5.5.4 Summary of Results

Due to the small population size, sample size here was limited and substructured to a known extent, therefore results should not be over interpreted. However, sample size was comparable to other larger populations previously analysed and genetic results undoubtedly confirm field observations that indicate a small and declining population. The Lower Zambezi population suffered from a heterozygote deficiency, low allelic richness, and there was significant evidence of a recent population bottleneck.

The population did not contain any new mtDNA haplotypes, nor any unique alleles on microsatellite loci, but was differentiated from African wild dog populations in other regions. There was evidence of historical and recent gene flow between the Lower Zambezi and neighbouring southern African populations Hwange and Okavango, shown in both the mtDNA DNA control region analysis and in the microsatellite loci assignment tests and proportion of shared alleles methods. Due to the size and status of the population the Lower Zambezi population should not be taken as representative of Zambian wild dog populations as a whole.

5.5.5 Implications for Management

Zambia is located midway between southern and eastern African wild dog populations, and thus may represent a key region of historical dispersal. African wild dogs have undergone rapid decline throughout their former ranges and it is therefore critical to conserve all remaining wild dog populations in-situ. This must incorporate maintenance of genetic variability to conserve population viability and evolutionary processes. Given the potential for the Lower Zambezi population to maintain the continuity of wild dogs' distribution between southern and eastern Africa, preservation of this population and others in Zambia should be an important part of species management. Although the conservation of the few large, stronghold populations in Africa is essential, incorporating a broader geographic and genetic range should be of equal priority for conservation of the species, and this must comprise the majority of smaller populations throughout Africa.

Low levels of genetic diversity in the Lower Zambezi population support direct field observations and suggest the population is in need of active management and augmentation if it is to remain viable. Anthropogenic mortality and demographic factors were shown to have contributed to this population's decline, however loss of genetic diversity is an important consideration for future management. Long-term conservation should be aimed at maintaining realistic levels of gene flow thereby increasing genetic diversity and, optimally, this would be achieved through increasing connectivity with larger populations and facilitating successful dispersal. Extensive sampling of the two remaining Zambian wild dog populations in the Luangwa valley complex and the Kafue National Park region would provide valuable data on the genetic diversity in Zambia, which would be valuable for management of the larger region. The South Luangwa population lies along a continuous river valley running northeast from the Lower Zambezi, and may be a potential source population for the area. Increasing connectivity with this area could provide sufficient gene flow to secure a viable meta-population of wild dogs in eastern Zambia. This would also secure a larger section of corridor, following natural river valleys, between the large and stable populations in eastern and southern Africa.

Second to increasing connectivity, alternative strategies could incorporate augmentation of the Lower Zambezi population through reintroduction, to increase

levels of heterozygosity and prevent further genetic effects on the population. Again, this should mimic realistic gene flow, and should involve dogs from nearby Zambian populations, or at least from populations within realistic historical dispersal distances (Pitra et al. 2002). Genetic data suggest the geographically neighbouring populations of Hwange and Okavango would provide the most suitable genetic stock for augmentation from the populations studied (Table 5.6 and Figure 5.5). Previous research of grey wolves showed a single immigrant recovered genetic diversity in a genetically depauperate, small population (Vila et al. 2003). In the case of the Lower Zambezi population, deterministic threats and demographic factors must also be considered, as discussed in the previous chapters.

Girman et al. (1993) found evidence of morphological differentiation between populations at the extreme ends of the eastern and southern wild dog clades, but more extensive comparisons of morphology and inheritable ecological adaptations over a larger number of populations in the admixture zone has not yet been carried out. Direct observational studies have also recorded different breeding seasons in populations (Frame et al. 1979; Maddock & Mills 1994; Malcolm 1979; McNutt 1996; Reich 1981; Schaller 1972), and other localised adaptations are likely given the geographic distance between populations and the diversity of habitat. For example, lifetime reproductive success has been shown to be heritable in cheetahs (Marcella 2001), and the relationship between disease resistance and the major histocompatability complex plus other loci has been well established (Frankham et al. 2002; Morton 2003; Singh et al. 1997).

The genetic evidence from both this study and Girman et al.'s (2001) suggests that although eastern and southern populations do not form distinctive monophylectic clades and therefore may fall under a single evolutionarily significant unit, at least two management units should fall within this category, namely eastern and southern. Mitochondrial DNA AMOVA analysis (Table 5.3), and microsatellite loci genetic distance analysis (Figure 5.4) showed that eastern and southern populations are differentiated, and translocations between the two regions would not be recommended. All populations were differentiated to some extent, therefore both genetic and ecological exchangeability (Crandall et al. 2000) should be an important consideration in any wild dog management program. Management should focus on maintaining

genetic diversity based on realistic gene flow, and avoiding the introduction of new alleles that might compromise the population's ability to adapt to local selection pressures (Amos & Balmford 2001).

CHAPTER 6: GENERAL DISCUSSION AND IMPLICATIONS FOR MANAGEMENT

6.1 CONCLUSIONS FROM PREVIOUS CHAPTERS

The previous chapters provided a comprehensive assessment of the demographics, population dynamics, ecology and genetic diversity of the Lower Zambezi wild dog population. This chapter combines these findings to assess the viability of the population, and to suggest options for management of wild dog populations in the region of the study area.

A summary of key findings from the previous chapters is presented below.

6.1.1 Demographics and Causes of Decline

I) Snaring was identified as the most important cause of adult mortality, and a threat to wild dog population persistence. This threat must be mitigated as an integral part of any program aimed at conserving a wild dog population in the area.

II) Inbreeding avoidance appeared to be a substantial contributor to population decline through emigration from the study area. Limited mate selection corresponded with neither sex displaying philopatry. When unrelated mates were available female philopatry was observed. Large dispersal distances effectively removed adults from the population. This result has important implications for the management of small populations (≤50 dogs); lack of mate choice may increase dispersal distances and thereby increase edge effects on populations, even when resident pack home ranges lie entirely within a protected area. No inbreeding was observed despite the small size of the population.

III) There was no significant bias in the population sex ratio. There was a trend for a higher proportion of females in all age groups, but the significance of this was limited by lack of power in analysis due to the small size of the population. This result may be a product of small population stochasticity, but it may also be a product of small population dynamics and Allee effects on maternal condition.

6.1.2 Ecology and Habitat Utilisation

I) The study area contained a diversity of habitats on the alluvial terraces of the river valley floor. There was a high overall density of impala, which formed the main prey base for the wild dog population. Prey availability was sufficient to sustain a larger population of African wild dogs than was present in the study area

II) Wild dog annual home range size varied. Range size was related to den locations in remote areas of the Zambian escarpment. Non-breeding packs remained on the river valley floor. Predator avoidance was related to long-distance movements of den sites in some pack years. The Zambezi River and the Zambian Escarpment appeared to be effective barriers to wild dog home range movements.

III) The wild dog population showed a strong preference for the high prey density open grassland habitats. All habitats were utilised but thicket was avoided during hunting and hunting success was reduced in this habitat.

6.1.3 Interpredator Competition

I) Densities of sympatric carnivores, lion and spotted hyaena, were moderate in relation to other study sites. Direct predation of adult wild dogs by lion and spotted hyaenas was rare. However, spotted hyaenas were likely to have affected pup and juvenile survival in two pack years. Kleptoparasitism of wild dog kills by either competing predator species was also rare.

II) In contrast to previous studies, wild dogs showed only temporal avoidance of high lion density areas. Low lion density areas were preferred during breeding periods, while moderate to high lion density areas were preferred during non-breeding periods. No relationship between lion and wild dog densities was detected across study sites, and the interaction of these two species appears to be site specific, contrary to previously published literature.

6.1.4 Genetic Analysis

I) This was the first study to show a loss of genetic variability in a free-ranging African wild dog population. The Lower Zambezi population suffered from a loss of heterozygosity, low allelic richness, and there was significant evidence of a recent population bottleneck.

II) The population did not contain any new mtDNA haplotypes, nor any unique alleles on microsatellite loci, but was differentiated from African wild dog populations in other regions. There was evidence of historical and recent gene flow between the Lower Zambezi and the neighbouring southern African populations of Hwange and Okavango, confirmed by both the mitochondrial DNA control region analysis and the microsatellite loci assignment tests and proportion of shared alleles analysis.

6.2 GENERAL DISCUSSION

6.2.1 Population Viability

There have been numerous modelling techniques developed for predicting population persistence and selecting the most appropriate management strategies (Cross & Beissinger 2001; Haydon et al. 2002; McCarthy et al. 2003; Morris et al. 2002; Parysow & Tazik 2002; Reed et al. 2002). The most common technique, population viability analysis (PVA), is widely used in endangered species recovery plans (Kinvall 2003; Morris et al. 2002). In the case of African wild dogs, PVA has been extensively used for modelling extinction risk and recommending management targets (Burrows et al. 1994; Ginsberg et al. 1995; Ginsberg & Woodroffe 1997; Vucetich & Creel 1999).

Ginsberg and Woodroffe (1997) used the PVA program VORTEX (Lacy 1993) and data from several study sites across sub-Saharan Africa to investigate the critical determinants to population persistence. They incorporated the effects of both mild and severe catastrophes on survival and fecundity into the model. The study determined that absolute population size and adult mortality were the most important variables affecting the persistence of African wild dog populations, whilst both adult and juvenile survival were important in small populations. This finding was supported with further analysis of demographic data from a large (>300) wild dog population in the Selous (Vucetich & Creel 1999). Competition with lions was also identified as an important determinant of wild dog population persistence; however the parameters used in that model were heavily influenced by Creel and Creel's (1996) negative correlation of lion and wild dog densities across study sites. This correlation was shown in the present study to be limited in its applicability for general species management, since on further investigation the effects of lions appear to be site-specific (see section 4.4.3). More recent analysis, based on data from the three largest known populations of wild dogs in Africa (Creel et al. 2004), focussed on identifying the most critical age-specific vital rates (survival and fecundity). They found that the survival of pups and yearlings had the greatest effect on population persistence for all three populations, a result which was also suggested by Cross and Beissinger (2001).

In contrast to these previous analyses on large and stable wild dog populations, there are other mechanisms important to the persistence of small populations. In

populations where pack sizes may drop below a certain threshold, pup and juvenile survival is strongly linked to adult survival through Allee effects, several of which were observed in this study population (see section 2.5.4 and 2.5.5). The Allee mechanism occurs when individual fitness is related to numbers of conspecifics in a positive manner (Stephens & Sutherland 1999), and occurs in wild dogs through dynamics related to pack size.

African wild dogs are obligate social cooperators and pack size has been correlated with both hunting and reproductive success in field studies (Creel 1997; Creel & Creel 1995, 1996; Fanshawe & Fitzgibbon 1993). Courchamp and MacDonald (2001) modelled an Allee effect in African wild dogs based on a critical minimum threshold of pack size, below which the probability of extinction increases. They found that statistically the critical pack size for breeding success is around five adults, which agreed with previous estimates from the field studies. This theory was further quantified by Courchamp et al. (2002) who assessed the trade-off costs of hunting against those of pup guarding. Based on five years of empirical data (n=13 denning periods and eight packs) they again found a critical threshold of five adults for pack size; packs of less than five were significantly less likely to leave a pup guard (Mann-Whitney $Z=-2.635$, $P=0.0084$) and thus risked higher pup mortality. This finding, combined with the correlations of pack size and reproductive success in other field studies, lends considerable empirical support to the theory of Allee effects in wild dogs.

Based on this theory, the poor pup survivorship recorded in the last two years of the Lower Zambezi population, 2003 and 2004, (see section 2.4.1.1, and Figure 6.1 below) may have been a result of low adult pack size, particularly in relation to defence of pups from spotted hyaenas in these two pack years (see section 2.4.1.2). There were three to five adults present throughout each pack year, but one pack also contained five yearlings. However, the yearlings in this pack were observed to make little contribution to the defence of kills and pups (see section 2.4.1.2 and 4.4.2.2), therefore the experience level of the individual and other fitness factors are likely to play a part in determining Allee effects (as suggested by Courchamp et al. 2002), rather than absolute numbers. More importantly in this population, the Allee effect of reduced mate selection played an important role in limiting the population by

removing adults through dispersal (see section 2.5.4 and 2.5.5). The poor pup survivorship observed in 2003 and 2004 was accompanied by emigration from the population.

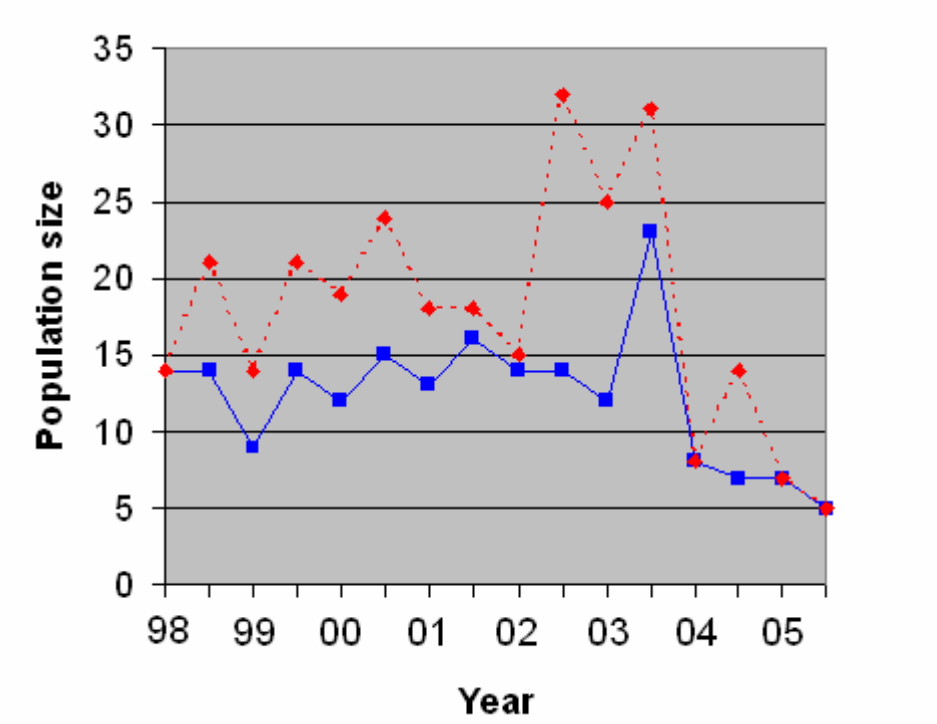


Figure 6.1 Graph of wild dog population size over time in the Lower Zambezi from 1998 to 2005. Solid line represents adult and yearling population, dotted line includes pups.

At the population level Allee effects are reflected by the presence of inverse density dependence at smaller population sizes. The most common population growth model incorporating Allee effects is the extinction-survival model, where there are two equilibria, a stable high equilibrium (direct density dependence) and an unstable lower equilibrium (Boukal & Berec 2002). There is a positive growth rate in between the two equilibria but a negative growth rate at very high or low population sizes. Below the lower equilibrium populations become extinct, while populations above the upper threshold become established at the stable equilibrium. This effect was modelled in African wild dogs by Courchamp and MacDonald (2001). Results showed inverse density dependence at low pack and population sizes, and direct density dependence at high densities and larger pack sizes where there were upper limits to group size,

caused by intra-group competition for feeding rates and breeding opportunities. They found from field studies of larger populations that the average pack size of ten lay in the middle balance between the two equilibria, giving a dome shaped distribution (as in Figure 6.2).

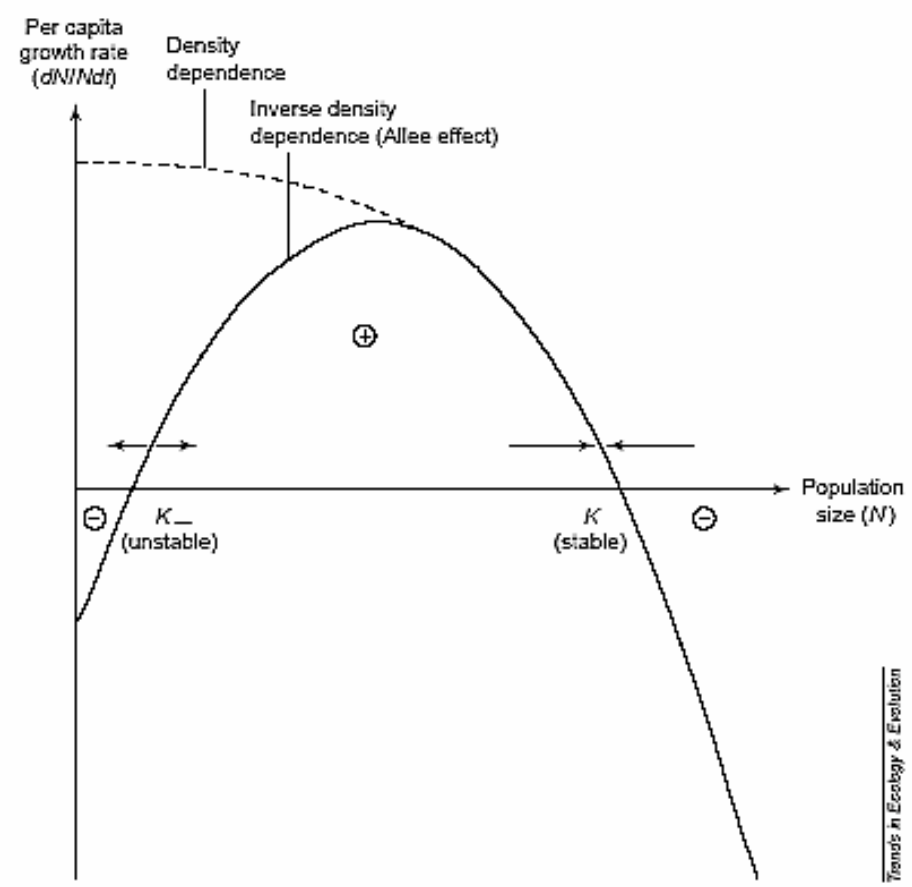


Figure 6.2 A basic diagram of the Allee effect, taken from Courchamp et al. (1999). Above the carrying capacity (stable K) the per capita growth rate (dN/ dt) is negative, while it is positive below K. In the presence of an Allee effect (inverse density dependence), there are two eqiulibria and the growth rate becomes negative below a critical population threshold (unstable K_) leading the population to extinction.

Based on this model, populations undergoing Allee effects have per capita growth rates much lower than predicted from the more common logistic growth models, with the biggest reductions in growth (negative growth) occurring at smaller population sizes (Stephens & Sutherland 1999).

In the case of the Lower Zambezi population viability modelling would be superfluous given the current state of the population (n=5 dogs) and the level of deterministic threats. Modelling with an initial population size of n=5 would give unreliable results: predictive reliability was shown to be poor in grey wolf populations when the population consisted of only one or two colonizing packs (Callaghan 2002). Although exact critical population sizes for Allee effects have not been identified in wild dog populations, previous PVA analysis confirmed the sensitivity of very small wild dog populations (≤20 adults) to any increase in mortality (Ginsberg & Woodroffe 1997), and pack sizes are likely to be reduced in smaller populations, thus increasing the likelihood of pack sizes of five or less. The decline of the Lower Zambezi population provides empirical support for the Allee effect model (Figure 6.1), where small population size was accompanied by low average pack size (7.2), long distance dispersal due to limited mate selection, and by low overall reproductive success.

There has been some debate over whether environmental and demographic stochasticity alone could have been the cause of decline in another small population of wild dogs (Burrows et al. 1994; Ginsberg et al. 1995), or whether catastrophic events (disease outbreaks) caused the local extinction of the population. There was no evidence of disease outbreaks or catastrophic events in the present study. The results from this study suggest that population decline was caused by a combination of increased adult mortality from anthropogenic causes, which interacted with the Allee effects from small population size. These factors combined to maintain a small population size below the critical lower equilibrium level, which suffered from negative growth.

6.2.2 Implications for Population Management

Whilst modelling is useful for a long-term outlook, recovery plans for declining populations often involve more immediate measures (Morris et al. 2002; Woodroffe & Ginsberg 1998). In any small population suffering from inverse density dependence, anthropogenic mortality will be additive (Courchamp et al. 2000), and reducing mortality should be the first priority for increasing the probability of population persistence.

Given its small size during the study period and the deterministic threats identified here, the Lower Zambezi population may have been a sink population which persisted due to limited immigration into the area, perhaps from a larger and more stable population. Source populations have a birth rate that exceeds the death rate, while in sink populations death rate exceeds birth rate (Pulliam 1988). Source populations can produce surplus animals that may emigrate to poorer quality sink areas, (Kreuzer & Huntly 2003; Pulliam 1988). If immigration offsets mortality populations can persist in sink areas (Holt 1985). In this case, there is no evidence of habitat quality restrictions, the sink dynamic was instead related to anthropogenic mortality, and lack of successful dispersal which would usually act to recolonise a population if edge effects were not in place (Tilman 1997). Results from this study indicate a high carrying capacity for the area. The data confirmed an abundance of prey, suitable habitat, and low levels of interpredator competition which was demonstrated by a preference for high prey density areas and only temporal avoidance of moderate to high lion densities. Thus if the critical factors involved in population decline can be mitigated, namely anthropogenic mortality and Allee effects from small population size, the Lower Zambezi area could plausibly sustain a much larger population of wild dogs than was observed during the course of this study.

With an estimate of only 3000-5000 wild dogs left in Africa, conservation priorities are difficult to set. Although not unique, all populations studied to date are genetically differentiated, thus conservation of all remaining populations should form part of efforts to preserve the remaining diversity of the species (Amos & Balmford 2001; Hedrick & Miller 1992; Wilson et al. 2000). The conservation of large and stable populations as strongholds for the species is of unquestionably high value, and the three largest known populations remain in the Selous Game Reserve, the Okavango National Park and adjoining areas, and the Kruger National Park. Given their size these populations require little additional management effort to maintain their viability (Woodroffe et al. 2004). However, Girman et al. (2001) suggested that there was evidence that the Kruger population suffered a recent loss of genetic diversity, and although large, these three populations do not represent the remaining diversity of the species. Where reserves and resources are available, conservation priorities should include the maintenance of networks of smaller populations, improving connectivity, or managing immigration (Reed et al. 2003).

Zambia is important for African wild dog conservation due to its large conservation areas. There are several clumps of adjacent protected areas that measure over 10,000 km^2 each, which has been estimated as the most effective area required to sustain a viable population of large carnivores such as the African wild dog (East 1981). Few countries contain protected areas of this size. A recent IUCN canid Status Survey and Conservation Action Plan (Woodroffe et al. 2004a) listed Zambia as one of 7 countries with the largest estimated extant wild dog populations (>400). Combined with the large protected areas available, Zambia is therefore one of the most potentially significant African wild dog conservation areas in Africa.

Wild dogs are a charismatic species and represent a potential tourist attraction and income source for both the local area and for Zambia in general. During the course of the present study the wild dogs became a flagship species for the Lower Zambezi area, attracting international visitors and thus playing an important role in ecotourism, as well as raising awareness of the African wild dogs' conservation value amongst the local community and government agencies. Tourism is one of the main sources of employment for communities around the National Parks in Zambia. Conservation targeted at land-intensive, flagship species is often the best course of action to protect not only one population but whole ecosystems (Reed et al. 2003; Roberge & Angelstam 2004; van Langevelde et al. 2000), and the African wild dog is an ideal candidate from this perspective. The coexistence of wild dogs and lions in the high prey density areas of the valley floor make the Lower Zambezi and adjoining river valley ecosystems a valuable asset for ecotourism.

6.2.2.1 Potential Management Strategies

The first step for management of the wild dog population would be to substantially reduce the rate of adult mortality caused by snaring. This threat was present within the protected area boundaries, and management would therefore fall under the jurisdiction of the Zambia Wildlife Authority. An increased allocation of resources into anti-poaching activities would be necessary. Since the GMAs contain settlement areas and border the National Park, community education and outreach programs would play an important part in this strategy, as well as addressing ways to increase the direct benefits of ecotourism to local communities.

I) Improve connectivity

In addition to mitigating the effects of snaring to directly reduce mortality, the most effective long-term management solution to maintain a Lower Zambezi wild dog population would be to improve connectivity with a larger, potential source population. The risk of extinction has been shown to be reduced by improving habitat connectivity and maintaining source populations in other large carnivore populations (Ferreras et al. 2001).

The Lower Zambezi National Park and adjoining Chiawa GMA form a combined protected area of approximately 6,400 km^2. However, field data from this study suggests that wild dog home ranges were limited by the steep mountains of the escarpment to the north, since even during denning periods the wild dogs returned to the valley floor to hunt. They were also limited by the low density human settlements in the western Chiawa GMA area, which all packs avoided. The settlement areas start immediately west of the study area (see Figure 6.3). Thus the effective area available to wild dogs, if restricted to the valley floor to the east of the village areas, is reduced to approximately 1700 km^2. If the geographically continuous valley floor area in the adjoining Rufunsa GMA to the east of the Lower Zambezi is included, the available area is over 2,500km^2. There may be additional suitable wild dog areas in the escarpment where slope is reduced and prey density increased; since emigrants were not collared the probability of detection of wild dogs in remote areas of the escarpment would have been low.

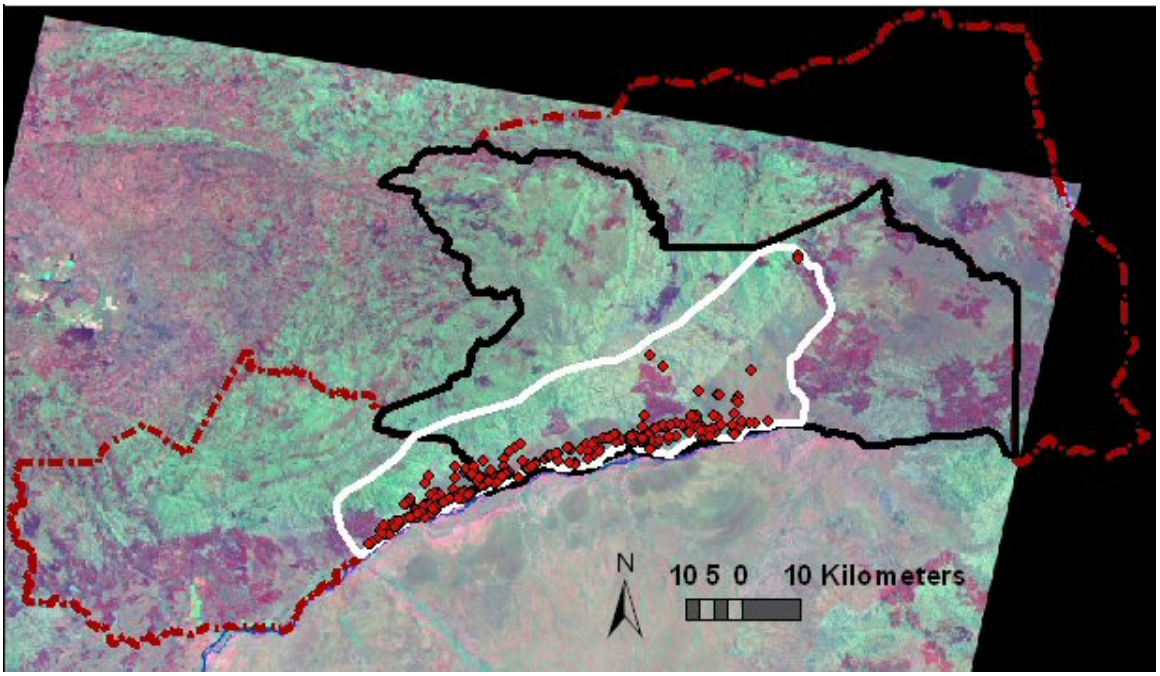


Figure 6.3 Map of protected area boundaries and core study area boundary, imposed over Landsat-7 satellite image of the area. The Lower Zambezi National Park is outlined by solid black line, a solid white line borders the core study area. Dashed red lines indicate GMA boundaries for the Chiawa and Rufunsa GMA's to the west and east respectively. GPS locations of wild dog records from 1999 to 2003 are indicated by red dots. The escarpment is visible as pale green areas on the satellite image.

The largest nearby populations of wild dogs occur in the Mana Pools National Park in Zimbabwe directly across the Zambezi River, and in the South Luangwa National Park in Zambia. The Zambezi River is likely to be an effective barrier to regular dispersal from Mana Pools due its size and regulated constant flow (section 3.4.2.1). The Lower Zambezi valley floor is continuous with the South Luangwa river valley, which runs south-west and meets the Zambezi River to the east of the Lower Zambezi National Park at the eastern border of the Rufunsa GMA. South Luangwa National Park thus lies along a potential river valley corridor that is joined to the Lower Zambezi area through existing GMAs (Figure 6.4). The area of these combined GMAs and National Parks is approximately 35,400 km^2, however this includes steep escarpment areas and wild dog movements may be concentrated in the river valley floors. Sightings reports collected from the South Luangwa safari area during the course of this study, and previous size estimates of the Zambian wild dog population (Buk 1995), suggest that the South Luangwa population may be large, although its

current status is unknown. The current study also received annual reports of wild dog packs in several of the adjoining GMAs.

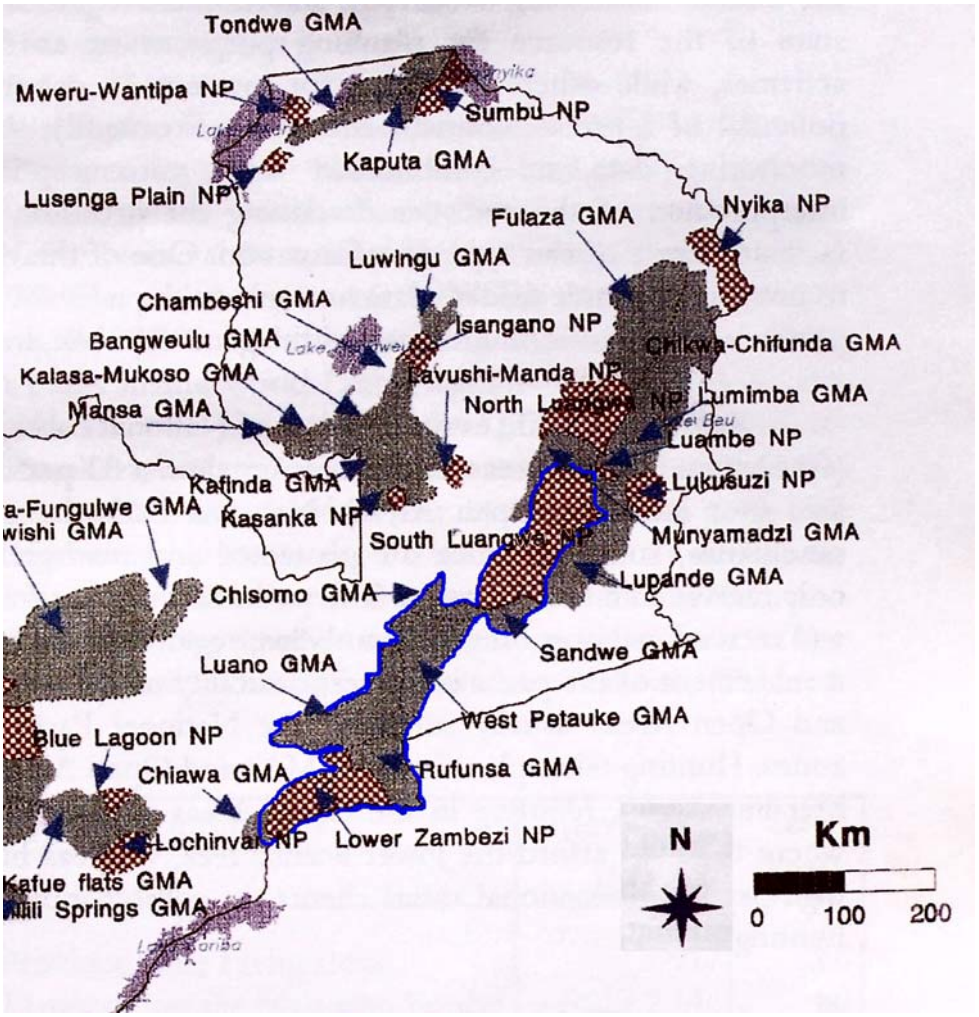


Figure 6.4 Potential wild dog dispersal corridor in eastern Zambia. The corridor area is outlined in blue, and includes the Lower Zambezi and South Luangwa National Parks, and several Game Management Areas, with the Luangwa River forming its eastern border. Figure modified from Jachman, 2000.

Utilising this natural geographic corridor to increase connectivity between the Lower Zambezi and South Luangwa National Parks would be an effective long-term strategy for wild dog conservation, and would only require more active management of existing designated protected areas. The level of human development and community attitudes in the GMA areas would have to be assessed and conservation actions must

include community education and reduction of human-wildlife conflicts where they exist.

Management priorities should include further research into:
1. The current size and distribution of the South Luangwa and GMA wild dog populations.
2. The home range and dispersal patterns of this population, and whether they utilize or are restricted by the continuous geographic river valley corridor.
3. The use of breeding refuges in the extended river valley area and correlations with home range requirements.
3. Potential threats to the population, including snaring, and any areas of human conflict within the GMAs.
4. The historical and recent genetic diversity of the population and whether it is differentiated from the Lower Zambezi population.

Even if the South Luangwa National Park wild dog population is threatened and not a self-sustaining source population, improving connectivity with the Lower Zambezi National Park and other nearby GMAs (Figure 6.4) may still prove beneficial. This type of metapopulation management can be an effective approach since an increased number of patches increases colonization, and therefore reduces local extinction and the threshold of patch occupancy below which all subpopulations may go extinct (Stephens & Sutherland 1999). A "metapopulation" PVA model could be used to guide data collection and determine minimum viable population sizes for this strategy; this PVA model follows the fates of multiple populations and the probabilities of recovery through colonization, extinction of sub-populations, and the likelihood of metapopulation persistence (Morris et al. 2002).

II) Augment the Lower Zambezi population.

Connecting and effectively protecting large areas of reserves in resource poor countries may be logistically difficult. Established land-use development and anthropogenic threats can limit feasibility. A second management strategy would maintain a wild dog population in the Lower Zambezi and immediately adjoining Chiawa and Rufunsa GMAs. Ginsberg and Woodroffe et al. (1997) predicted that populations of around 50 dogs remain resilient to stochasticity and could persist if

well protected, but are susceptible to increases in mortality. Based on the highest density recorded in the study area (2.2 adults/100km^2) the valley floor area of the Lower Zambezi National Park and immediately adjoining GMAs could support at least 50 dogs. However, a population this size would persist only if snaring and any other constant causes of mortality were removed.

The area's high prey densities and other ecological factors (section 6.1.1) suggest that a larger and more stable population could exist at higher densities than recorded here. The diversity of vegetation and presence of breeding refuge habitats in the escarpment enabled temporal avoidance of competing predators in the valley floor, and this may reduce the home range area requirements of wild dogs in this ecosystem. The Zambezi River and Zambian Escarpment provide physical deterrents to pack movements, and if the population were large enough and unrelated mates were made available for emigrants, dispersal out of the area may be reduced. The maximum density of wild dogs recorded in any study area to date was 4 adults/100km^2, and based on this density an area this size could hold up to 100 wild dogs, a far more robust population with a greater probability of persistence (Ginsberg and Woodroffe 1997). However, a more comprehensive vegetation survey of the eastern section of the protected area would be required, to assess the prevalence of thicket habitats (see Figure 3.2), particularly in Rufunsa GMA. Wild dog hunting success was shown to be low in this habitat, thus a high proportion of thicket could limit the suitability of this area to resident wild dog packs, and further limit the potential size of the population. More ground-truthing and investigation of habitat types in the GMA would be needed.

To increase the current population several strategies might be considered, including soft-release of whole packs initially, followed by the augmentation of the population by the introduction of single sex dispersing groups, to mimic natural dispersal (Vucetich & Creel 1999). Once established the population could be maintained with the occasional introduction of new immigrants. Low immigration rates have been shown to increase a population's probability of persistence (Vucetich & Creel 1999), and even the introduction of one individual was shown to enable outbreeding behaviour and recover genetic diversity in a population of wolves (Vila et al. 2003). The artificial increase of pack sizes would also be an important consideration in initial management strategies to reduce Allee effects (Courchamp & MacDonald 2001;

Stephens & Sutherland 1999). Smaller packs have previously been shown to adopt pups in artificial pack formation in captivity (McNutt 1996b).

Captive dogs have been used successfully in reintroductions in the past, when combined with wild caught dogs who taught them how to hunt and avoid competing predators (Bauman et al. 2004). However, the majority of wild dogs bred in captivity have South African origins, while one group is Tanzanian (Woodroffe et al. 2004). Based on the genetic results presented in this study these dogs would be the least suitable option for reintroduction into Zambia. Wild caught dogs of suitable origins would be a sounder option. There has previously been concern over the likelihood of finding free-ranging populations which can afford to lose individuals for translocation without compromising their own probability of persistence (Vucetich & Creel 1999; Woodroffe & Ginsberg 1997). However, based on the findings here, other small populations may be losing dispersers to edge effects when mates are not available, and if this is the case artificial translocation of dispersing groups to and from other populations in Zambia would be worth investigating. Dispersers or "problem packs" inhabiting farmlands and with origins from Hwange or Okavango could also be suitable for translocation.

6.3 CONCLUSIONS

In conclusion, with the allocation of resources into appropriate management strategies, the Lower Zambezi wild dog population could be restored to viability, and this population could make a valuable contribution to the conservation of the species. The critical contributors to population decline were identified as increased adult mortality from anthropogenic causes, interacting with Allee effects on dispersal and reproductive success, which lead to a lack of recruitment into the population. Environmental and ecological factors suggest the study region could support a much larger population of wild dogs than was observed during the course of this research.

In addition to assessing population status and causes of decline, this study provided new insights into wild dog population dynamics. In contrast to previous wild dog studies, the wild dogs in the Lower Zambezi preferred areas of high prey density, and during non-breeding periods preferred areas of high lion density. The effect of sympatric lion population density on wild dog population density was shown to be inconsistent across study sites, and the direct effects of competition from lions were site-specific.

Despite outbreeding behaviour, there was evidence of a loss of genetic diversity and of a population bottleneck in the Lower Zambezi wild dog population. This loss of genetic variability is an important consideration for the long-term management of wild dog populations.

6.4 ADDITIONAL RESEARCH REQUIRED

In addition to the research areas listed under management above, further research is recommended in the following areas:

I) Assessment of dispersal distances in wild dog populations, and correlations with mate availability. Previous research in larger populations has suggested that sex ratio bias may be an important mechanism in determining differences in dispersal distance and dispersal frequency between the sexes (McNutt 1996). For small populations, lack of mate choice for both sexes may increase dispersal distances and act to increase edge effects on the population, regardless of reserve size. Research should include an assessment of how pack sizes are correlated to population size, to determine the threshold levels at which inverse density dependence may come into play in wild dog populations.

II) Investigation of mate selection mechanisms and outbreeding behaviour in wild dogs, including olfactory imprinting and possible MHC linkage. Further research should investigate the underlying biochemical and physiological mechanisms which affect mate selection and inbreeding avoidance behaviour in wild dogs.

III) Further studies of the genetic diversity of fragmented wild dog populations where dispersal mechanisms are increasingly compromised by human settlements. This study has shown that despite the presence of outbreeding behaviour in wild dogs, population decline can lead to loss of genetic diversity and increased chances of inbreeding depression, particularly in small populations. Further research into the genetic diversity of the remaining small populations distributed across Africa is required to assess the present diversity of the species, and to assist in prioritising conservation strategies.

REFERENCES

Alexander, K. A., and M. Appel. 1994. African wild dogs (*Lycaon pictus*) endangered by a canine distemper epizootic among domestic dogs near the Masai Mara National Reserve, Kenya. Journal of Wildlife Diseases **30:**481-485.

Alexander, K. A., P.A Conrad, I.A Gardner, C. Parish, M. Appel, M.G. Levy, and P. K. N. Lerche. 1993. Serologic survey for selected microbial pathogens in African wild dogs (*Lycaon pictus*) and sympatric domestic dogs (*Canis familiaris*) in Maasai Mara, Kenya. Journal of Zoo and Wildlife Medicine **24(2):**140-144.

Alexander, K. A., P.W Kat, L.A Munson, A. Kalake, and M. J. G. Appel. 1996. Canine distemper-related mortality among wild dogs (*Lycaon pictus*) in Chobe National Park, Botswana. Journal of Zoo and Wildlife Medicine **27(3)**:426-427.

Amos, W., and A. Balmford. 2001. When does conservation genetics matter? Heredity **87**:257-265.

Andelman, S., and W. F. Fagan. 2000. Umbrellas and flagships: Efficient conservation surrogates or expensive mistakes? Proceedings of the National Academy of Sciences of the United States of America **97(11):**5954-5959.

Andersson, R., and S. Bergstrom. 1998. Is maternal condition associated with a low sex ratio at birth? Human Biology **70**:1101-1106.

Andreka, G., I.J. Linn, M. R. Perrin, and A. H. Maddock. 1999. Range use by wild dogs in the Hluhluwe-Umfolozi Park, South Africa. South African Journal of Wildlife Research 29(1); 1-9.

Ashley, M. V., D. J. Melnick, and D. Western. 1990. Conservation genetics of the black rhinoceros (*Diceros bicornis*). I Evidence from the mitochondrial DNA of three populations. Conservation Biology **4**:71-77.

Avise, J. C., and S. Nelson. 1989. Molecular genetic relationships of the extinct dusky seaside sparrow. Science **243**: 646-648.

AWF. 2004. Zambezi Heartland: Regional Context. AWF Spatial Analysis Laboratory. http://www.awf.org/heartlands/zambezi/map.php, accessed September 2004.

Ballou, J., and K. Ralls. 1982. Inbreeding and juvenile mortality in small populations of ungulates; a detailed analysis. Biological Conservation **24:**239-272.

Bauman, K. L., C.S. Asa, J. Grisham, and W. Verberkmoes. 2004. Captive Canid Conservation. Pages 280-288 in C. Sillero-Zubiri, M. Hoffman, and D. MacDonald, editors. Canids: Foxes, Wolves, Jackals and Dogs - 2004 Status Survey and Conservation Action Plan. IUCN, Gland, Switzerland.

Beketov, S. V., and S. N. Kashtanov. 2002. A variation of secondary sex ratio in blue fox *Alopex lagopus L.* Genetika **38**:1417-1421.

Bingham, M. 1995. Zambia's vegetation. Zambia National Tourist Board, Accessed July 2005 from http://www.zambiatourism.com/travel/general/vegetati.htm.

Bingham, M. G. 1998. The ecology of the Lower Zambezi National Park. Chiawa camp, Chongwe Area. Unpublished report.

Bjørnstad, O. N., H. P. Andreassen, and R. A. Ims. 1998. Effects of habitat patchiness and connectivity on the spatial ecology of the root vole *Microtus oeconomus*. Journal of Animal Ecology **67**:127-140.

Boudjemadi, K., J. Lecomte, and J. Clobert. 1999. Influence of connectivity on demography and dispersal in two contrasting habitats: an experimental approach. Journal of Animal Ecology **68**:1207-1224.

Boukal, D. S., and L. Berec. 2002. Single-species models of the Allee Effect: Extinction boudaries, sex ratios and mate encounters. Journal of Theoretical Biology **218**:375-394.

Bradshaw, C. J. A., R. G. Harcourt, and L. S. Davis. 2003. Male-biased sex ratios in New Zealand fur seal pups relative to environmental variation. Behavioral Ecology and Sociobiology **53**:297-307.

Brook, B. W., D. W. Tonkyn, J. J. O'Grady, and R. Frankham. 2002. Contribution of inbreeding to extinction risk in threatened species. Conservation Ecology **6**:16.

Buk, K. 1995. African wild dog survey in Zambia. Canid News **3**.

Burrows, R. 1992. Rabies in wild dogs. Nature **359**:277.

Burrows, R. 1995. Demographic changes and social consequences in wild dogs 1964 -1992. in A. R. E. Sinclair, and P. Arcese, editors. Serengeti II: research, management, and conservation of an ecosystem. Chicago University Press, Chicago.

Burrows, R. 1998. What was the scientific basis of IUCN Canid Specialist Group's claim that handling of African wild dogs (*Lycaon pictus*) has no effect on their mortality, survival or longevity? IUCN/SSC Specialist Group Newsletter **15:**6-8.

Burrows, R., H. Hofer, and M. L. East. 1995. Population dynamics, intervention and survival in the African wild dogs (*Lycaon pictus*). Proceedings of the Royal Society of London B **262:**235-245.

Burrows, R., H. Hofer, and M. East. 1994. Demography, extinction and intervention in a small population: the case of the Serengeti wild dogs. Proceedings of the Royal Society of London - Series B: Biological Sciences **256:**281-292.

Bwangamoi, O., T.A Ngatia, and J. D. Richardson. 1993. Sarcocystis-like organisms in the musculature of a domestic dog (*Canis familiaris*) and wild dogs (*Lycaon pictus*) in Kenya. Veterinary Parasitology **49(2/4):**201-205.

Callaghan, C. J. 2002. The ecology of the grey wolf (*Canis lupus*) habitat use, survival and persistence in the Central Rocky Mountains, Canada. PhD Dissertation, University of Guelph, Canada.

Carbone, C., J.T. du Toit, and I. J. Gordon. 1997. Feeding success in African wild dogs: does kleptoparasitism by hyaenas influence pack size? Journal of Animal Ecology **66(3):**318-326.

Caro, T. M. 2003. Umbrella species: Critique and lessons from East Africa. Animal Conservation **6(2):**171-181.

Caughley, G. 1994. Directions in conservation biology. Journal of Animal Ecology **63:**215-244.

Chen, Y., X. Z. Y. Zhang, F. Dong, and J. Wang. 2000. Molecular phylogeny of canidae using mitochondrial cytochrome b DNA sequences. Acta Genetica Sinica **27(1):**7-11.

Childes, S. L. 1988. The past history, present status and distribution of the hunting dog *Lycaon pictus* in Zimbabwe. Biological Conservation **44:**301-316.

Clark, A. B. 1978. Sex ratio and local resource competition a prosimian primate. Science **201**:163-165.

Collett, D. 1994. Modelling Survival Data in Medical Research. Chapman & Hall, London.

Colly, L. P., and J. W. Nesbit. 1992. Fatal acute babesiosis in a juvenile wild dog (*Lycaon pictus*). Journal of the South African Veterinary Association **63 (1):**36-38.

Cornuet, J. M., S. Piry, G. Luikart, A. Estoup, and M. Solignac. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics **153**:1989-2000.

Courchamp, F., T. Clutton-Brock, and B. Grenfell. 1999. Inverse density dependence and the Allee effect. Trends in Ecology and Evolution **14**:405-410.

Courchamp, F., T. Clutton-Brock, and B. Grenfell. 2000. Multipack dynamics and the Allee affect in the African wild dog, *Lycaon pictus*. Animal Conservation **3**:277-285.

Courchamp, F., and D. W. MacDonald. 2001. Crucial importance of pack size in the African wild dog *Lycaon pictus*. Animal Conservation **4:**169-174.

Courchamp, F., G. S. A. Rasmussen, and D. W. Macdonald. 2002. Small pack size imposes a trade-off between hunting and pup-guarding in the painted hunting dog *Lycaon pictus*. Behavior Ecology **13**:20-27.

Crandall, K. A., O.R.P Bininda-Emonds, G.M. Mace, and R. K. Wayne. 2000. Considering evolutionary processes in conservation biology. Trends in ecology and evolution **15:**290-295.

Creel, S. 1997. Cooperative hunting and group size:assumptions and currencies. Animal Behaviour **54**:1319-1324.

Creel, S. 2001. Four factors modifying the effect of competition on carnivore population dynamics as illustrated by African wild dogs. Conservation Biology **15(1):**271-274.

Creel, S. R., and N. M. Creel. 1995. Communal hunting and pack size in African wild dogs, *Lycaon pictus*. Animal Behaviour **50:**1325-1339.

Creel, S. R., and N. M. Creel. 1996. Limitation of African wild dogs by competition with larger carnivores. Conservation Biology **10:**1-15.

Creel, S.R., and N. M. Creel. 1997. Lion density and population structure in the Selous Game Reserve: evaluation of hunting quotas and offtake. African Journal of Ecology **35:**83-93.

Creel, S.R., and N. M. Creel. 1998. Six ecological factors that may limit African wild dogs, *Lycaon pictus*. Animal Conservation **1:**1-9.

Creel, S.R., and N. M. Creel 2002. The African wild dog. Behaviour, ecology and conservation. Princeton University Press, Princeton, New Jersey.

Creel, S., and D. W. MacDonald. 1992. Cause of wild dog deaths. (African wild dogs). Nature **360(6405):**633-635.

Creel, S., J. W. McNutt, and M. G. L. Mills. 2004. Demography and Population Dynamics of African Wild Dogs in Three Critical Populations. Pages 337-350 in D. W. Macdonald, and C. Sillero-Zubiri, editors. Biology and Conservation of Wild Canids. Oxford University Press.

Creel, S., N.M. Creel, J.A. Matavelo, M.M.A. Mtambo, E. K. Batamuzi, and J. E. Cooper. 1995. The effects of anthrax on endangered African wild dogs (*Lycaon pictus*). Journal of Zoology (London) **236:**199-209.

Creel, S., N.M Creel, and S. L. Monfort. 1997a. Radiocollaring and stress hormones in African wild dogs. Conservation Biology **11(2):**544-548.

Creel, S., N.M. Creel, L. Munson, D. Sanderlin, and M. J. Appel. 1997b. Serosurvey for selected viral diseases and demography of African wild dogs in Tanzania. Journal of Wildlife Diseases **33(4):**823-832.

Creel, S. R., N.M. Creel, M.G.L. Mills, and S. L. Monfort. 1997c. Rank and reproduction in cooperatively breeding African wild dogs: behavioural and endocrine correlates. Behavioural Ecology **8(3):**298-306.

Creel, S., N.M. Creel, and S. L. Monfort. 1998. Birth order, estrogens and sex-ratio adaptation in African wild dogs (*Lycaon pictus*). Animal Reproduction Science **53(1-4):**315-320.

Creel, S., G. Spong, J. L. Sands, J. Rotella, J. Zeigle, L. Joe, K. M. Murphy, and D. Smith. 2003. Population size and estimation in Yellowstone wolves with error-prone noninvasive microsatellite genotypes. Molecular Ecology **12**:2003-2009.

Cross, P. C., and S. R. Beissinger. 2001. Using logistic regression to analyse the sensitivity of PVA models: A comparison of methods based on African wild dog models. Conservation Biology **15(5):**1335-1346.

Du Toit, R. F. 1982. A Preliminary assessment of the environmental implications of the proposed Mupata and Batoka hydro-electric schemes (Zambezi River, Zimbabwe). Natural Resources Board of Zimbabwe, Harare.

Duncan, P. 1983. Determinants of the use of habitat by horses in a mediterranean wetland. Journal of Animal Ecology **52**:93-109.

Dunham, K. 1994. The effect of drought on the large mammal populations of Zambezi riverine woodlands. Journal of Zoology London **234(3):**489-526.

Durchfeld, B., W. Baumgartner, W. Herbst, and R. Brahm. 1990. Vacccine-associated Canine Distemper Infection in a litter of African Hunting Dogs (*Lycaon pictus*). Journal of Veterinary Medicine **B37:**202-212.

East, M. L. 1996. Wild dogs in the Serengeti. Science 271.

East, R. 1981. Species-area curves and populations of large mammals in African savanna reserves. Biological Conservation **21:**111-126.

Engen, S., R. Lande, and B. Saether. 2003. Demographic stochasticity and Allee effects in populations with two sexes. Ecology **84**: 2378-2386.

Erdfelder, E., F. Faul, and A. Buchner. 1996. GPOWER: A general power analysis program. Behavior Research Methods, Instruments, & Computers **28**:1-11.

Ernest, H. B., M. C. T. Penedo, B. P. May, M. Syvanen, and W. M. Boyce. 2000. Molecular tracking of mountain lions in the Yosemite Valley region in California: genetic analysis using microsatellites and faecal DNA. Molecular Ecology **9**:433-441.

Estes, R. D. 1991. The behaviour guide to African mammals; including hoofed mammals, carnivores, primates. University of California Press, Berkeley.

Estes, R. D., and J. Goddard. 1967. Prey selection and hunting behaviour of the African wild dog. Journal of Wildlife Management **31:**52-70.

Excoffier, L., P.E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics **131**:479-491.

Fanshawe, J. H., and C. D. Fitzgibbon. 1993. Factors influencing the hunting success of an African wild dog pack. Animal Behaviour **45:**479-490.

Fanshawe, J. H., L.H. Frame, and J. R. Ginsberg. 1991. The wild dog - Africa's vanishing carnivore. Oryx **25(3):**137-146.

Fekadu, M., F. W. Chandler, and A. K. Harrison. 1982. Pathogenesis of rabies in dogs inoculated with an Ethiopian rabies virus strain. Immunoflourescence, histologic and ultrastructural studies of the central nervous system. Archives of Virology **71:**109-126.

Felsenstein, J. 2004. PHYLIP (version 3.6): Phylogeny Inference Package. University of Washington, Seattle, USA. Available from: http://evolution.gs.washington.edu/phylip.html.

Fenner, F. J., E.P.J. Gibbs, F.A. Murphy, R. Rott, M.J. Studdert, and D. O. White 1993. Veterinary Virology, 2nd Ed. Academic Press Inc., California.

Ferreras, P., P. Gaona, F. Palomares, and M. Delibes. 2001. Restore habitat or reduce mortality? Implications from a population viability analysis of the Iberian lynx. Animal Conservation **4:**265-274.

Fisher, D. O. 1999. Offspring sex ratio variation in the bridled nailtail wallaby, *Onychogalea fraenata*. Behavioral Ecology and Sociobiology **45**: 411-419.

Frame, L. H., and G. W. Frame. 1976. Female wild dogs emigrate. Nature **263:**227-229.

Frame, L. H., J.R. Malcolm, G.W. Frame, and H. v. Lawick. 1979. Social organisation of African wild dogs (*Lycaon pictus*) on the Serengeti Plains, Tanzania 1967-1978. Zeitschrift fur Tierpsychologie **50:**225-249.

Frankham, R., J. D. Ballou, and D. A. Briscoe 2002. Introduction to Conservation Genetics. Cambridge University Press, Cambridge, UK.

Frantzen, M. A. J., J.B. Silk, J.W.H Ferguson, R. K. Wayne, and M. H. Kohn. 1998. Empirical evaluation of preservation methods for faecal DNA. Molecular Ecology **7:**1423-1428.

Fraser, D. J., and L. Bernatchez. 2001. Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Molecular Ecology **10**:2741-2752.

Freitag, S., and T. J. Robinson. 1993. Pylogenetic patterns in mitochondrial DNA of the Ostrich (*Struthio camelus*). Auk **110**:614-622.

Fuller, T. K., and P. W. Kat. 1990. Movements, activity, and prey relationships of African wild dogs (*Lycaon pictus*) near Aitong, southwestern Kenya. African Journal of Ecology **28:**330-350.

Fuller, T. K., and P. W. Kat. 1993. Hunting success of African wild dogs in southwestern Kenya. Journal of Mammology **74(2):**464-467.

Fuller, T. K., M.G.L. Mills, M. Borner, K. Laurenson, and P. W. Kat. 1992a. Long distance dispersal by African wild dogs in East and Southern Africa. Journal of African Zoology **106:**535-537.

Fuller, T. K., P.W Kat, J.B. Bulger, and e. al. 1992b. Population dynamics of African wild dogs. in D. R. Barret, and R. H. McCulloghIn, editors. Wildlife 2001: Populations. Elsevier Science,, London.

Gascoyne, S. C., M.K. Laurenson, S. Lelo, and M. Borner. 1993. Rabies in African wild dogs (*Lycaon pictus*) in the Serengeti region, Tanzania. Journal of Wildlife Diseases **29(3):**396-402.

Gautestad, A. O., and I. Mysterud. 1993. Physical and biological mechanisms in animal movement processes. Journal of Applied Ecology **30**:523-535.

GenStat Release 8 2005. VSN International Ltd, Hemel Hempstead, UK.

Gilpin, M. E., and M. E. Soulé. 1986. Minimum Viable Populations: Processes of Species Extinction. Pages 19-34 in M. E. Soulé, editor. Conservation Biology; the Science of Scarcity and Diversity. Sinauer, Sunderland, MA.

Ginsberg, J. R. 1993. Mapping wild dogs. IUCN/CSG Canid News **1:**2-6.

Ginsberg, J. R. 1994. Conservation biology and status of the African wild dogs, *Lycaon pictus*. Endangered Species Update **11(10):**1-6.

Ginsberg, J. R., G.M. Mace, and S. Albon. 1995a. Local extinction in a small and declining population: wild dogs in the Serengeti. Proceedings of the Royal Society of London, Series B **262:**221-228.

Ginsberg, J. R., K.A. Alexander, S. Creel, P.W Kat, J. W. McNutt, and M. G. L. Mills. 1995b. Handling and survivorship of African wild dogs (*Lycaon pictus*) in five ecosystems. Conservation Biology **9(3):**665-674.

Ginsberg, J. R., and R. Woodroffe. 1997. Extinction risks faced by remaining wild dog populations. Pages 75-87 in R. Woodroffe, J. R. Ginsberg, and D. W. Macdonald, editors. The African Wild Dog: Status Survey and Conservation Action Plan. IUCN, Gland, Switzerland.

Girman, D. J., C. Vila, S. C. E. Geffen, M.G.L. Mills, J.W. McNutt, J.R Ginsberg, P.W Kat, K. H. Mamiya, and R. K. Wayne. 2001. Patterns of population subdivision, gene flow and genetic variability in the African wild dog (*Lycaon pictus*). Molecular Ecology **10(7):**1703-1723.

Girman, D. J., M.G.L. Mills, E. Geffen, and R. K. Wayne. 1997. A molecular genetic analysis of social structure, dispersal, and interpack relationships of the African wild dog (*Lycaon pictus*). Behavioural Ecology and Sociobiology **40:**187-198.

Girman, D. J., P.W. Kat, M.G.L. Mills, J.R Ginsberg, M. Borner, V. Wilson, J.H. Fanshawe, C. Fitzgibbon, L.M Lau, and R. K. Wayne. 1993. Molecular genetic and morphological analyses of the African wild dog (*Lycaon pictus*). Journal of Heredity **84:**450-459.

Gorman, M. L., M.G. Mills, J.P. Raath, and J. R. Speakman. 1998. High hunting costs make African wild dogs vulnerable to kleptoparasitism by hyaenas. Nature **391:**479-480.

Gorman, M. L., M.G.L. Mills, and J. French. 1992. Satellite Tracking of the African Wild Dog *Lycaon pictus*. in I. G. Priede, and S. M. Swift, editors. Wildlife telemetry: Remote Monitoring and Tracking of Animals. Ellis Horwood,, London.

Goudet, J. 2002. FSTAT (version 2.9.3.2) A program to estimate and test gene diversities and fixation indices. Available from: http://www.unil.ch/izea/softwares/fstat.html.

Hansen, M. M., E. E. Nielsen, and K. L. D. Mensberg. 1997. The problem of sampling families rather than populations: relatedness among individuals in samples of juvenile brown trout *Salmo trutta* L. Molecular Ecology **6**469-474.

Harcourt, A. H., S.A. Parks, and R. Woodroffe. 2001. Human density as an influence on species/area relationships: double jeopardy for small African reserves? Biodiversity and Conservation **10:**1011-1026.

Hardy, I. C. W. 1997. Possible factors influencing vertebrate sex ratios: an introductory overview. Applied Animal Behaviour Science **51**: 217-241.

Haydon, D. T., M.K. Laurenson, and C. Sillero-Zubiri. 2002. Integrating epidimiology into population viability analysis: Managing the risk posed by rabies and canine distemper to the ethiopian wolf. Conservation Biology **16(5):**1372-1385.

Hedrick, P. W., and S. T. Kalinowski. 2000. Inbreeding depression in conservation biology. Annual Review of Ecology and Systematics **31**139-162.

Hedrick, P. W., and P. S. Miller. 1992. Conservation genetics: Techniques and fundamentals. Ecological Applications **2**:30-46.

Hewitt, G. 2000. The genetic legacy of the Quarternary ice ages. Nature **405**:907-913.

Hofmeyer, M., J. Bingham, E.P. Lane, A. Ide, and L. Nel. 2000. Rabies in African wild dogs (*Lycaon pictus*) in the Madikwe Game Reserve, South Africa. Veterinary Record **146:**:50-52.

Holsinger, K. E., and R. J. Mason-Gamer. 1996. Hierarchical analysis of nucleotide diversity in geographically structured populations. Genetics **142**:629-639.

Holt, R. D. 1985. Population dynamics in two-patch environments: some anomalous consequences of an optimal habitat distribution. Theoretical Population Biology **28**:181-208.

Hradecky, P. 1985. Possible pheromonal regulation of reproduction in wild carnivores. Journal of Chemical Ecology **11(2):**:241-250.

IUCN. 2002. IUCN Red list of Threatened Species. IUCN, Gland, Switzerland.

Jachmann, H. 2000. Zambia's Wildlife Resources: A Brief Ecology. Wildlife Resources monitoring Unit, Environmental Council of Zambia, Lusaka, Zambia.

Jarman, M. V. 1979. Impala social behaviour. Beihefte Z. Tierpsychol. 21: 1-92.

Kalbfleisch, J. D., and R. L. Prentice 1980. The Statistical Analysis of Failure Time Data. Wiley, New York.

Kat, P. W., K.A Alexander, J.S. Smith, and L. Munson. 1995. Rabies and African wild dogs in Kenya. Proceedings of the Royal Society of London B **262:**:229-233.

Kelt, D. A., and J. H. Brown. 2000. Species as units of analysis in ecology and biogeography: Are the blind leading the blind? Global Ecology & Biogeography **9**:213-217.

Kie, J. G., J.A Baldwin, and C. J. Evans 1994. CALHOME: Home Range Analysis Program - Electronic Users Manual. U.S Forest Service, Fresno and Albany, California.

Kieser, J. A., and H. T. Groeneveld. 1992. Mandibulodental allometry in the African wild dog, *Lycaon pictus*. Journal of Anatomy **181(1):**133-137.

Kim, K. S., Y. Tanabe, C. K. Park, and J. H. Ha. 2001. Genetic variability in East Asian dogs using microsatellite loci analysis. The Journal of Heredity **92(5):**398-403.

Kinvall, O. 2003. Temporal extrapolation of PVA results in relation to the IUCN Red List criterion E. Conservation Biology 17(1):316-321.

Knobel, D. L., and J. T. d. Toit. 2003. The influence of pack social structure on oral rabies vaccination coverage in captive African wild dogs (*Lycaon pictus*). Applied Animal Behaviour Science **80:**61-70.

Kocher, T. D., W. K. Thomas, S. A. Meyer, S. V. Edwards, S. Paabo, F. X. Villablanca, and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences, USA **86**:6196-6200.

Kohn, M., F. Knauer, A. Stoffella, W. Schroder, and S.Paabo. 1995. Conservation genetics of the European brown bear - a study using excremental PCR of nuclear and mitochondrial sequences. Molecular Ecology **4:**95-103.

Kohn, M. H., E. C. York, D. A. K. , G. Haught, R. M. Sauvajot, and R. K. Wayne. 1997. Estimating population size by genotyping faeces. Proceedings of the Royal Society of London - Series B: Biological Sciences. **266(1420):**657-663.

Kreuzer, M. P., and N. J. Huntly. 2003. Habitat-specific demography:

evidence for source-sink population structure in a mammal, the pika. Oecologia **134**:343-349.

Kruger, S. C., M.J. Lawes, and A. H. Maddock. 1999. Diet choice and capture success of wild dog (*Lycaon pictus*) in Hluhluwe-Umfolozi Park, South Africa. Journal of Zoology (London) **284(4):**543-551.

Kruuk, H. 1972. The Spotted Hyaena: a Study of Predation and Social Behaviour. University of Chicago Press, Chicago.

Kruuk, L. E. B., T. Clutton-Brock, S. D. Albon, J. M. Pemberton, and F. E. Guinness. 1999. Population density affects sex ratio variation in red deer. Nature **399**:459-461.

Kumar, S., K. Tamura, I. Jakobsen, and M. Nei. 2001. MEGA 2: Molecular Evolutionary Genetics Analysis software. Bioinformatics **17**:1244-1245.

Lacy, R. C. 1993. Vortex: a computer simulation model for population viability analysis. Wildlife Research **20**:45-65.

Laikre, L., and H. Ryman. 1991. Inbreeding depression in a captive wolf (*canis lupus*) population. Conservation Biology **5:**33-40.

Lambeck, R. J. 1997. Focal Species: A multi-species umbrella for nature conservation. Conservation Biology **11(4):**849-856.

Laurenson, K., J. Esterhuysen, P. Stander, and J. V. Heerdan. 1997. Aspects of rabies epidemiology in Tsumkwe District, Namibia. Onderstepoort Journal of Veterinary Research **64(1):**39-45.

Lawson, E. J., and A. R. Rodgers. 1997. Differences in home-range size computed in commonly used software programs. Wildlife Society Bulletin **25**:721-729.

Lehman, N., A. Eisenhawer, K. Hansen, L. D. Mech, R. O. Peterson, P. J. P. Gogan, and R. K. Wayne. 1991. Introgression of coyote mitochondrial DNA into sympatric North American wolf populations. Evolution **45**:104-119.

Lehman, N., and R. K. Wayne. 1991. Analysis of coyote mitochondrial DNA genotype frequencies: Estimation of the effective number of alleles. Genetics **128:**:405-416.

Leuthold, W. 1970. Observations on the social organisation of impala. Z. Tierpsychol. 27:693-721.

Linnell, J. D. C., J.E. Swenson, and R. Anderson. 2000. Conservation of biodiversity in Scandanavian boreal forests: Large carnivores as flagships, umbrellas, indicators, or keystones? Biodiversity and Conservation **9(7):**857-868.

Luikart, G., and J. M. Cornuet. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Conservation Biology **12**:228-237.

MacDonald, D. W. 1983. The ecology of carnivore social behaviour. Nature, London, **301:**379-383.

Maddock, A. H. 1993. Wild Dog Project - a success story. Custos **19(4)**

Maddock, A. H., and M. G. L. Mills. 1994. Population characteristics of African Wild dogs *Lycaon pictus* in the Eastern Transvaal Lowveld, South Africa, as revealed through photographic records. Biological Conservation **67:**57-62.

Malcolm, J. R. 1979. Social Organisation and Communal Rearing in African Wild Dogs. Harvard University, Cambridge, Massachusetts.

Malcolm, J. R. 1980. Food caching by African wild dogs (*Lycaon pictus*). Journal of Mammology **61(4):**7434-7744.

Malcolm, J. R., and K. Marten. 1982. Natural selection and the communal rearing of pups in African wild dogs *Lycaon pictus*. Behavioural Ecology and Sociobiology **10:**1-13.

Manel, K., M.K. Schwartz, G. Luikart, and P. Taberlet. 2003. Landscape genetics: combining landscape ecology and population genetics. Trends in Ecology and Evolution **18**:189-197.

Marcella, K. 2001. Lineage loss in Serengeti cheetahs: Consequences of high reproductive variance and heretibility of fitness on effective population size. Conservation Biology **15**:137-147.

Maynard-Smith, J. 1980. A new theory of sexual selection. Behavioral Ecology and Sociobiology **7**:247-251.

McCarthy, M. A., S.J. Andelman, and H. P. Possingham. 2003. Reliability of relative predictions in population viability analysis. Conservation Biology **17(4):**982-989.

McCormick, A. E. 1983. Canine distemper in African cape hunting dogs (*Lycaon pictus*) - possibly vaccine induced. Journal of Zoo Animal Medicine **14:**66-71.

McCreery, E. K., and R. L. Robbins. 2001. Proximate explanations for failed pack formation in *Lycaon pictus*. Behaviour **138:**1467-1479.

McElroy, D., P. Moran, E. Bermingham, and I. Kornfield. 1991. REAP: the restriction enzyme analysis package, version 4.0. Journal of Heredity **83**:157-158.

McNutt, J. W. 1996a. Sex-biased dispersal in African wild dogs, *Lycaon pictus*. Animal Behaviour **52:**1067-1077.

McNutt, J. W. 1996b. Adoption in African wild dogs, *Lycaon pictus.* Journal of Zoology (London) **240**:163-173.

Mills, M. G. L. 1985. Hyena survey of Kruger National Park: August - October 1984. In Newsletter 2 Hyena Specialist Group. International Union for the Conservation of Nature, Oxford, England.

Mills, M. G. L. 1995. Notes on wild dog *Lycaon pictus* and lion *Panthera leo* population trends during a drought in the Kruger National Park. Kodoe **38**:95-99.

Mills, M. G. L., and H. C. Biggs. 1993. Prey apportionment and related ecological relationships between large carnivores in Kruger National Park. Symposia of the Zoological Society of London **65:**:253-268.

Mills, M. G. L., and M. L. Gorman. 1997. Factors affecting the density and distribution of wild dogs in the Kruger National Park. Conservation Biology **11(6):**1397-1406.

Mills, M. G. L., J. M. Juritz, and W. Zucchini. 2001. Estimating the size of spotted hyaena (*Crocuta crocuta*) populations through playback recordings allowing for non-response. Animal Conservation **4**:335-343.

Minch, E., A. Ruiz-Linares, D. Goldstein, M. Feldman, and L. L. Cavalli-Sforza 2004. MICROSAT (version 2). A computer program for calculating various statistics on microsatellite data. Stanford University, Standford, CA. Available from: http://hpgl.stanford.edu/projects/microsat/.

Moerhrenschlager, A., and M. J. Somers. 2004. Canid reintroductions and metapopulation management. Pages 289-298 in C. Sillero-Zubiri, M. Hoffman, and D. MacDonald, editors. Canids: Foxes, Wolves, Jackals and Dogs -2004 Status Survey and Conservation Action Plan. IUCN, Gland, Switzerland.

Moritz, C. 1994. Defining "evolutionary significant units" for conservation. Trends in Ecology and Evolution **9**:373-375.

Moritz, C. 1999. Conservation units and translocations: Strategies for conserving evolutionary processes. Hereditas **130**:217-228.

Morris, W. F., P.L. Bloch, B.R. Hudgens, L.C. Moyle, and J. R. Stinchcombe. 2002. Population viability analysis in endangered species recovery plans: past use and future improvements. Ecological Applications **12(3):**708-712.

Morton, N. E. 2003. Genetic epidemiology, genetic maps, and positional cloning. Philosophical Transactions of the Royal Society of London B Biological Sciences **358**:1701-1708.

Mugdadi, A., and I. Ahmad. 2004. A bandwidth selection for kernel density estimation of functions of random variables. Computational Statistics and Data Analysis **47**:49-62.

Myers, J. H. 1978. Sex ratio adjustment under food stress: maximization of quality of numbers of offspring. American Naturalist **112**:381-388.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89**:583-590.

Nei, M. 1982. Evolution of human races at the gene level. Pages 167-181 in B. Bonn&Tamir, T. Cohen, and R. M. Goodman, editors. Human Genetics, Part A: The Unfolding Genome, Proceedings of the Sixth International Congress of Human Genetics. Alan R. Liss Incorporated, New York.

Nei, M., and A. K. Roychoudoury. 1974. Sampling variances of heterozygosity and genetic distance. Genetics **76**:379-390.

Nicholas, K. B., and H. B. J. Nicholas. 1997. GeneDoc: a tool for editing and annotating multiple sequences alignments. Distributed by the author.

Oetting, W. S., H. K. Lee, D. J. Flanders, G. L. Wiesner, T. A. Sellers, and R. A. King. 1995. Linkage analysis with multiplexed short tandem repeat polymorphisms using infrared flourescence and M13 tailed primers. Genomics **30**:450-458.

Packer, C., and L. Ruttan. 1988. The evolution of cooperative hunting. American Naturalist **132:**159-198.

Palgrave, K. C. 1997. Trees of Southern Africa. Second revised edition. Struik Publishers Ltd., Cape Town, RSA.

Parysow, P., and D. J. Tazik. 2002. Assessing the effect of estimation error on population viability analysis: an example using the black-capped vireo. Ecological Modelling **155:**217-229.

Pienaar, U. d. V. 1969. Predator prey relationships amongst the mammals of the Kruger National Park. Kodoe **12**:108-176.

Pierce, M. A., M.K. Laurenson, and S. C. Gascoyne. 1995. Hepatozoonosis in cheetahs and wild dogs in the Serengeti ecosystem. African Journal of Ecology **33(3):**273-275.

Piggot, M. P., and A. C. Taylor. 2003a. Extensive evaluation of faecal preservation and DNA extraction methods in Australian and introduced species. Australian Journal of Zoology **51**:341-355.

Piggot, M. P., and A. C. Taylor. 2003b. Remote collection of animal DNA and its applications in conservation management and understanding the population biology of rare and cryptic species. Wildlife Research **30**:1-13.

Pilgrim, K. R., D. K. Boyd, and S. H. Forbes. 1998. Testing for wolf-coyote hybridization in the Rocky Mountains using mitochondrial DNA. Journal of Wildlife Management **62**:683-689.

Piry, S., A. Alapetite, J. M. Cournuet, D. Paetkau, L. Baudouin, and A. Estoup. 2004. GENECLASS2: A software for genetic assignment and first-generation migrant detection. Journal of Heredity **95**:536-539.

Piry, S., G. Luikart, and J. M. Cornuet. 1999. BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data . Journal of Heredity **90**:502-503.

Pitra, C., A. J. Hansen, D. Lieckfeldt, and P. Arctander. 2002. An exceptional case of historical outbreeding in African sable antelope. Molecular Ecology **11**:1197-1208.

Poiani, K. A., M.D. Merril, and K. A. Chapman. 2001. Identifying conservation-priority areas in a fragmented Minnesota landscape based on the umbrella species concept and selection of large patches of natural vegetation. Conservation Biology **15:**513-522.

Posada, D., and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics **14**:817-818.

Post, E., M.C. Forchhammer, N.C. Stenseth, and R. Langvatn. 1999. Extrinsic modification of vertebrate sex ratios by climatic variation. American Naturalist **154**:194-204.

Pritchard, J. K., M. Stephens, and P. Donelly. 2000. Inference of population structure from multilocus genotype data. Genetics **155**:945-959.

Pulliam, H. R. 1988. Sources, sinks and population regulation. American Naturalist **132**:652-661.

Ralls, K., J.D. Ballou, and A. Templeton. 1988. Estimates of lethal equivalents and the cost of inbreeding in mammals. Conservation Biology **2(2):**185-193.

Randi, E., V. Lucchini, M. J. Christensen, N. Mucci, S. M. Funk, G. Dolf, and V. Loeschcke. 2000. Mitochondrial DNA variability in Italian and East European wolves: Detecting the consequences of small population size and hybridization. Conservation Biology **14**:464-473.

Rannala, B., and J. L. Mountain. 1997. Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences USA **94**:9197-9201.

Rasmussen, G. S. A. 1996. Predation on bat-eared foxes Octocyon megalotis by Cape hunting dogs *Lycaon pictus*. Koedoe **39(1):**127-129.

Rasmussen, G. S. A. 1999. Livestock predation by the painted hunting dog. Biological Conservation. 88: **133-139**.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 2.1): population genetics software for exact tests and ecumenicism. Journal of Heredity **86**:248-249.

Reed, D. H., J.J. O'Grady, B.W. Brook, J.D. Ballou, and R. Frankham. 2003. Estimates of minimum population sizes for vertebrates and factors influencing those estimates. Biological Conservation **113:**23-24.

Reed, J. M., L.S. Mills, J.B. Dunning Jr, E.S Menges, K.S. McKelvey, R. Frye, S.R. Beissinger, M. Anstett, and P. Miller. 2002. Emerging issues in population viability analysis. Conservation Biology **16(1):**7-19.

Reich, A. 1981. The Behaviour and Ecology of the African Wild Dog (*Lycaon pictus*) in the Kruger National Park. Yale University, New Haven, Conneticut.

Roach, J. L., P. Stapp, B. V. Horne, and M. F. Antolin. 2001. Genetic structure of a metapopulation of black-tailed prairie dogs. Journal of Mammology **82**:946-959.

Roberge, J., and P. Angelstam. 2004. Usefulness of the umbrella species concept as a conservation tool. Conservation Biology **18(1):**76-85.

Roelke-Parker M.E., L. Munson, C. Packer, R. Kock, S. Cleaveland, C. M., S.J. O'Brien, A. Pospischil, R. Hofmann-Lehmann, and H. Lutz. et al. 1996. A canine distemper virus epidemic in Serengeti lions (Panthera leo). Nature 1 **379(6564):**441-445.

Russell, A. F., P. N. M. Brotherton, G. M. McIlrath, L. L. Sharpe, and T. Clutton-Brock. 2003. Breeding success in cooperative meerkats: Effects of helper number and maternal state. Behavioral Ecology **14**:486-492.

Ryder, O. A. 1986. Species conservation and systematics: the dilemma of subspecies. Trends in Ecology and Evolution **1**:9-10.

Saitou, N., and M. Nei. 1987. The neighbour-joining method. A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution **4:**406-425.

Sambrook, J., E.F Fritsch, and T. Maniatis 1989. Molecular Cloning: a Laboratory Manual, 2nd edn. Cold Spring Harbor Laboratory Press, New York.

Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proceeding of the National Academy of Science, USA **74**: 5463-5467.

Schaller, G. B. 1972. The Serengeti Lion: A Study of Predator-Prey Relations. University of Chicago Press, Chicago.

Scheepers, J. L., and K. A. E. Venzke. 1995. Attempts to reintroduce African wild dogs *Lycaon pictus* into Etosha National Park, Namibia. South African Journal of Wildilfe Research **25:**138-140.

Schenkel, R. 1966. On sociology and behaviour in impala (*Aepyceros melampus*). Z Saugetierk 31: 177-205.

Schneider, S., and L. Excoffier. 1999. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: Application to human mitochondrial DNA. Genetics **152**:1079-1089.

Schneider, S., J.M. Kueffer, D. Roessli, and E. L. 1997. ARLEQUIN (Version 2). A software for population genetic data analysis. Available from: http://anthropologie.unige.ch/arlequin/.

Seaman, D. E., and R. A. Powell. 1996. An evaluation of the accuracy of kernel density estimators for home range analysis. Ecology **77**:2075-2085.

Seaman, D. E., and R. A. Powell. 1998. KernelHR: A program for estimating animal home ranges. Wildlife Society Bulletin **26**:95-100.

Simberloff, D. 1998. Flagships, umbrellas, and keystones: Is single-species management passé in the landscape era? Biological Conservation **83(3):**247-257.

Singh, N., S. Agrawal, and A. K. Rastogi. 1997. Infectious dieases and immunity, special reference to major histocompatability complex. Emerging Infectious Diseases **3**: 41-49.

Smith, B. L., R. L. Robbins, and S. H. Anderson. 1996. Adaptive sex ratios: Another example? Journal of Mammology **77**:818-825.

Smithers, R. H. N. 1983. The Mammals of the Southern African Subregion. University of Pretoria, Pretoria, South Africa.

Spencer, J., and R. Burroughs. 1992. Antibody response to canine distemper vaccine in African wild dogs. Journal of Wildlife Diseases **28(3):**443-444.

Spencer, J. A. 1991. Antibody response to modified-live canine adenovirus vaccine in African hunting dogs (*Lycaon pictus*). Journal of Veterinary Medicine Series B **38(6):**477-479.

Spong, G. 2002. Space use in lions, *Panthera leo*, in the Selous Game Reserve: Social and ecological factors. Behavioural Ecology and Sociobiology 52:303-307.

Spong, G., J. Stone, S. Creel, and M.Bjorkland. 2002. Genetic structure of lions (*Panthera leo*) in the Selous Game Reserve: implications for the evolution of sociality. Journal of Evolutionary Biology **15:**945-953.

Stander, P. E. 1991. Demography of lions in the Etosha National Park, Namibia. Madoqua **18**:1-9.

Stander, P. E. 1993. Hunting success of lions in a semi-arid environment. In: Mammals as predators. Symposia of the Zoological Society of London **65:**127-143.

Stephens, P. A., and W. J. Sutherland. 1999. Consequences of the Allee effect for behaviour, ecology and conservation. Trends in Ecology and Evolution **14**:401-405.

Stow, A. J., and P. Sunnucks. 2004. Inbreeding avoidance in Cuningham's skinks (*Egernia cunninghami*) in natural and fragmented habitat. Molecular Ecology **13**:443-447.

Swafford, D. L. 2000. PAUP* Phylogenetic analysis using parsimony (*and other methods) Version 4. Sinauer Associates, Sunderland, Massachusetts.

Taberlet, P., S. Griffin, B. Goossens, S. Questiau, V. Manceau, N. Escaravage, L. P. Waits, and J. Bouvet. 1996. Reliable genotyping of samples with very low DNA quantities using PCR. Nucleid Acids Research **24(16)**:3189-3194.

Tamura, K., and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution **10**:512-526.

Taylor, C. R., K. Schmidt-Nielsen, R. Dmiel, and M. Fedak. 1971. Effect of hyperthermia on heat balance during running in the African hunting dog. American Journal of Physiology **220(3):**823-827.

Telfer, S., S. B. Piertney, J. F. Dallas, W. A. Stewart, F. Marshall, J. L. Gow, and X. Lambin. 2003. Parentage assignment detects frequent and large scale dispersal in water voles. Molecular Ecology **12**:1939-1949.

Temminck, M. C. J. 1820. Sur le genre Hyéne, et description d'une espéce nouvelle, découverte en Afrique. Annls. Gen. sci. phys.Brusslels **3:**46-57.

Thomas, L., S. T. Buckland, K. P. Burnham, D. R. Anderson, J. L. Laake, D. L. Borchers, and S. Strindberg. 2002. Distance Sampling. Pages 544-552 in A. H. El-Shaarawi, and W. W. Piegorsch, editors. Encyclopedia of Envirometrics. John Wiley and Sons, Ltd., Chichester.

Thomas, L., J. L. Laake, S. Strindberg, F. F. C. Marques, S. T. Buckland, D. L. Borchers, D. R. Anderson, K. P. Burnham, S. L. Hedley, J. H. Pollard, and J. R. B. Bishop 2003. Distance 4.1. Release 2. Research Unit for Wildlife Population Assessment, University of St Andrews, UK.

Tilman, D. 1997. Community invasibility, recruitment limitation, and grassland biodiversity. Ecology **78**:81-92.

Trivers, R. L., and D. E. Willard. 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science **179**:90-92.

Tufto, J., R. Anderson, J. Linnell. 1996. Habitat use and ecological correlates of home range size in a small cervid: The roe deer. Journal of Animal Ecology **65**:715-724.

Turnbull, F. C. B., R.H.V Bell, K. Saigawa, F.E.C. Munyenyembe, C.K Mulenga, and L. H. C. Makala. 1991. Anthrax in wildlife in the Luangwa Valley, Zambia. Veterinary Record **128:**399-403.

Turnbull, P. C. B. 1990. Anthrax. in G. R. Smith, and C. S. F. Easmon, editors. Topley and Wilson's Principles of Bacteriology, Virology and Immunity - Vol 3 - Bacterial Diseases. Edward Arnold, London.

Tuyttens, F. A. M., and D. W. MacDonald. 2000. Consequences of social perturbation for wildlife management and conservation. in L. M. Gosling, W.J Sutherland, editor. Conservation Biology Series 2: Behaviour and Conservation. Cambridge University Press, Cambridge, UK.

Van Heerdan, J. 1979. The transmission of canine erlichiosis to the wild dog (*Lycaon pictus*) and black-backed jackal (*Canis mesomelas*). Journal of the South African Veterinary Association **50(4):**245-248.

Van Heerdan, J., M.G.L. Mills, M.J. van Vuuren, P. J. Kelly, and M. J. Dreyer. 1995. An investigation into the health status and diseases of wild dogs (*Lycaon pictus*) in the Kruger National Park. Journal of the South African Veterinary Association **66(1)**:18-22.

Van Heerdan, J., N. Bainbridge, R. E. J. Burrows, and N. P. J. Kriek. 1989. Distemper-like disease and encephalitozoonosis in wild dogs (*Lycaon pictus* Temminck, 1820). Onderstepoort Journal of Veterinary Research **48:**19-21.

Van Heerdan, J., W.H. Swart, and D. G. A. Meltzer. 1980. Serum antibody levels before and after administration of live canine distemper vaccine to the wild dog *Lycaon pictus*. Journal of the South African Veterinary Association **51:**283-284.

Van Langevelde, A. S. F., F. Claassen, and G. Sparenburg. 2000. Competing land use in the reserve site selection problem. Landscape Ecology 15:243-256.

Van Oosterhout, C., W.F. Hutchinson, D.P.M. Wills, and P. F. Shipley 2003. Micro-Checker User Guide. University of Hull, Hull, UK. Available from: http://www.microchecker.hull.ac.uk/.

Van Valkenburgh, B., and K. Koepfli. 1993. Cranial and dental adaptations for predation in Canids. 65: pages 15-37 in N. Dunstone, and M. L. Gorman, editors. Mammals as Predators. Series Symposia of the Zoological Society. Oxford University Press, Oxford.

Vila, C., A. Sundqvist, O. Flagstad, J. Seddon, S. Bjornfeldt, I. Kojola, A. Casulli, H. Sand, P. Wabakken, and H. Ellegren. 2003. Rescue of a severely bottlenecked wolf (*Canis lupus*) population by a single immigrant. Proceedings of the Royal Society of London -Series B **270**:91-97.

Vila, C., I. R. Amorim, J. A. Leonard, D. Posada, J. Castroviejo, F. Petrucci-Fonesca, K. A. Crandall, H. Ellegren, and R. K. Wayne. 1999. Mitochondrial DNA phylogeography and population history of the grey wolf *Canis lupus*. Molecular Ecology **8**:2089-2103.

Vucetich, J. A., and S. Creel. 1999. Ecological interactions, social organisation, and extinction risk in African wild dogs. Conservation Biology **13(5):**1172-1182.

Walker, J., and M. S. Hopkins. 1990. Vegetation in R. C. McDonald, R. F. Isbell, J. G. Speight, J. Walker, and M. S. Hopkins, editors. Australian Soil and Land Survey Field Handbook. 2nd Edition. Australian Collaborative Land Evaluation Program, CSIRO Land and Water., Canberra.

Waser, P. M. 1975a. Diurnal and nocturnal strategies of the bushbuck Tragelaphus scriptus (Pallas). E. Afr. Wildl. J. 13: 49-63.

Waser, P. M. 1975b. Spatial associations and social interactions in a “solitary” ungulate: the bushbuck *Tragelaphus scriptus* (Pallas). Z Tierpsychol. 37: 24-36.

Waser, P. M. 1996. Pattern and Consequences of Dispersal in Gregarious Carnivores. Pages 267-295 in J. L. Gittleman, editor. Carnivore behaviour, ecology and evolution, Vol 2. Cornell University Press, Ithaca New York:.

Watson, J., D. Freudenberger, and D. Paull. 2001. An assessment of the focal-species approach for conserving birds in variegated landscapes in southeastern Australia. Conservation Biology **15(5):**1364-1373.

Wayne, R. K., M.W. Allard, and R. L. Honeycutt. 1992. Mitochodrial DNA variability in the gray wolf: Genetic consequences of population decline and habitat fragmentation. Conservation Biology **6(4):**559-569.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution **38**:1358-1370.

Whitman, K., A.M. Starfield, H.S. Quadling, and C. Packer. 2004. Sustainable trophy hunting of African lions. Nature **428**:175-178.

Wieczorek, A. M., R.C. Drewes, and A. Channing. 2000. Biogeography and evolutionary history of *Hyperolius* species: Application of molecular phylogeny. Journal of Biogeography **27**:1231-1243.

Wilson, G. M., R.A. van Den Bussche, P.K. Kennedy, A. Gunn, and K. Poole. 2000. Genetic variability of wolverines (*Gulo gulo*) from the northwest territories, Canada: Conservation implications. Journal of Mammology **81**:186-196.

Woodroffe, R. 2001. Assessing the risks of intervention: immobilisation, radio-collaring and vaccination of African wild dogs. Oryx **35(3):**234-244.

Woodroffe, R., and J. R. Ginsberg. 1997. The role of captive breeding and reintroduction in wild dog conservation. Pages 100-111 in R. Woodroffe, J. R. Ginsberg, and D. W. Macdonald, editors. The African Wild Dog: Status Survey and Conservation Action Plan. IUCN, Gland, Switzerland.

Woodroffe, R., and J. R. Ginsberg. 1998. Edge effects and the extinction of populations inside protected areas. Science **280:**2126-2128.

Woodroffe, R., and J. R. Ginsberg. 1999a. Conserving the African wild dog *Lycaon pictus*. I. Diagnosing and treating causes of decline. Oryx **33(2):**132-142.

Woodroffe, R., and J. R. Ginsberg. 1999b. Conserving the African wild dog *Lycaon pictus*. II. Is there a role for reintroduction? Oryx **33(2)**: 143-151.

Woodroffe, R., J. R. Ginsberg, and D. W. Macdonald 1997. The African Wild Dog: Status Survey and Conservation Action Plan. IUCN, Gland, Switzerland.

Woodroffe, R., J. W. McNutt, and M. G. L. Mills. 2004. Species status accounts: African wild dog. Chapter 6. Pages 174-183 in C. Sillero-Zubiri, M. Hoffman, and D. MacDonald, editors. Canids: Foxes, Wolves, Jackals and Dogs - 2004 Status Survey and Conservation Action Plan. IUCN, Gland, Switzerland.

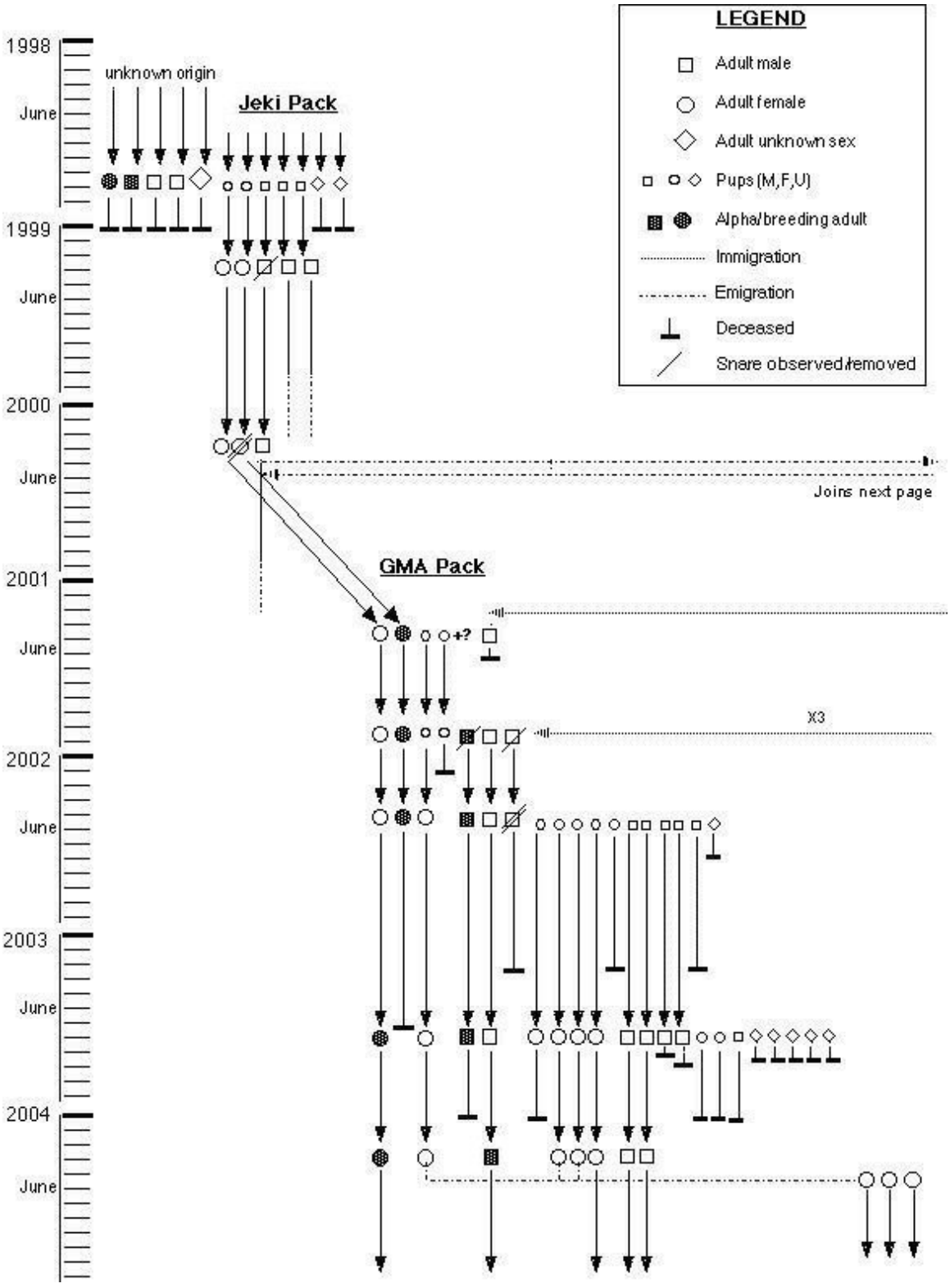
Worton, B. J. 1987. A review of models of home range for animal movement. Ecological Modelling **38**:277-298.

Worton, B. J. 1989. Kernel methods for estimating the utilization distribution in home-range studies. Ecology **70**:45-57.

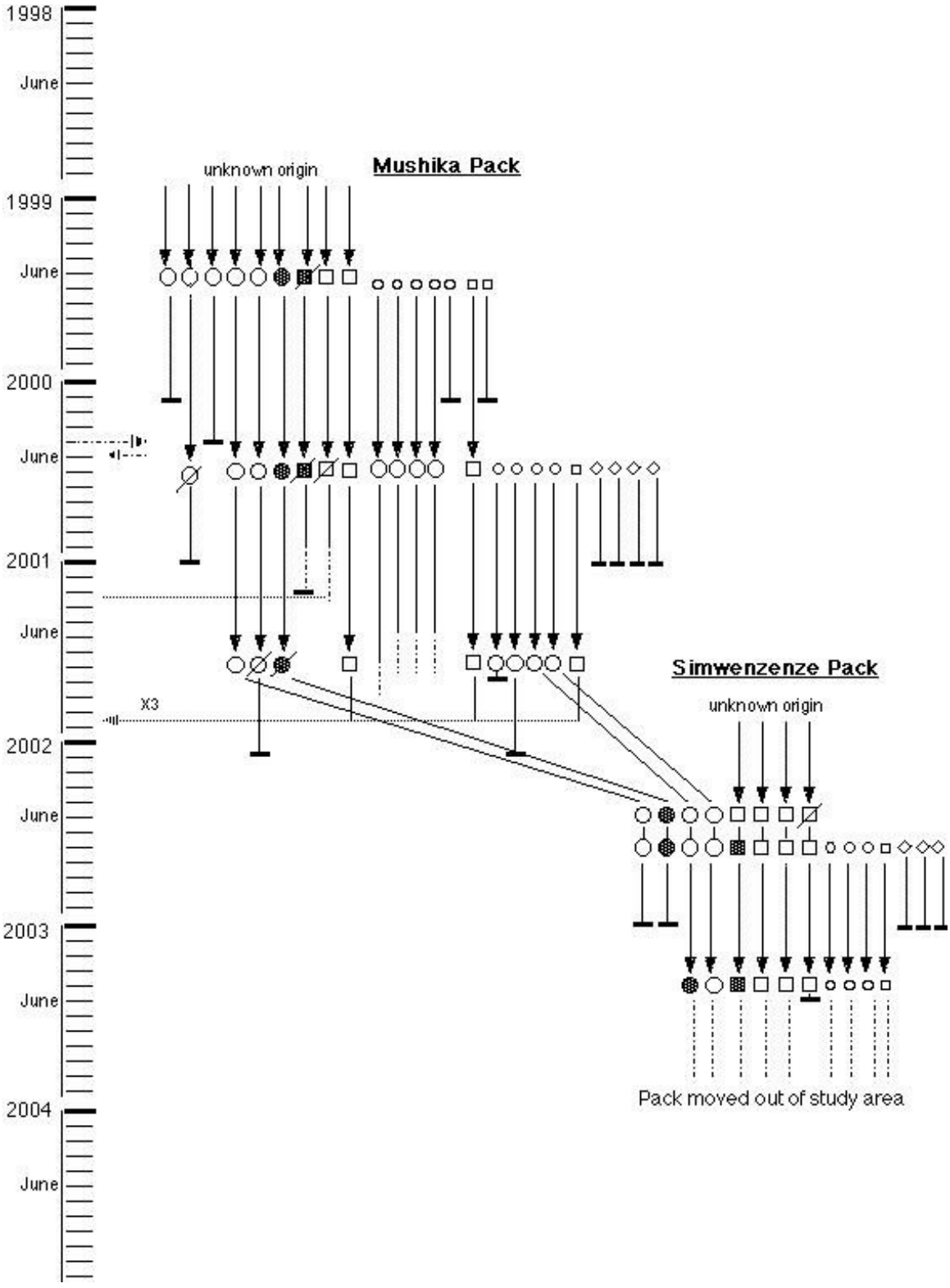
Wozencraft, W. 1989. The phylogeny of the recent carnivora. Pages 495-535 in J. L. Gittleman, editor. Carnivores: Behaviour, Ecology and Evolution. Cornell University Press, Ithaca, New York.

Zenger, K. R., M. D. B. Eldridge, and D. W. Cooper. 2003. Intraspecific variation, sex biased dispersal and phylogeography of the eastern grey kangaroo (*Macropus giganteus*). Heredity **91:**153-162.

Appendix 1


LEGEND
Adult male
Adult female
Adult unknown sex
Pups (M,F,U)
Alpha/breeding adult
Immigration
Emigration
Deceased
Snare observed/removed
1998
June
1999
June
2000
June
2001
June
2002
June
2003
June
2004
June
unknown origin
Jeki Pack
Joins next page
GMA Pack
+?
X3

Appendix 1



Appendix 1. Lower Zambezi wild dog population pedigree tree and pack composition changes for the period 1998 to 2004.

APPENDIX 2

Table 1 Common plant growth forms (adapted from Walker & Hopkins, 1990).

Growth form	Description
Tree	Single stemmed woody plant > 2 m tall.
Shrub	Woody plant multi-stemmed at base, or single stemmed < 2 m tall.
Ground cover forms include:	
Tussock grass	Discrete but open tussock usually with distinct individual shoots.
Hummock grass	Coarse grass with a mound-like form.
Sod grass	Short to medium height grass forming compact tussocks. Leaves form dense canopy.
Sedge	Herbaceous erect plant usually with a tufted habit.
Rush	Herbaceous erect plant.
Forb	Herbaceous or slightly woody non-grass, annual or sometimes perennial.

Table 2 Vegetation cover classes (adapted from Walker & Hopkins, 1990).

Cover class	Trees/shrubs	Ground cover and shrubs *Foliage cover % of groundcover*
Closed or dense	Crowns touching to overlapping	> 70%
Mid-dense	Crowns touching or slightly separated	30 - 70%
Sparse	Crowns clearly separated	10 - 30%
Very sparse	Crowns well separated	2.5 - 9%
Isolated plants	Trees about or greater than 100 m apart	<2.5
Isolated clumps	Clump of 2 - 5 woody plants 200 m or further apart	-

Table 3 Simplified structural formation classes used to describe habitats (adapted from Walker & Hopkins, 1990).

Growth form			***Structural formation classes***			
Crown separation	***Closed or dense***	***Mid-dense***	***Sparse***	***Very sparse***	***Isolated plants***	***Isolated clumps***
Tree	Closed forest	Open forest	Woodland	Open woodland	Isolated trees	Isolated clump of trees
Shrub	Closed shrubland	Shrubland	Open shrubland	Sparse shrubland	Isolated shrubs	Isolated clump of shrubs
Ground cover	Closed grassland	Grassland	Open grassland	Sparse grassland	Isolated grasses	Isolated clump of tussock grasses

Table 4 List of plant species identified in each habitat type.

Veg type	Common name	*Latin Name*
Albida woodland		
	Yellow paperbark acacia	*Acacia sieberiana*
	Foam bush	*Aerva leucura*
	Y-thorn balanites	*Balanites maughamii*
		Blumea spp.
		Bothiscline laxa
	Wild cabbage bush	*Calatropsis procera*
		Crossandra spinescus
	Fever berry	*Croton megalobotrys*
	Fertility plant	*Cyathula orthocantha*
	Bush acorn	*Diospyrus sinsensis*
	Salt bush	*Duospermum quadrangularis*
	Winterthorn acacia	*Feidherbia albida*
		Heliotropium ovalifolium
	Sausage tree	*Kigelia africana*
	Wild dagga	*Leonotis nepetifolia*
	Wild lavender	*Ocimum canum*
	Rain tree	*Philenoptera violacea*
	Winter cassia	*Senna singueana*
	Senna	*Senna obtusifolia*
	Snake apple	*Solanum panduriforme*
		Sphaeranthus flexuosus
	Natal mahogany	*Trichelia emitica*
	Epsom daisy	*Vernonia glabra*
Ecotone	Elephants ear	*Abutalon angulatum*
	Knob-thorn acacia	*Acacia nigrescens*
	Yellow paperbark acacia	*Acacia sieberana*
	Umbrella thorn	*Acacia tortilis*
	Baobab	*Adansonia digitata*
	Foam bush/lambs tail	*Aerva leucura*
	Purple hook-berry	*Artabotrys brachypetalous*
		Asparagus africanus
	Y-thorn balanites	*Balanites maughamii*
	Broad-leaved shepherds tree	*Boscia mossambicensis*
	Woolly caper bush	*Capparis tomentosa*
	Cardiogyne	*Cardiogyne africana*
	Thorny bone-apple	*Catunaregam sp*
	Four-leaved combretum	*Combretum adenogonium*
	Leadwood tree	*Combretum imberbe*
	Spiny combretum	*Combretum obovatum*
		Crossandra spinescus
	Fertility plant	*Cyathula orthocantha*
	Chinese latern bush	*Dichrostachys cinerea*
	Rhino thorn	*Dicoma anomela*
		Diospryrus quiloensis
	Bush acorn	*Diospyros sinensis*
	Salt bush	*Duopsermum quadrangularis*

Veg type	Common name	*Latin Name*
Ecotone cont.		*Elaeodendron schlechterianum*
	Snow berry	*Flueggea virosa*
	Velvet-leaved paddle pod	*Hippocratea buchananii*
	Indigo plant	*Indigophora sp*
	Wild Dagga	*Leonotis nepetifolia*
	Bean tree	*Markhamia zanzibarica*
	Cork bush	*Mundulia sericea*
	Wild lavender	*Ocimum canum*
		Ocimum americanus
	Rain tree	*Philenoptera violacea*
		Pterocaulon decurrins
	Winter cassia	*Senna singueana*
	Long-tail cassia	*Senna abbreviata*
	Senna	*Senna obtusifolia*
	Snake apple	*Solanum panduriforme*
		Sphaeranthus flexuosis
	Star chesnut	*Sterculia africana*
		Trichodesma physaloides
		Trichodesma zeylanicum
	Epsom daisy	*Vernonia glabra*
		Vernonia pertersii
	Nyala tree	*Xanthocercis zambesiaca*
	Small leaved sour plum	*Ximenia americana*
		Ziziphus abbyssinica
	Buffalo-thorn	*Ziziphus mucronata*
<u>Grassland</u>	knob thorn acacia	*Acacia nigrescens*
	Umbrella thorn acacia	*Acacia tortilis*
	Baobab	*Adansonia digitata*
		Blumea spp.
	Woolly caper bush	*Caparis tomentosa*
		Carbornia glauca= Maerua edubilis
	Baloon pea	*Crotelaria Spp.*
	Salt bush	*Duospermum quadrangularis*
	Ilala palm	*Hyphaenae petersiana*
	Wild dagga	*Leonotis nepetifolia*
	Wild lavender	*Ocimum canum*
	Adrenaline grass	*Panicum maximum*
	Mustard tree	*Salvadora persica*
	Senna	*Senna obtusifolia*
		Sphaeranthus flexuosus
	Epsom daisy	*Vernonia glabra*
<u>Thicket</u>		*Acacia ataxacantha*
	Knob-thorn acacia	*Acacia nigrescens*
	Umbrella thorn	*Acacia tortilis*
		Albizia antelminthica
	Baobab	*Andansonia digitata*
	Y-thorn balanites	*Ballanytes*
	Broad-leaved shepherds tree	*Boscia mossambicensis*
		Cadaba kirkii

Veg type	Common name	*Latin Name*
Thicket cont.		*Caparis sinensis*
	Woolly caper bush	*Caparis tomentosa*
		Catunaregam sp
	Mopane	*Colophospermum mopane*
	Four-leaved combretum	*Combretum adenogonium*
		Combretum elaeagnoides
	Leadwood tree	*Combretum imberbe*
		Combretum obovatum
		Crossandra spinescus
		Crossandra spinescus cyathula orthacantha
	Chinese lantern bush	*Dichrostachys cinerea*
		Dicoma anomela
		Diospyros quiloensis
	Bush acorn	*Diospyros sinensis*
	Salt bush	*Duospermum quadrangularis*
		Euphorbia vine
	Monkeys finger	*Friesodielsia obovata*
	Wild Chinese hats	*Karomia tettensis*
		Maerua edubilis= carbornia glauca
	Bean tree	*Markhamia zanzibarica*
		Ocimum spp. (canum and americanus)
	Woody pear tree	*Schrebera trichoclada*
		Senna abreviata
	Pink jacaranda	*Stereospermum kunthianum*
		Stropanthus kombe
		Xeroderris stuhlmanii

APPENDIX 3

AWDC

AFRICAN WILD DOG CONSERVATION

Lower Zambezi National Park, Zambia

LOWER ZAMBEZI NATIONAL PARK LION SURVEY 2003

Please complete by 30th October 2003

To: Safari Camp Operators
AWDC is currently collecting identification and home range data on lions in the Lower Zambezi National Park (LZNP) and eastern Chiawa GMA. This information will be used to investigate the effect of lion densities and movements on the LZNP African wild dog population. This a request for all the safari guides in your camp to confer and fill out the form below together, based on their knowledge of the local lion population in your area. The information is for the 2003 season only. If you have noticed any marked differences in the lion population since 2002, such as deaths or disappearances, please fill out the last section. Thank you very much for your help.

QUESTIONNAIRE:

Name of Safari Camp:

Names of guides completing survey:

Date:

Please include any nicknames you have given individual lions in the section below. If any animals are known to move between groups include them in the group they spend the most time with and indicate which other group(s) they have been seen with. Include cubs in the appropriate sex group.

Total Number of lion groups/prides in your area:

Number of lions in group (1):
No. of Dominant males: .....
Approx. ages: .....
Number of other males:.....
Approx. ages: .....
.....
Number of females: .....
Approx ages: .....
Number of Cubs:.....
Approx. ages:.....

Would you describe this as a discrete pride or an occasional association?
.....
Description of home range area the group uses:.....
.....
.....
.....

Number of lions in group (2):
No. of Dominant males: .....
Approx. ages: .....
Number of other males:.....
Approx. ages: .....
.....
Number of females: .....
Approx ages: .....
Number of Cubs:.....
Approx. ages:.....

Would you describe this as a discrete pride or an occasional association?
.....

Description of home range area the group uses:.....
.....
.....
.....
.....

Number of lions in group (3):
No. of Dominant males: .....
Approx. ages: .....
Number of other males:.....
Approx. ages: .....
.....
Number of females: .....
Approx ages: .....
Number of Cubs:.....
Approx. ages:.....

Would you describe this as a discrete pride or an occasional association?
.....
Description of home range area the group uses:.....
.....
.....
.....
.....
TOTAL NUMBER OF LIONS IN YOUR AREA: .....

Additional notes (including changes in population since 2002):
.....
.....
.....
.....
.....
.....
.....
.....**Thank you.**

APPENDIX 3

Table 1. Lion density values for home range areas shown in Figures 4.2a,b, and c (Chapter 4), from three survey years.

Area ID	Lion Range Area (Km^2)	Lion Density (adults/km^2)		
		2001	2002	2003
1	76.8	0.024	0.024	0.024
2	7.0	0.073	0.049	0.049
3	34.5	0.159	0.110	0.129
4	9.4	0.100	0.086	0.105
5	60.9	0.173	0.148	0.126
6	35.8	0.098	0.064	0.021
7	17.0	0.037	0.029	0.029
8	53.1	0.015	0.032	0.032
9	102.6	0.047	0.044	0.040
10	149.3	0.035	0.015	0.011

Table 2. Spotted hyaena density estimates (adults per km^2) for each calling station over four surveys.

Calling Station No.	UTM GPS Location	Location Description	Hyaena Density (adults/km^2)			
			2000	2002_1	2002_2	2003
1	734268: 8254021	Kayila		0.33	0.66	0.11
2	745832: 8259818	Royal airstrip		0.28	0.19	0.09
3	753988: 8263250	Nkalangi	0.28	0.57	0.00	0.19
4	762344: 8266880	Fridays Corner	0.28	0.85	0.12	0.12
5	772170: 8269604	Out of Africa	0.23	0.15	0.27	0.11
6	780128: 8270994	Jeki East	0.46	0.39	0.46	0.50
7	790030: 8271954	Back Plain	0.83	0.39	0.12	0.04
8	796336: 8272360	Mushika River	0.05	0.54	0.18	0.27

APPENDIX 4

Table 1 Mitochondrial DNA nucleotide divergence (d_A) between wild dog populations across Africa

Population	Mara/Serengeti	Selous	Zambia	Hwange	Namibia	Okavango
Mara/Serengeti						
Selous	0.0268					
Zambia	0.0397	0.0058				
Hwange	0.0310	0.0031	0.0009			
Namibia	0.0356	0.0027	0.0037	0.0023		
Okavango	0.0064	0.0090	0.0142	0.0094	0.0132	
Kruger	0.0375	0.0034	0.0028	0.0017	0.0004	0.0139

Table 2 Allelic Richness per locus and population, based on a minimum sample size of 5 diploid individuals.

Locus	Kruger	Hwange	Okavango	Namibia	Selous	Mara/Seren	Lower Zambezi
155	3.728	3.979	3.126	2.833	5.452	4.514	2.762
173	2.961	3.305	3.880	2.985	2.625	3.789	2.744
250	2.889	4.466	3.963	2.833	4.018	3.521	3.401
263	3.516	3.520	3.958	3.818	5.075	3.964	2.817
366	2.107	3.360	3.026	3.000	3.739	2.870	2.720
423	3.747	4.038	2.872	3.818	4.480	3.455	3.382
442	1.809	1.999	1.982	1.833	1.988	1.987	1.979
453	2.847	2.797	2.901	3.667	3.511	3.116	2.856
606	2.051	2.657	1.994	2.833	1.937	1.993	1.985
671	4.030	4.299	4.348	5.000	3.848	3.349	4.025
677	2.579	2.389	1.992	2.833	2.908	2.956	1.996
Mean	**2.933**	**3.346**	**3.095**	**3.223**	**3.598**	**3.229**	**2.788**
SE	**0.755**	**0.811**	**0.863**	**0.819**	**1.159**	**0.771**	**0.648**

APPENDIX 4

Table 3 Microsatellite allele frequencies for Zambian African wild dogs. Sample KAF-1A is from the Kafue NP, sample SL-2A is from South Luangwa NP, all other samples are from the Lower Zambezi NP.

	Primer Identification Number.										
Sample ID	**L155**	**L173**	**L250**	**L263**	**L366**	**L423**	**L442**	**L453**	**L606**	**L671**	**L677**
SA5	135 135	073 073	091 089	142 136	130 128	082 080	118 116	107 089	121 121	158 150	123 121
PIC.BL.1	135 135	073 073	095 091	142 142	130 130	082 080	118 118	107 089	121 121	158 150	123 121
BAT.BL1A	141 135	073 071	095 095	142 136	130 130	000 000	118 116	089 079	121 121	158 158	123 121
SA8	141 135	073 071	091 089	142 136	132 128	076 076	118 116	089 079	123 121	156 148	121 121
SA3	141 141	073 071	095 091	142 136	132 128	082 080	118 116	089 079	123 121	156 148	123 121
BIL.BL1A	135 135	000 000	091 089	136 136	130 130	000 000	118 118	000 000	123 121	156 150	123 123
SNP.BL.1A	135 135	071 069	089 087	136 136	130 130	082 080	118 116	089 079	121 121	148 148	121 121
LU1	137 137	069 069	000 000	000 000	130 130	000 000	118 118	000 000	000 000	150 150	121 121
PUP1A	141 135	073 071	000 000	000 000	130 128	082 080	118 118	079 079	121 121	000 000	123 121
MUN2A	000 000	073 073	095 091	000 000	000 000	080 080	118 116	107 089	121 121	158 158	121 121
Q1	000 000	073 071	091 091	136 136	130 130	082 076	118 116	079 079	123 121	158 158	121 121
SCP1A	000 000	073 073	095 095	136 136	000 000	000 000	000 000	000 000	121 121	158 150	123 121
RID1A	000 000	073 069	091 091	136 136	000 000	000 000	118 118	000 000	123 121	156 156	123 121
RING1A	000 000	071 071	091 091	142 136	000 000	080 080	118 118	079 079	000 000	158 156	121 121
BLZ1A	000 000	073 071	091 091	142 142	000 000	000 000	118 116	000 000	123 121	158 156	121 121
NIMB1A	000 000	073 073	091 091	142 136	000 000	078 076	118 118	000 000	123 121	000 000	123 121
BOR1A	000 000	071 071	000 000	136 136	000 000	082 080	118 116	089 079	123 121	150 150	121 121
KAF 1A	000 000	071 071	089 087	138 138	000 000	000 000	118 116	000 000	121 121	146 158	123 123
SL2A	141 141	071 071	089 091	138 138	000 000	000 000	118 116	000 000	123 121	150 150	123 123