FACTORS WHICH MAY BE PREVENTING THE RECOVERY OF POPULATIONS OF HELMETED GUINEAFOWL IN THE MIDLANDS OF KWAZULU-NATAL

by

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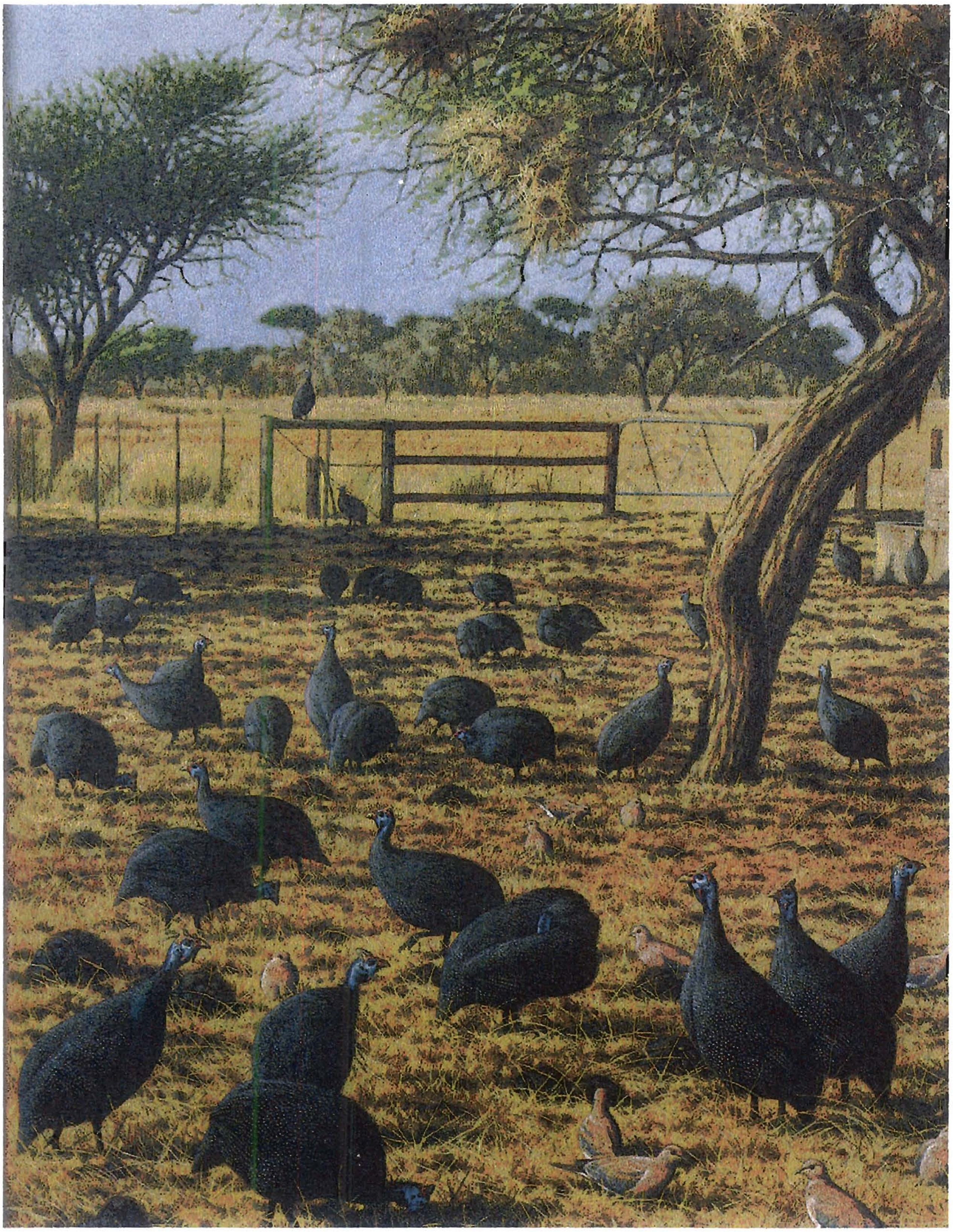
DECLARATION

This thesis reports the results of original research I conducted under the auspices of the Percy FitzPatrick Institute of African Ornithology, Department of Zoology, University of Cape Town, between 1997 and 2000. All assistance that I received has been fully acknowledged. This work has not been submitted for a degree at any other University.

Signed by candidate

Charles S. Ratcliffe



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Special thanks must go to friends Lionel Song and June Wilmot for their hospitality throughout my stay within the Midlands. I would also like to thank the KwaZulu-Natal Conservation Service, in particular Dr David Johnson, for providing accommodation. In addition, thanks must go to the farmers on whose properties I conducted research, in particular, Alan, Graham & James Gibson, Trevor & Denise of Chelmsford Reserve, Ian Mitchell-Innes, Brian & Owen Geekie, Tex Turton, Graham Acutt and Pat Eustace. Thank you also to Dr Gerard Malan for introducing me to the Midlands and its farmers, to Leon Kruger, Mark Haldane, John Perrings and Mathew Prophet for advice and assistance in the field, and to Graham Bennett for supplying domesticated guineafowl for experiments.

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Abstract: The Helmeted Guineafowl *Numida meleagris* is, naturally, a species of open savannas. However, since the mid-18th century, it has undergone the most extensive range expansion of any African gamebird. In southern Africa, this expansion has been mostly due to a combination of deliberate introductions and the natural expansion into areas transformed by agriculture and urban development, which supply key resources such as food, cover, roosting sites and watering points. The Midlands of KwaZulu-Natal province have been no exception in this regard. With the advent of, in particular, crop agriculture, large populations of guineafowl have occupied, and increased numerically in, this variegated landscape since the turn of the 20th century.

It was thus of some concern when, during the early 1980s, local farmers, wingshooters and other conservationists noticed marked declines in populations across a broad geographical front in the Midlands. A project was thus initiated in 1995 by African Gamebird Research, Education and Development Trust (AGRED), KwaZulu-Natal, in consultation with the Gamebird Research Programme of the Percy FitzPatrick Institute, to investigate the factor/s that may have caused these declines and how to remedy them. The project was conducted in three phases: first an assessment of the status of guineafowl populations and potential socio-economic causal factors based on interviews and questionnaires; second, the acquisition of detailed knowledge of aspects of the biology of the bird; and third, the proposal of management strategies to resuscitate diminished populations. Phase 1 suggested that illegal hunting and/or negative attitudes towards wildlife had occurred and continues to occur within the Midlands, but that it is prevalent mostly

on farms with poor labour relations. Illegal hunting may thus have an impact on a local scale, but there is no evidence to support a widespread impact on a regional basis. Statistical analysis conducted during Phase 1 and early Phase 2 pointed to two significant factors that, at first, seemed to contradict one another. Declining and near extinct populations were correlated with the intensive use of pesticides, whereas healthy populations were correlated with the presence of extensive edge habitats. The apparent contradiction is explained by the changes in production of crop agriculture from the 1970s through to the late 1980s, and by detailed research on movements of, and habitat use by, guineafowl fitted with radio transmitters. Production of maize - the dominant commercial crop in the study area - doubled between the late 1970s and early 1980s. Other crops, such as wheat and soya, also increased significantly during the same period. The negative correlation found with pesticides probably merely reflects the loss and polarisation of guineafowl habitat due to increasing crop agriculture and subsequent indirect effects of pesticides (reduction in food and cover), and not the deliberate misuse of agrochemicals in causing widespread declines. In addition, the results of radio-telemetric studies revealed the importance of small maize fields in supporting healthy populations of guineafowl, whereas near extinct populations were associated with less diverse habitats that were farmed more extensively.

Further investigation during Phase 2 supported the notion that poisoning was also a local phenomenon, since the Allerton Provincial Veterinary Laboratory recorded only 42 such cases since 1985. Liver samples obtained from specimens throughout the study area revealed only infinitesimally low (parts per billion), residual traces of DDT and Dieldrin. Thus, there is no evidence to support the notion that agrochemicals are having ongoing, direct negative effects on populations. Additional research included aspects such as diseases, habitat utilisation, dietary constraints and genetics. Twelve diseases were tested for, of which only two have the potential to decimate a species at a population level - Avian influenza and Newcastle disease. Neither of these two diseases were found in the population.

An investigation of habitat utilisation was undertaken through radio-tracking 15 flocks representing three different population scenarios - stable, declining and near extinct. Flock home ranges increased in size as one moved from stable through to near extinct populations. Habitat preferences included fallow lands and small maize fields, but it was not the nature of the habitat types themselves that distinguished between populations, but more the availability of a variety of habitats and resources over a continuous area. The greater the mosaic of habitats over a large area, the healthier the population.

One of these 'habitats', soya bean cropland, was thought to have an adverse effect on populations since raw soya has various negative nutritional constraints for commercial poultry. A digestibility study was conducted on captive birds to investigate this and it was found that nutritionally, soya is comparable with other grain crops such as maize. Finally, a genetic study investigated the degree to which wild populations were interbreeding with feral, domestic guineafowl which have the ability to reduce the viability of the wild strain through undermining successful reproduction. Results indicate that interbreeding has certainly occurred which may have inhibited the recovery of local populations. However, many of the 'contaminated' specimens originated from apparently healthy populations, suggesting that impaired breeding success as a result of interbreeding, may not be important in terms of affecting the viability of the wild population.

The findings in Phase 2 suggested that the increases in crop agriculture from the 1970s - primarily of maize - lead to a loss of suitable habitats and the fragmentation of guineafowl populations throughout the Midlands. In addition, the indirect effects of pesticides, in combination with modern farming techniques, exacerbated the situation through reducing the availability of food resources and edge habitats. Once populations have become fragmented, they become vulnerable to extinction as they cannot be resuscitated from the now more distant neighbouring populations in the event of large, localised mortality. They are thus susceptible to a variety of more proximal causal factors such as disease, poisoning and illegal hunting and drop below the Minimal Viable Population (MVP - defined as the number of individuals in the population capable of breeding in order to sustain the population in the face of normal negative pressures such as predation, disease, and unavailability of food) leading to extinctions.

The results of Phase 3 of the project suggest that returning populations to viable levels requires re-creating suitable, interconnected habitats across landscapes on a conservancy level through management strategies which enhance landscape connectivity. Some of these strategies include the creation of a mosaic of habitats with a large edge component. Numerous small maize fields are more suitable than one large monoculture. Apart from small maize fields, other important habitats include fallow lands and a winter green crop such as lucern. Agrochemicals should be applied according to manufacturers instructions, while users need to be well educated in their safe application. Guineafowl populations need to be monitored and rural communities and farm labourers educated and included in the benefits of this potentially sustainable resource. Current populations cannot support a biologically and economically viable wingshooting operation, and their recovery lies at the level at which appropriate management policies are undertaken. Populations will only recover if there is suitable habitat over an extensive area so that fragmented populations can once again be connected, and return to numbers that previously supported sustainable wingshooting.

CHAPTER 1

GENERAL INTRODUCTION

1.1. RATIONALE AND AIMS

The rural environment of South Africa has experienced many changes in its diverse history. From the establishment of the first wine farms in the Western Cape, to the development of extensive sugar cane fields and maize monocultures in KwaZulu-Natal, land has been progressively transformed to accommodate the needs of a burgeoning humanity. Changes in land use have often been to the detriment of bird species, leading to declining numbers and, on occasion, local extinctions. There are, however, some species that have expanded their ranges in southern Africa by exploiting these transformed habitats. Hockey et al. (1989) note that in the southern Western Cape province, 85 bird species have benefitted from the modification of the rural landscape. More specifically, the moderate fragmentation of habitats has promoted species, including gamebirds, that thrive in a mosaic of agriculture and natural vegetation types (Malan & Benn, 1999). One such gamebird that has benefited from this transformation of the landscape is the Helmeted Guineafowl *Numida meleagris*.

The one thing common to all gamebirds is that they are, at least potentially, sufficiently abundant and productive to withstand local 'harvesting' year after year. The utilisation of this production by wingshooters has been particularly well developed in the Northern Hemisphere, and has become a commercially viable industry. However, commercial wingshooting is still very much in its infancy in southern Africa. Nonetheless, in certain parts of South Africa, it has been embraced by various rural communities with considerable success (Little & Crowe 1993a; Little & Crowe 1993b; Little & Crowe 1993c). Pioneering work in the mid-1980s in KwaZulu-Natal (Mentis & Bigalke, 1985), and subsequent research on the Stormberg plateau in the Eastern Cape province (Little, 1992; Little & Crowe 1993a; b; c), has shown that populations of Greywing Francolin, *Francolinus africanus*, can be harvested on a basis that is both biologically sustainable, and economically viable. Apart from promoting an appreciation of fauna and flora, wingshooting has a role in fostering the development of a 'wise-use' conservation ethic in these communities (Pero & Crowe, 1996). Furthermore, gamebird utilisation provides a potentially valuable source of additional income to farmers (Smith, 1994).

Helmeted Guineafowl have traditionally been sought after by wingshooters since well into the last century. Their range expansion, resilience to habitat transformation and the ability to produce large numbers of eggs over consecutive seasons, has made this species an important asset to commercial wingshooting industries. It was thus with some concern that farmers, wingshooters and other conservationists noticed declines in guineafowl populations within the Midlands of KwaZulu-Natal province in the early 1980s. Prof. Tim Crowe of the FitzPatrick Institute was therefore contacted in this regard, and his advice was to wait for the return of years of above-average rainfall, since previous research (Crowe & Siegfried, 1978; Berry & Crowe, 1985) had shown a positive correlation between guineafowl population size and rainfall. Thus, populations should recover after the drought years. This did not happen. At the invitation of the KwaZulu-Natal branch of the African Gamebird Research, Education and Development Trust (AGRED), Prof. Crowe and Dr Rob Little thus embarked on a reconnaissance trip in 1994 to assess the status of guineafowl populations. They found widespread collapse and, in some areas, local extinction of guineafowl. Therefore, in 1995, a project was initiated by AGRED KwaZulu-Natal to investigate potential factors that had lead to these declines and possible remedies to resuscitate guineafowl populations to levels which could be utilised sustainably.

The project was divided into three phases - Phase 1, an initial assessment of the status of the population; Phase 2, a more detailed study relating to the biology of the bird; Phase 3, management recommendations relating to resuscitating populations within the Midlands. M.Sc. students Lionel Pero and Luthando Maphasa working on Phase 1. Through the analysis of questionnaires, Pero ascertained the status of populations in KwaZulu-Natal, as well as the period over which declines occurred. Furthermore, Pero identified significant correlations between various land-use practices, and guineafowl population size (Pero & Crowe, 1996). The most significant of these correlations was between intensive pesticide use and extinct or declining populations suggesting intensive, modern farming techniques as a probable cause.

Maphasa (1996) investigated the cultural and socio-economic aspects that may have lead to the declines. Illegal hunting was certainly occurring within the Midlands, but it was localised and did not explain the widespread collapse in populations. The work done by these two students lead to a third M.Sc. project which investigated the molecular phylogeography of Helmeted Guineafowl (Rossouw, 1996). Amongst various findings were the occurrence of DNA from domesticated guineafowl, *Numida meleagris galeata*, in wild birds which, morphologically, appeared to be pure wild type guineafowl. Thus, there appeared to be morphologically undetectable genetic introgression from domestic guineafowl into wild populations.

Phase 2 moved away from a broad geographical approach, to one of more intensive studies relating to the biology of the birds. Dr Gerard Malan completed the initial aspects of Phase 2 through a detailed analysis of available cover (Malan, 1998) and the agricultural land-use practices of various representative farms, in terms of their correlation with associated guineafowl populations (Malan & Benn, 1999). The findings emphasised the importance of edge habitats in providing food, cover and nesting sites.

The remainder of Phase 2 was completed by the author and involved investigating many other potential factors suggested by farmers, conservationists and wingshooters alike. These included agrochemicals, disease, genetics, habitat utilisation, diet and associated species diversity. Intensive studies were thus conducted to ascertain if these factors are continuing to suppress populations, and what measures could be undertaken to return the species to viable populations. The aim of this dissertation is thus to address these aspects in particular, and to provide insight into the cause/s of population declines. This dissertation also seeks to complete Phase 3 of the project involving management policies and recommendations needed to resuscitate diminished populations.

1.2. GENERAL DESCRIPTION

1.2.1. Description

The name guineafowl stems from the general area from which Portuguese explorers transported wild birds, the Gulf of Guinea in west Africa, to Europe in the 15th century for domestication (Donkin, 1991). More than 2000 years ago, the ancient Greeks and Romans also domesticated guineafowl from North Africa and, possibly, present-day Sudan, for meat and egg production. A very successful guineafowl broiler industry still persists in France.

The South African form of the Helmeted Guineafowl *N. m. coronata* can be distinguished by having a bony casque or 'helmet' on top of its head and long, pennant-shaped wattles which are blue with red-tips and hang down from its jaw. Of the nine recognised African subspecies, three occur in southern Africa, *N. m. damarensis* in the drier parts of western Botswana and Namibia, *N. m. mitrata* in Mozambique, Zimbabwe and northern Botswana and *N. m. coronata* in eastern Botswana and South Africa. The most striking differences among subspecies are in size and shape of the helmet and, in the colouration of the head and wattles. There are no obvious differences in appearance between the sexes, although males tend to be slightly larger (Crowe et al., 1986). There are, however, marked differences in behaviour. Only males exhibit the characteristic hump-backed display with their wings raised and held close to their bodies (Crowe et al., 1986). Females can be distinguished by the two-noted 'buck-wheat' call used during the breeding season to keep in contact with their mates, who often respond to it with a single-noted 'cheeng', in what seems like a three-noted call from a single bird (Elbin et al., 1986). Females also tend to walk flat-footed and appear slouched in posture, whereas males tend to walk erect and on their toes (Crowe, 2000a).

1.2.2. Distribution and Habitat

The Helmeted Guineafowl is Africa's most widespread upland gamebird (Crowe et al., 1986). It is locally common to abundant in virtually all open-country terrain from near-desert to the edges of forests and the bases of high mountains, especially in savannas mixed with cultivation for maize and wheat (Crowe et al., 1986). It will, however, not normally penetrate deep into extensive plantings of either crop (Little, 1997). It is currently absent from the deserts and sub-deserts of southern Namibia and the Northern Cape, presumably because these areas lack suitable drinking water and elevated roosts (Little, 1997). This guineafowl has actually expanded its range enormously in southwestern South Africa where humans have added these missing habitat requirements such as roosts, cover and watering points, to the landscape, with even telephone poles serving as nightly roosts (Little, 1997). Humans have further promoted this range expansion by capturing wild birds and releasing them into new areas (Crowe, 2000a). Locally, numbers of guineafowl can fluctuate dramatically, exploding in years when there is good rainfall in the months prior to breeding (Crowe & Siegfried, 1978).

1.2.3. Habits

Crowe (2000a) has revised the natural history of the Helmeted Guineafowl. During the non-breeding period, guineafowl form relatively stable flocks of 15-40 birds. Individually ringed birds have remained together for as long as four years. The much larger aggregations of as many as 2000 birds are most likely many flocks converging on some superabundant resource. During the non-breeding season, flocks typically descend off the roost and move to water to drink and socialize. Feeding takes place up until mid-morning, whereupon birds will seek thick cover for the heat of the day. Feeding re-commences in the late afternoon after which birds will return to their arboreal roost just prior to sunset. Roosts can be used for many years as indicated by large accumulations of faeces below.

Guineafowl fly rarely and then only if pressed or to mount their roost. Flock members keep in contact by emitting a single-noted call "cheenk". Only males apparently maintain a dominance hierarchy (pecking order). Females, however, support the males in between-flock 'gang fights', helping them to drive out intruders from the group territory. Much of the serious aggression within flocks appears to be older males attacking first-year males, presumably to drive them out of the flock.

As the breeding season approaches, flock size steadily drops and the first several days with heavy rainfall stimulates pairing. Males compete for females through chasing one another while in the hump-backed display. The chases appear to be contests of fitness rather than aggressive interactions, since the pursuer will slow down if his quarry does so as well. Chasing sometimes leads to blood-letting fights with the beaks, wings and claws used as weapons. Fights end generally when the loser assumes a crouch similar to that of a sexually receptive hen or simply runs away from the scene.

Once the pairbond is established, the male guards and defends his hen vigilantly and aggressively, spending much of his time in upright posture. He will even forego feeding himself, rather engaging in courtship feeding, catching and dropping protein-rich grasshoppers and other prey in front of his mate, while assuming the hump-backed display and uttering a soft 'chip, chip, cheree' call. Paired hens spend most of their time feeding and preening. This is reflected in their weights, with males dropping to about 85% of their non-breeding mass while hens increase in weight accordingly to produce a clutch of eggs.

1.2.4. Food and Feeding

Guineafowl are very opportunistic in their feeding habits (Crowe, 2000a). More than 80% of the guineafowl's diet can, in one way or another, be attributed to agriculture (Grafton, 1971; Mentis et al., 1975) with the main components including arthropods, many of which are agricultural pests (Skead, 1962; Ayeni, 1983a; Little et al., 1995; Witt et al., 1995), weeds and tubers of cultivated areas, pasture greens and disused agricultural grain (Grafton, 1971; Mentis et al., 1975). During the non-breeding season, they focus on underground bulbs and the stems of plants, primarily *Cyperus* spp., but will readily shift to grass seed when this is abundant. They will even take seeds of plants such as dubbeltjies *Tribulus terrestris* which are protected by prickly thorns. As the breeding season approaches, they shift to invertebrates, especially insects such as grasshoppers and termites. This provides the hens with protein essential to produce eggs. Unlike Swainson's Spurfowl *Pternistis swainsonii* (Crowe, 2000b), they do not normally take growing maize plants or maize cobs still attached to healthy plants. Nearly all the maize taken is from fallen or discarded cobs.

1.2.5. Breeding

In the predominantly summer rainfall regions of the eastern and southern South Africa, highest breeding activity for Helmeted Guineafowl is during summer (October- March) and during late summer and early autumn (January-March) in the north, e.g. Botswana and Namibia (Little, 1997). It is largely determined by the timing of regular heavy rainfall during the warmer months (Crowe & Siegfried, 1978). In the winter rainfall regions, e.g. the Western Cape and the western half of the Eastern Cape province, peak breeding is during September-December to take advantage of food fostered by winter rains. Nests are extremely well-concealed and are simple scrapes in the earth lined lightly with feathers and grass stems. Six to 20+ eggs are laid. Larger clutches are almost certainly the result of egg dumping by more than one hen into a single nest (Crowe, 2000a).

CHAPTER 2

THE PRINCIPAL STUDY AREA

The study area is about 3 000 km^2 in extent (Fig. 2.1) and is located in KwaZulu-Natal Midlands between Newcastle (29°55'E; 27°42'S) in the north, Bergville (29^{0}20'E; 28^{0}43'S) in the west, Underberg (29°30'E; 29°47' S) in the south, and Greytown (30^{0}35'E; 29^{0}S) in the east. Altitude varies from 800 to 1750m with a summer rainfall of 600-1600mm per annum, primarily between October and March. The Midlands is an intensively farmed area and livestock farming (mainly of cattle), predominates over maize (*Zea* spp.), wheat (*Triticum* spp.) and pastures (for grazing and fodder) (Pero & Crowe, 1996). The bioclimatic characteristics of the region are summarized in Table 2.1.

Table 2.1. Bioclimatic and land-use characteristics (modified from Department of Agriculture, 1991) of the sampling areas.

Location within study area	Vegetation	Land-use	Mean annual rainfall	Topography
Northwest	Moist tall grassveld / dry tall Grassveld and *Acacia* thickets	Stock, & various crops	800 - 1000	Undulating with hills
West to central	Dry tall grassveld and *Acacia* thickets	Stock, maize & pasture	600 - 800	Undulating with hills
Central to east	Secondary grassland with upland evergreen forests and woodland	Stock, timber & maize	800 - 1600	Rolling and hilly
Southeast	Short grassland, relic pockets of *Podocarpus* forest and mixed evergreen woodland	Stock, vegetables & pasture	800 - 1500	Rolling, occasionally undulating, with hills and deep valleys

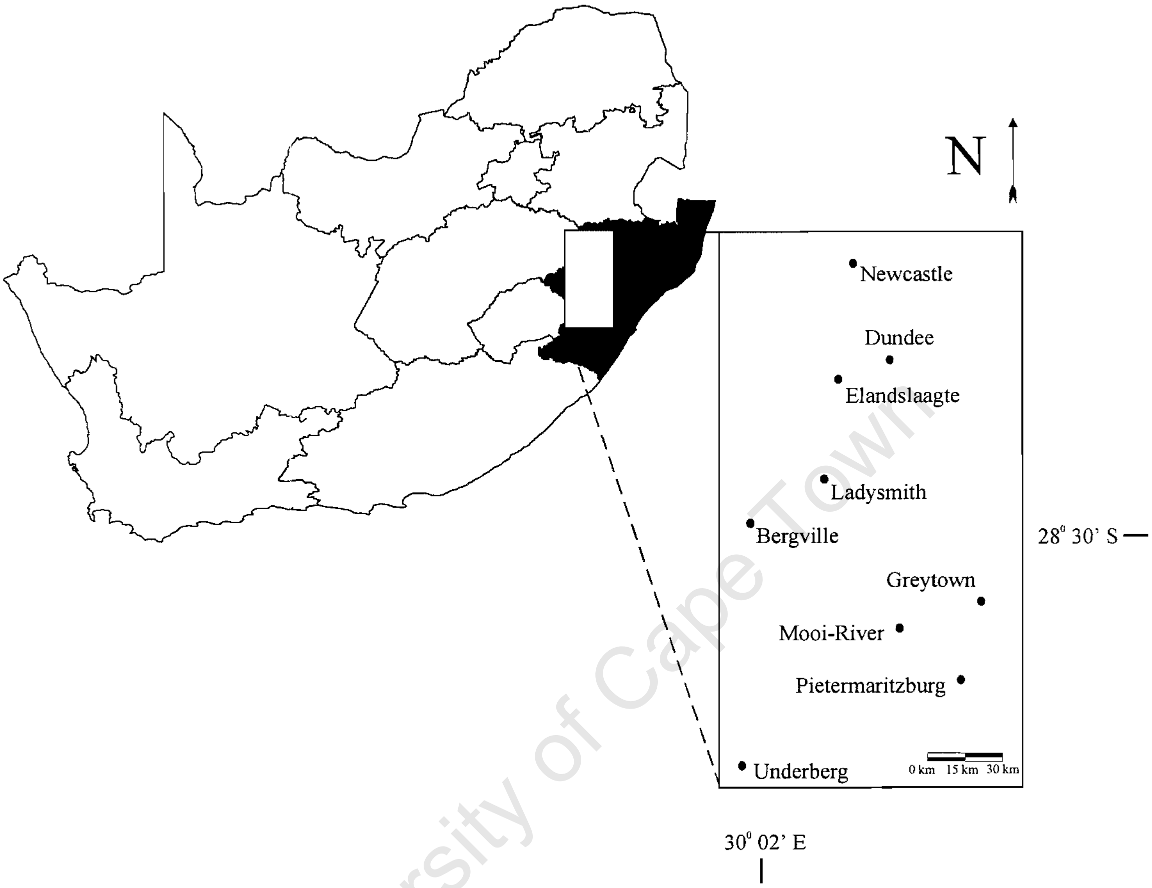

Fig. 2.1. The principal study area and location of major towns.

CHAPTER 3

AGROCHEMICALS

Status as of 18/9/2000: in press. Pesticide residues in Helmeted Guineafowl *Numida meleagris* livers collected on crop farms in the Midlands of KwaZulu-Natal province, South Africa. *South African Journal of Wildlife Research*. Co-authors: T.M. Crowe & S.K.C. Peall.

SUMMARY

The livers of 36 Helmeted Guineafowl *Numida meleagris* were collected during July 1997 to August 1998 from 10 farms with varying intensities of pesticide use within the Midlands of KwaZulu-Natal province, South Africa. Residues (ppb) of Dieldrin and p,p' isomers of DDE were detected in eight (22 %) samples representing three farms with high and two with moderate pesticide-use. No chemical residues were associated with farms with no pesticide application. The ranges of the residue concentrations detected were: Dieldrin 2-32 ppb and p,p' DDE 8-208 ppb. These residues represent historical rather than recent contamination, indicating that current populations are not being affected by sub-lethal accumulations of pesticides.

3.1. INTRODUCTION

In the early 1980s, Helmeted Guineafowl *Numida meleagris* populations declined dramatically in the Midlands of KwaZulu-Natal province of South Africa (Pero & Crowe, 1996). The area is farmed intensively and is characterised by a combination of stock and crop agriculture - predominantly maize (Chapter 2). There has been an increasing demand for agricultural produce caused by increasing human population growth in this region which has resulted in the modernisation of agriculture in order to sustain sufficiently high yields (Myers & Simon, 1994). This modernisation has resulted in an increased dependence on agrochemicals (Pero & Crowe, 1996). The intensive use of herbicides and insecticides is a potentially limiting factor for guineafowl populations, both indirectly and directly (Grafton, 1971; Ayeni, 1981; Johnson, 1984). The indirect effects of pesticides may reduce food and suitable edge habitats for various species (Andrews & Rebane, 1994), whereas sub-lethal doses of 2,4-D, paraquat and monocrotophos, all used within the region, are known to lower the reproductive success and survival of gamebird chicks (Stromborg, 1986; Potts, 1986; Orians & Lack, 1992).

Within its range in southern Africa, the Helmeted Guineafowl is often abundant in savannas mixed with cultivation, where important resources such as food, cover, roosts and water occur (Crowe et al., 1986). More than 80% of this guineafowl's diet can, in one way or another, be attributed to agriculture (Grafton, 1971; Mentis et al., 1975), with the main components including arthropods, many of which are agricultural pests (Skead, 1962; Ayeni, 1983a; Little et al., 1995; Witt et al., 1995), weeds and tubers of cultivated areas, pasture greens and disused agricultural grain (Grafton, 1971; Mentis et al., 1975). The consumption of large quantities of agricultural grains in winter, and arthropods in summer (Grafton, 1971; Mentis et al., 1975) results in guineafowl populations being exposed to a host of agrochemicals. In the study area, a 65 % increase in the average expenditure on pesticides per hectare of maize, was recorded between 1980 and 1990 (Pero & Crowe, 1996). Previous studies have found accumulations of pesticide residues in various tissues of Helmeted Guineafowl which may have physiological implications (Wiese & Basson, 1967; Little et al., 1997).

The aim of this chapter is to investigate the direct effects of both historical and recent pesticide contamination - either through sub-lethal accumulations or direct poisonings - to ascertain if pesticides were instrumental in the demise of populations, or are continuing to suppress their recovery. Historical data were obtained from the Allerton Provincial Veterinary Laboratory in Pietermaritzburg, whereas analysis of the levels of pesticide residues in the livers of Helmeted Guineafowl from farms with varying degrees of pesticide use, provided insight into both recent and historical contaminations.

3.2. METHODS

Data regarding poisoning cases involving guineafowl since 1985 were obtained from Allerton laboratory. The livers of 36 Helmeted Guineafowl were collected from July 1997 to August 1998 from 10 farms with varying intensities of pesticide use within the study area (Fig. 3.1). Farms were classified in terms of the number of pesticides utilised, namely: high (> 5), moderate (< 5) or none (Appendix 3.1). The agrochemicals utilised on the individual farms are listed in Appendix 3.2., however, it must be noted that this inventory may not be complete due to poor recollection by individuals, lack of transparency regarding chemical use, or through changes in farm management.

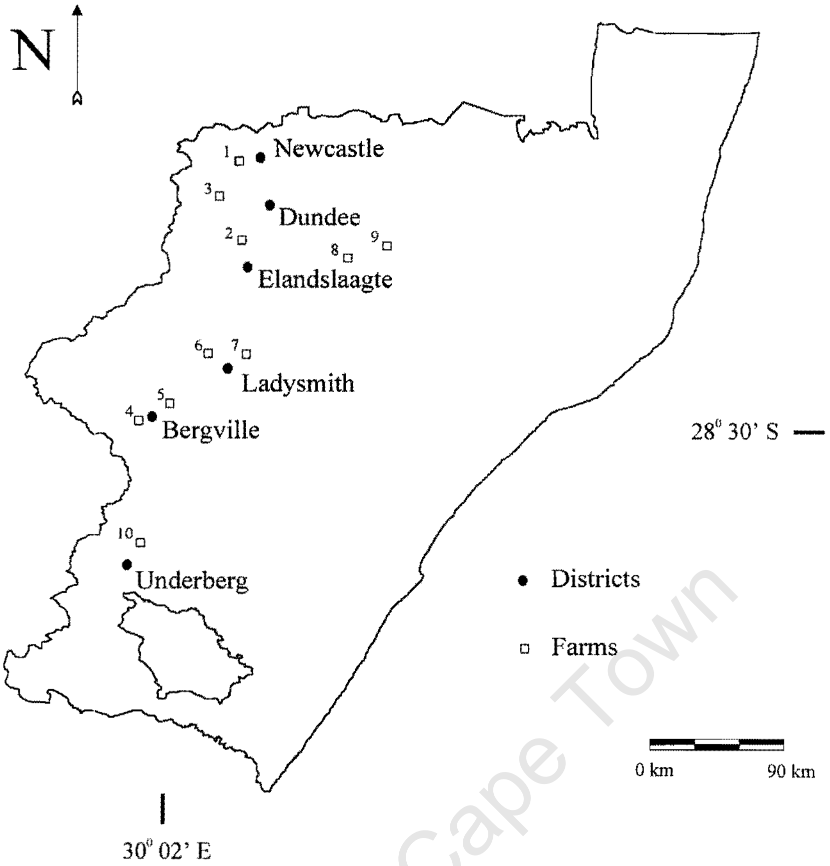

Fig. 3.1. Location of farms (numbered) within the study area from which liver samples were obtained - see Appendix 3.1. and 3.2. for farm details.

The livers were frozen at -20^0 C and subsequently analysed for various monograms, such as pyrethroids, organochlorines and organophosphates. A 5-12 g sample was transferred into a 150 ml beaker and was extracted with 25 ml n-hexane for 30 s using an Utra-Turrax blender. The n-hexane was filtered through Whatman 1PS filter paper and the filtrate was collected in a separating funnel. The liver was extracted again in the same way and the n-hexane fractions combined. The n-hexane in the separating funnel was then extracted with 50 ml acetonitrile. The separating funnel was shaken for 2 min and left until the layers separated. The lower acetonitrile layer was collected in a 250 ml round-bottom flask. The n-hexane layer was extracted twice more with 35 ml acetonitrile and all the acetonitrile aliquots were combined. The acetonitrile fractions were then evaporated to dryness in a Buchi model R-124 rotary evaporator at 40^0 C. The dried residue was dissolved in 5 ml of n-hexane.

Column chromatography was used to clean up a 4 ml aliquot of the re-dissolved residue. A glass column, 30 cm x 19 mm internal diameter, was half-filled with n-hexane, and 5 g of 1.5 % deactivated silica gel was added, 1 g of anhydrous sodium sulphate was added to the top of the column. The hexane was drained until it reached the top of the sodium sulphate. The 4 ml aliquot was added to the column. The analytes were eluted with 100 ml of toluene. The toluene was collected in a 250 ml round-bottom flask and evaporated in a rotary evaporator at 40^0 C. The residue was redissolved in 1 ml of n-hexane. Organochlorine and organophosphate pesticides were determined by gas chromatography.

The samples were analysed by gas chromatography using two different systems as in Little et al. (1997). The DDE and dieldrin levels were quantified using a gas chromatograph mass spectrometer. An internal standard was not added to the samples because it could mask an analyte where analytes were unknown and sometimes occurred in small quantities. Reagent blanks were run through the entire analytical process to establish background interferences. The specifications for the laboratory apparatus are as in Little et al. (1997). Recoveries in spiked guineafowl livers were 100% for dieldrin at a level of 22 ppb, and 117% for DDE at a level of 97 ppb. All results found assume a 100% recovery.

3.3. RESULTS

Residues (ppb) of Dieldrin and p,p' isomers of DDE were detected in 8 (22 %) samples representing three farms with high and two with moderate pesticide-use (Appendix 3.1). These farms occurred in four of the six districts within the study area. No chemical residues were detected on farms without pesticide application. Of the eight positive samples, six contained p,p' DDE while Dieldrin occurred in five. The ranges of the residue concentrations detected were: p,p' DDE 8-208 ppb, and Dieldrin 2-32 ppb. None of the samples tested positive for organophosphates or pyrethroids which were used during the study. Pesticides previously detected by the above methods are: endosulfan, pyrethroids, DDT(DDE) and chlorfenvinphos.

The direct poisoning cases (historical data) are represented in Fig.3.2.

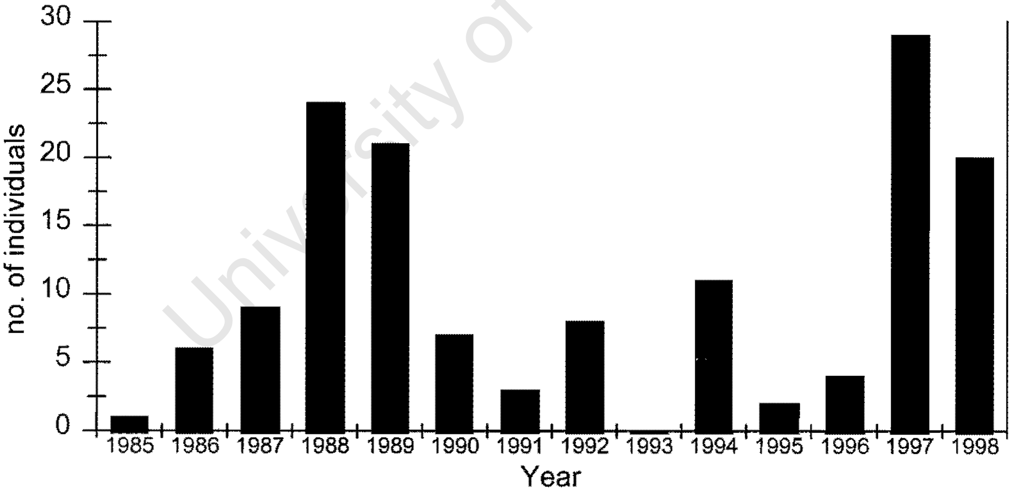

Fig. 3.2. Guineafowl poisoning cases since 1985 (from Allerton Provincial Veterinary Laboratory, KwaZulu-Natal).

3.4. DISCUSSION

DDE is one of the most frequently detected metabolites of 1,1,1-trichloro-2,2-bis(*P*-chlorophenyl) ethane commonly referred to as DDT (Peakall & Kemp, 1980; Henny et al., 1984). Both DDT and dieldrin are organochlorines which are persistent in the environment, dissolve readily in fat and therefore accumulate in animal tissues (L. Lotter pers. comm.). Through lipid metabolism, organochlorine residues can be mobilised, resulting in their distribution and concentration in other tissues (Ecobhicon & Saschenbrecker, 1968; Ruiz et al., 1984), which may result in death (Clark, 1978).

In southern Africa, organochlorines have been found to bioaccumulate in Little Stints *Calidris minuta*, Threebanded Plovers *Charadrius tricollaris* (Lotter & Bouwman, 1997), Cape Vultures *Gyps coprotheres* (Van Wyk et al., 1993), Pied Kingfishers *Ceryle rudis* (Evans & Bouwman, 1993) as well as Helmeted Guineafowl (Wiese & Basson, 1967; Little et al., 1997; L. Lotter, unpubl. data.). In liver samples analysed from healthy guineafowl populations (20-30 % recruitment rate over past five years) (R. Little, pers. comm.) on deciduous fruit farms in the Western Cape province, residues of β-endosulfan, endosulfan sulphate, p,p' DDE and p,p' DDD were detected (Little et al., 1997). The p,p' DDE concentrations ranged from 0.011-0.719 ppm (11-719 ppb), which is substantially higher than residues detected in this study (8-208 ppb) involving declining populations. The range of dieldrin residues (2-32 ppb) was similar to residues detected in specimens from the North West province (3.6-55 ppb) (L. Lotter, unpubl. data.).

In terms of seasonality, the majority of samples were obtained during winter months - traditionally a period of low agrochemical activity - yet of the 10 samples from the summer months, only one sample revealed traces of Dieldrin and DDT. DDT and dieldrin, under special conditions, have a half life of 11,2 and 5-25 years respectively (Cooke & Stringer, 1982; Wingo, 1966), and therefore it is reasonable to expect to find traces of these elements 20 years after the local ban on the use of these insecticides in 1976 and 1983 respectively (Anon, 1976; A. Nel pers. comm.1999). The DDE and dieldrin residues therefore represent historical rather than recent contamination which would continue to affect populations through sub-lethal accumulations and therefore suppress their recovery. Apart from Elandslaagte and Newcastle, all districts contained farms with small traces of both DDT and dieldrin. The intensity with which these chemicals were applied 20 years ago (pre-ban) is unknown, and therefore although there was little difference between farms of currently high and moderate pesticide use, little can be deduced due to this time elapsed and numerous local factors which may have affected these residues. No other chemicals were detected in the tissue samples suggesting very little recent contamination.

The direct effect of agrochemicals through deliberate poisoning similarly, does not seem to be having adverse effects on the population. Historically, only 42 cases of poisoning involving 145 birds have been reported since 1985 (Fig. 3.2) - this amounts to three large flocks over a period of 13 years! Furthermore, over 80 % of these direct poisoning cases were attributed to organophosphates - 54.5 % of which were cases involving monochrotophos (Ratcliffe & Crowe, 1999a). These chemicals are used widely in the study area, but were not detected in the liver tissues of the guineafowl sampled. This is possibly due to organophosphates hydrolyzing rapidly in birds and therefore do not accumulate as original compounds (Layher et al., 1985). The lack of pesticide residues detected is most likely due to many pesticides becoming increasingly degraded within a few days, after contact with air or soil, into relatively harmless chemicals (Riley, 1990). It is also well known that, in many cases, the half-lives of chemicals under moderate conditions are longer than those experienced under conditions characteristic of Africa (e.g. temperature and UV). Higher organic soil content than in arid areas may result in increased microbial action and thus shorten expected half-lives (L. Lotter pers. comm.). Additional factors, such as high rainfall, may also serve in reducing the direct and delayed direct effects of these chemicals.

3.5. CONCLUSIONS

For the most part, the effects of the accumulation of sub-lethal residues of pesticides in birds is unknown (Linger, 1994). However, in gamebirds, these accumulations have mostly been linked to impairing reproductive success (Potts, 1980; Bauer, 1985; O'Conner & Shrubb 1986; Stromborg, 1986). In this study, there was no marked contrast in sub-lethal accumulations of pesticides between farms with varying pesticide intensity. Furthermore, the residues detected were very small traces and were in contrast with previous findings which found significantly higher concentrations in areas that are under intensive pesticide regimes, and where guineafowl populations were relatively stable. This suggests that, in terms of the delayed direct effects through sub-lethal contamination of vital tissues, Helmeted Guineafowl are not currently being affected adversely by pesticides. Similarly, the relatively few historical cases of direct poisoning supports this. Admittedly, many cases would not have been reported but, assuming that official cases are representative of any trends within the population, then there is little evidence to substantiate that direct poisoning is an ongoing major and widespread factor in population declines. Of greater concern, however, are the indirect effects of these chemicals. Increases in intensive crop agriculture and subsequent chemical use, may have resulted in a reduction in important resources - notably food and cover - thus depressing guineafowl populations (Pero & Crowe, 1996; Malan & Benn, 1999; Ratcliffe & Crowe, 1999a).

Appendix 3.1. Pesticide residue concentrations (ppb wet weight) recovered from 36 Helmeted Guineafowl *Numida meleagris* livers collected during July 1997 to August 1998 from ten farms with varying intensities of pesticide use within the study area.

District	Farm #	Date collected	Pesticide-use			Liver mass (g)	Chemical detected	
			High	Moderate	None		DDE	Dieldrin
Newcastle	1	24/2/98	*			15.52	n/d	n/d
		"	*			11.53	n/d	n/d
Elandslaagte	2	7/7/97			*	8.93	n/d	n/d
		"			*	9.79	n/d	n/d
	3	23/8/97		*		11.20	n/d	n/d
		"		*		7.58	n/d	n/d
		"		*		9.58	n/d	n/d
		"		*		7.48	n/d	n/d
		24/8/97			*	8.62	n/d	n/d
		"			*	9.72	n/d	n/d
		"			*	9.25	n/d	n/d
Bergville	4	17/8/97	*			10.88	n/d	n/d
		"	*			11.21	n/d	n/d
		"	*			10.70	n/d	n/d
		"	*			11.00	n/d	n/d
		30/8/97	*			8.75	n/d	n/d
		"	*			8.35	n/d	n/d
		"	*			6.80	n/d	n/d
		"	*			8.93	n/d	n/d
	5	5/2/98	*			8.56	12	2
		12/3/98	*			9.35	n/d	n/d
		"	*			10.18	n/d	n/d
		"	*			10.14	n/d	n/d
Ladysmith	6	2/8/97		*		8.65	n/d	n/d
		"		*		8.85	n/d	32
		"		*		9.36	n/d	14
	7	20/3/98			*	8.64	n/d	n/d
		"			*	7.29	n/d	n/d
		"			*	9.71	n/d	n/d
		"			*	10.80	n/d	n/d
Dundee	8	11/6/98	*			8.18	32	3
		"	*			9.28	n/d	n/d
	9	12/6/98	*			5.48	208	n/d
		"	*			8.98	76	n/d
Underberg	10	26/8/98		*		11.71	8	n/d
		"		*		12.71	15	13
Totals			18(3)[1]	9(2)[1]	9		(6)[2]	(5)[2]

n/d = not detected

[1] number of farms with residues detected

[2] # of birds with residue

Appendix 3.2. Agrochemicals applied on the 10 farms from which samples were obtained.

District	Farm #	Chemicals	Active ingredient	Monogram
Newcastle	1	Cypermethrin	Cypermethrin	Pyrethroid
		Guardian S	Acetochlor	Organonitrogen
		Ratel	Acetochlor	Organonitrogen
		Lasso	Alachlor	Organonitrogen
		Sencor	Metribuzin	Organonitrogen
		Spotaxe	2,4D	Chlorophenoxy
		Classic	Chlorimuron-ethyl	Organonitrogen
		2,4-D Amine	2,4-D Amine	Chlorophenoxy
		TMTD	Thiram	Dithiocarbamate
Elandslaagte	2	None		
	3	Galleon	Atrazine	Organonitrogen
		Karate	Lambda - cyhalothrin	Pyrethroid
		Falcon	Metolachlor	Organonitrogen
		Kombat	2,4D / MCPA	Chlorophenoxy
Bergville	4	Eptam	EPTC	Dithiocarbamate
		Cypermethrin	Cypermethrin	Pyrethroid
		Dual	Metolachlor	Organonitrogen
		Gesaprim Super	Atrazine	Organonitrogen
		Decis	Deltamethrin	Pyrethroid
		Lindstof	Gamma-BHC	Organochlorine
		Punch extra	Carbendazim	Carbamate
	5	Sting	Glyphosate	Glyphosate
		2,4-D Amine	2,4-D Amine	Chlorophenoxy
		Lasso	Alachlor	Organonitrogen
		Dual	Metolachlor	Organonitrogen
		Fenom	Cypermethrin	Pyrethroid
		Galleon	Atrazine	Organonitrogen
		Gromoxone	Paraquat	Dipyridyl
		Punch C	Carbendazim	Carbamate
		Punch extra	Flusilazole	Triazole
Ladysmith	6	Ratel	Acetochlor	Organonitrogen
		Guardian	Acetochlor	Organonitrogen
		Cypermethrin	Cypermethrin	Pyrethroid
	7	None		
Dundee	8 & 9	Cypermethrin	Cypermethrin	Pyrethroid
		Guardian S	Acetochlor	Organonitrogen
		Ratel	Acetochlor	Organonitrogen
		Spotaxe	2,4D	Chlorophenoxy
		Lasso	Alachlor	Organonitrogen
Underberg	10	Dursban	Chlorpyrifos	Organophosphate
		Dual	Metolachlor	Organonitrogen
		Decis	Deltamethrin	Pyrethroid

CHAPTER 4

DISEASES

Status as of 18/9/2000: in review. A serological survey of wild Helmeted Guineafowl *Numida meleagris* in KwaZulu-Natal province, South Africa. *Avian Pathology*. Co-authors: R. F. Horner*, M. E. Parker & T. M. Crowe. * First author.

SUMMARY

Sera were collected from wild Helmeted Guineafowl *Numida meleagris* from six geographical areas of KwaZulu-Natal province, South Africa over a 16-month period from March 1997 to July 1998. The sera were examined for the presence of antibodies against a variety of disease entities which are known to affect domestic and commercial chickens *Gallus gallus*. Antibodies were detected against six of the 12 disease-causing agents assayed. The highest antibody prevalence, 28%, was against avian encephalomyelitis virus. No antibodies were detected against the viruses causing Newcastle disease or avian influenza. There is no evidence that a pathogen is suppressing the recovery of guineafowl populations within the Midlands of KwaZulu-Natal.

4.1. INTRODUCTION

The poultry industry in South Africa is based on chickens *Gallus gallus* (Order Galliformes), and is comprised of the highly intensive and well-developed commercial-broiler and table-egg producer flocks on the one hand, and the free-range mainly indigenous breed subsistence "village chicken" population on the other. Commercial flocks are managed along modern lines and whether great grand parent-breeder flocks, or commercial-broiler or layer-flocks, they are subjected to specifically designed vaccination programmes and varying degrees of biosecurity (fences, sanitation, quarantine facilities, etc.) to protect them against important disease-causing agents. In contrast, village chickens are not vaccinated, mix freely with other chickens in free-range situations, and are traded live between villages. Thus, they are not subjected to any biosecurity measures.

During outbreaks of disease, e.g. Newcastle disease, indigenous bird species may be subjected to varying degrees of pathogen exposure, depending on their proximity to, or remoteness from, village chicken populations. Indeed, many diseases which affect the domestic chicken, also occur in other Galliformes such as pheasants (Phasianinae), partridges (Perdicini), quail (*Coturnix* spp.), francolins (*Francolinus* spp.) and guineafowl (Numididae). The Helmeted Guineafowl has a wide geographical distribution and is often associated with cultivated lands in rural areas. Flocks that therefore occur in close proximity to village chicken populations have a greatest risk of infection than other populations.

Wild Helmeted Guineafowl populations have declined dramatically in KwaZulu-Natal Midlands over the past two decades (Pero & Crowe, 1996). The fact that some populations still appear to be in decline, suggests that, if this collapse were a result of some pathogen, that it should still reside within such populations, thus preventing any widespread recovery. To investigate this possibility and ascertain the disease status of guineafowl, sera were submitted to Allerton Provincial Veterinary Laboratory over a 16 month period and subjected to antibody analyses to detect a variety of common poultry diseases.

4.2. MATERIALS AND METHODS

4.2.1. Guineafowl

Six populations of wild guineafowl were identified within the study area. Localities were chosen by either their proximity to, or remoteness from, large village chicken populations (Fig. 4.1). Roosting sites of particular guineafowl flocks in each area were detected, and walk-in baited funnel-traps erected close to these sites. Chopped maize kernels were used to bait the traps. Birds under 35 weeks of age were termed sub-adult and birds over 35 weeks were termed as adult (Siegfried, 1966).

4.2.2. Sample collection

A blood sample from each captured bird was collected into a plain glass tube by venipuncture of the brachial vein. In some cases, a thin blood smear was made on a glass slide and air dried. Blood samples were allowed to clot at ambient temperature before being transported in a chilled

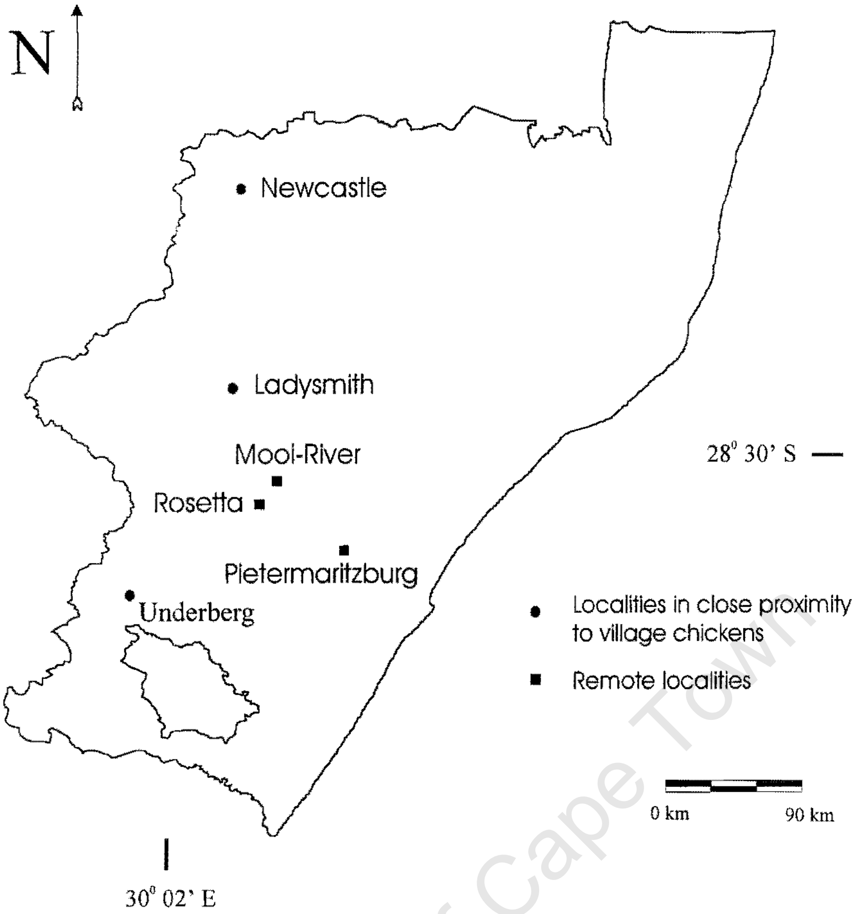

Fig. 4.1. Localities of sampling sites with regards to their proximity to village chicken populations within the study area.

environment to the laboratory together with details of the area, farm name, number of birds sampled and their age. In the laboratory, blood samples were stirred gently with a metal rod, incubated at 37^0 C for one hour and the serum poured off into plastic microtubes. They were then stored at - 20^0 C until serological testing. Blood smears were fixed in methanol for three minutes and stained with 10% Giemsa for 30 minutes. Each slide was examined at 400 times magnification over 25 fields of view.

4.2.3. Serological tests

Serum samples were subjected to a series of antibody detection tests as follows and each according to the manufacturers instructions. A brief description of each disease is in Appendix 4.1.

Mycoplasma gallisepticum (Mg) and *Mycoplasma synoviae* (Ms)	Plate Agglutination test using stained antigen from Intervet, Boxmeer, Holland.
Bacillary white diarrhoea (BWD)	Rapid plate agglutination test using stained antigen from Onderstepoort Biological Products, Pretoria.
Newcastle disease (NCD), Egg drop syndrome (EDS) and Avian influenza (AI)	Haemagglutination inhibition test (HIT) performed as described in The Official Journal of the European Communities, Annexure 111 (1992), using 4 HA units of antigen. All HI titres were expressed as log 2 of the reciprocal of the highest serum dilution showing complete haemagglutination inhibition.

Infectious bronchitis (IB) and Infectious bursal disease (IBD)	Sera were assayed at a 1:40 dilution using a commercial antibody ELISA kit from Delta Bioproducts, Kempton Park, Johannesburg.
Infectious laryngotracheitis (ILT).	Sera were assayed at a 1:100 using a commercial antibody ELISA kit from Kirkegaard and Perry Laboratories, Gaithersburg, Md. USA.
Chicken anaemia virus (CAV) and Avian encephalomyelitis (AE)	Sera were assayed at a 1:9 dilution for CAV and 1:500 for AE using commercial antibody ELISA kits from IDEXX Corporation, Westbrook, Maine, USA.
Avian pneumovirus (APV).	Sera were assayed at a 1:40 dilution using a commercial antibody ELISA kit (Avian rhinotracheitis) from Pathasure, Cambridge Veterinary Sciences Ltd. Ely UK

4.3. RESULTS

4.3.1. Serological examination

No antibodies were detected against BWD, NCD, IB, IBD, AI or EDS (Fig. 4.2). One bird out of 70 (1.4%) was positive for MG and two birds (2.9%) positive for MS. One bird out of 40 (2.5%) was positive for CAV, three out of 17 (17.6%) were positive for ILT and these involved two separate geographical areas. Seven birds out of 42 (16.7%) were positive for APV and involved three separate geographical areas. The highest prevalence of antibody was against AE in which 10 out of 36 (27.8%) birds were positive involving three separate geographical areas (two near village chicken populations and one remote). Of the 12 disease entities assayed, four occurred in proximity to village chicken populations whereas five occurred in remote populations (Table 4.1).

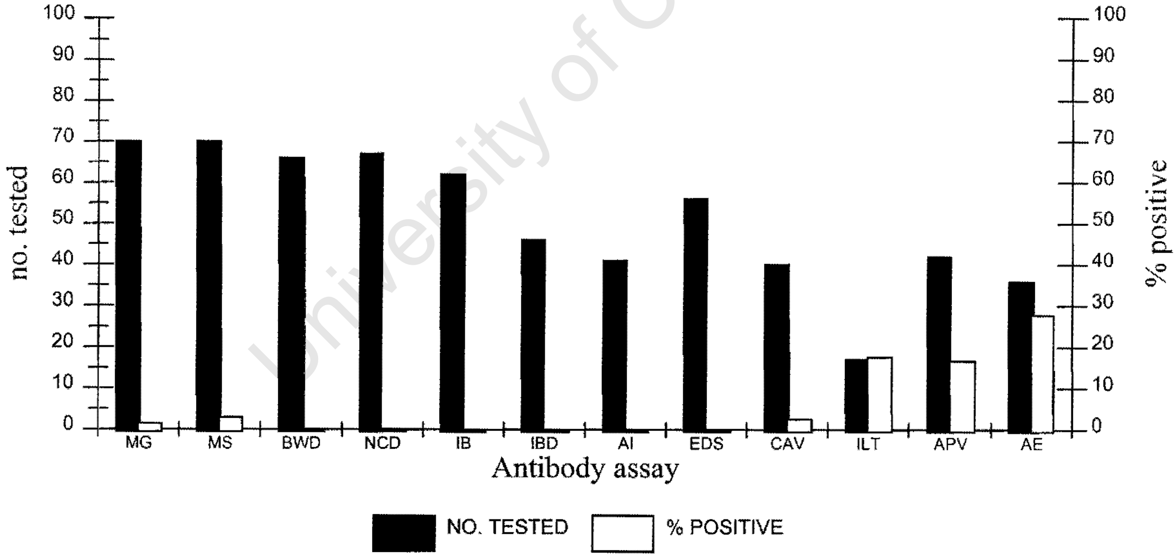

Fig. 4.2. Pathological tests of 70 guineafowl from KwaZulu-Natal Midlands assayed for diseases.

Table 4.1. Detection of antibodies in wild Helmeted Guineafowl *Numida meleagris* from various geographical areas in KwaZulu-Natal. March 1997 to July 1998.

	Detection of antibodies against											
	Mg	Ms	BWD	NCD	IB	IBD	AI	EDS	CAV	ILT	APV	AE
No. tested	70	70	66	67	62	46	41	56	40	17	42	36
No. positive	1	2	0	0	0	0	0	0	1	3	7	10
% positive	1.4	2.9	0	0	0	0	0	0	2.5	17	16.7	27.8
No. of areas sampled [a]	6	6	6	6	6	5	5	5	5	6	5	5
No. of areas positive	1	1	0	0	0	0	0	0	1	2	3	3

[a] Geographically separate areas of KwaZulu-Natal

4.3.2. Blood smear examination

Ninety eight blood smears were examined of which five (5.1%) involving two geographical areas (both in proximity to village chicken populations) were positive for *Haemoproteus* spp. No other protozoan parasites were seen.

4.4. DISCUSSION

Should rural village chickens or commercial poultry operations suffer an outbreak of infectious disease, the surrounding populations of indigenous species may well be prone to that particular pathogen. Despite the duration of this survey extending over a 16-month period and involving six geographical areas of the province, no antibodies were detected against six of the disease entities assayed.

Infection with MG can occur naturally in chickens and turkeys *Meleagris gallopavo*. The organism has also been isolated from naturally infected pheasants *Phasianus colchicus*, Chukar Partridge *Alectoris chukar* and quail *Coturnix coturnix*. The situation regarding natural MG infection in guineafowl is less clear and in this survey only one bird showed a positive serological reaction. In contrast with this, guineafowl are natural hosts of MS (Pascucci et al., 1976) but, despite this infection being reasonably widespread in village chicken populations in this province, only two guineafowl from one area tested positive.

BWD was mostly eliminated in this province 30 years ago and, although rare in most commercial poultry producing areas of the world, can occur in guineafowl (Snoeyenbos & Williams, 1991). Newcastle disease has been recorded in 27 of the 50 orders of birds (Kaleta & Baldauf, 1988) and does occur in guineafowl (Alexander, 1988). Paramyxovirus Type-1 has been isolated from wild Helmeted Guineafowl in this province on at least three occasions since 1991, with one isolate having an intra-cerebral pathogenicity index of 1.81. Of interest is that, during the latter part of this survey, field strain Newcastle disease problems were being experienced in both village and commercial chicken flocks, but no antibodies were detected in any of the 67 guineafowl tested.

It is generally accepted that the domestic chicken is the only bird that is naturally infected by IBV. However sero-conversion and respiratory symptoms of the disease were seen in pheasants in England (Spackman & Cameron, 1983) and the virus was isolated from guineafowl in South America (Ito Nair et al., 1991). Infectious bursal disease is essentially a disease of the domestic chicken, although virus has been isolated from ducks *Anas* spp. (McFerran, 1993) and turkeys (Jackwood et al., 1982). Antibodies against IBD have been found in some species of waterfowl and seabirds McFerran, (1993) but Nawathe et al. (1978) did not find any reactors out of 156 guineafowl tested in Nigeria.

Influenza A viruses infect a large variety of animal and bird species worldwide. The greatest variety and largest quantities of virus have been detected in birds, especially migratory waterfowl, however, it is the domestic turkey and chicken that have experienced most disease problems caused by this virus. Influenza A virus has been isolated from commercially reared guineafowl (Alexander, 1993). This potentially devastating virus does not however appear to have played a role in the decline of the KwaZulu-Natal guineafowl population.

Although ducks are considered the natural host for EDS virus, the disease and infection has occurred in commercial laying hens in many areas of the world, and guineafowl may be infected with the resulting production of typical soft shelled eggs (Guittet et al., 1981).

The chicken is considered the natural host of CAV (McNulty, 1991) and the virus has been isolated from chickens in many countries throughout the world. Antibodies to CAV were not found in a small survey of United Kingdom turkey and duck sera (McNulty et al., 1988). In this survey, only one guineafowl out of 40 tested showed antibodies to CAV.

Infectious laryngotracheitis is mainly a disease of the domestic chicken, but infection has occurred in the pheasant and peafowl *Pavo cristatus* (Crawshaw & Boycott, 1982). Guineafowl seem to be refractory to clinical disease (Jordan, 1993). Three out of 17 guineafowl showed antibodies to ILT which may indicate their potential to act as a carrier of this virus.

Avian pneumovirus can infect chickens, turkeys, guineafowl and pheasants whereas ducks, geese and pigeons (Columbidae) appeared refractory (Gough et al., 1988). Antibodies to APV have been demonstrated in naturally infected chickens and turkeys in many countries (Alexander, 1993) and in experimentally infected guineafowl (Picault, 1987; Gough et al., 1988). Heffels-Redmann et al. (1998) showed the presence of antibodies to APV in up to 50% of Herring gulls *Larus argentatus argentatus* sampled from northern Germany. In our survey of wild guineafowl, seven birds out of 17 sampled were antibody-positive to APV. The positive birds came from three of the five geographical areas sampled. Guineafowl may thus play a role in the transmission of this disease.

Avian encephalomyelitis occurs in chickens, pheasants, quail and turkeys. Ducks, pigeons and guineafowl have been experimentally infected and naturally occurring antibodies have been found in sera from partridges *Alectoris rufa*. (Calnek, 1993). Ten out of 36 guineafowl, involving three of the five areas sampled, were positive for antibody to AE in this study. This represents the highest prevalence of antibody found for any of the 12 disease entities assayed. Because AE virus is relatively resistant to environmental degradation, virus in the faeces of infected village chickens may be easily spread by horizontal transmission to adjacently feeding guineafowl populations.

4.5. CONCLUSIONS

The scale at which guineafowl populations collapsed within the Midlands of KwaZulu-Natal, would suggest the action of a pathogen that has the ability to depress populations on a regional basis. Newcastle disease and avian influenza are two such pathogens, both of which had negative results for the 70 and 56 birds tested respectively. There is little discrepancy between the number and type of disease entities that were detected in remote populations of guineafowl in comparison to those close to village chickens. Five disease entities (MG, CAV, AE, ILT, APV) occurred in remote populations, whereas four (MS, AE, ILT, APV) occurred in populations adjacent to village chickens. Three disease entities were common to both groups of populations. Considering that village chickens are potentially the greatest source of infection for wild guineafowl, there is no indication that flocks in close proximity to these populations, have had greater exposure to various pathogens than more remote populations. Indeed, one of the sample sites in close proximity to village chickens (Ladysmith), is representative of a healthy population with over 2000 birds (Ratcliffe & Crowe, in press)!

It is unlikely that an infectious disease resulted in the collapse of guineafowl populations in the 1980s. Reports and or actual submissions of sick, dying or dead birds would have occurred from farmers or provincial conservation organisations at some time during this period. However, failing this and because of the time elapsed, antibodies in infected but surviving birds would now be undetectable. In addition, guineafowl may live up to six years in the wild (T.M. Crowe, unpubl.data), and therefore no individuals exist from this time period. There has been little recovery in populations since this decline however, and thus some mechanism or mechanisms must still be holding populations in check. From the above survey though, there is no evidence to suggest that an infectious disease is suppressing current populations.

Appendix 4.1. Description of pathogens tested for between 1997 and 1999.

Mycoplasma gallisepticum **(MG)**

A respiratory disease transmitted through eggs (vertically) and may also be passed laterally directly or indirectly. It is an important commercial disease that spreads slowly.

Mycoplasma synoviae **(MS)**

A very quick spreading disease passed laterally and vertically affecting the joints (essentially muscular) or respiratory system.

Bacillary White Diarrhoea **(BWD)**

This disease was mostly eliminated 30 years ago. Tests are now done on domestic fowl as a screening process. It is caused by a bacterium of the *Salmonella spp.* group D (this group is responsible for a wide range of avian and mammalian diseases), and is transmitted through the droppings or vertically.

Newcastle Disease **(NCD)**

This is the only notifiable disease (the state needs to know of any outbreaks). It is a virus that is transmitted laterally (orally or breathed in), and may lie dormant for up to six months in the bone marrow and muscle tissue of a frozen carcass, or up to 2 months under favourable conditions. There are various strains of differing severity - virulent (velogenic), medium (mesogenic) and low (lentogenic). There are also different manifestations of the disease - respiratory, nervous

Appendix 4.1. (continued)

system or visceral, thus an array of symptoms can be found depending on the particular strain. Outbreaks of the disease have been reported in the early 1970s, late 1989 and early 1994/95 & 1998 amongst commercial poultry within the Midlands. A few isolated cases involving guineafowl - from Richmond - have been reported, but nothing on an epidemic scale. 70-80% of a flock may get wiped out should an outbreak occur.

Infectious Bronchitis (**IB**)

An infectious disease (inflammation of the bronchi tubes) transmitted laterally - but Allerton Research Laboratories are not sure whether the birds are susceptible to it. It is caused by a virus that affects domestic chickens but not turkeys and hence the testing in guineafowl.

Infectious Bursal Disease (**IBD**)

Very common in South Africa. Also known as Gumboro disease, this virus is very tough (can survive up to 6 months) and virulent strains occur. In 1989,1990 & 1991, there was a major outbreak in both Africa and Europe, eventually spreading into Asia and the Far East. It is highly infectious, and attacks the immune system of the bird. It is passed laterally (directly or indirectly), and should guineafowl be susceptible to it, it would affect birds at 6-8 weeks (adults would show no visible signs). It has a limited host range with quails showing no sign of infection under controlled conditions.

Appendix 4.1. (continued)

Avian Influenza (**AI**)

This is a virus that is passed laterally (directly and indirectly) and may have similar characteristics and symptoms as virulent NCD. It has only been recorded in ostriches in this country and is generally locally rare. It is potentially the second greatest potential threat to guineafowl populations in that it could prove fatal for entire populations.

Egg Drop Syndrome (**EDS**)

This is caused by a virus that is most common in waterfowl and involves a drop in egg production.

Chicken Anaemia Virus (**CAV**)

According to Dr Horner, this has never been tested for in guineafowl. Commercial poultry have tested positive for this disease. It is passed horizontally (directly and indirectly) and vertically. It causes anaemia in chicks of about 14-20 days through destroying bone marrow producing cells.

Avian Encephalomyelitis (**AE**)

This is caused by a virus and attacks the central nervous system especially the brain. In adults it may result in a drop in egg production if picked up prior/during the breeding season. It can be transmitted vertically - through the egg - with mortality in chicks being experienced within the first 6 weeks with symptoms of paralysis and "paddling". Histopathological examination of the brain will reveal typical pathology. If birds survive exposure, they will be immune and the disease will not be transmitted further. This disease is thus potentially devastating if picked up by the adults prior/during the breeding season (timing important).

Infectious Laryngotracheitis (**ILT**)

This is caused by an uncommon respiratory virus that is passed horizontally (directly and indirectly) and results in severe respiratory symptoms which may result in death. Symptoms include coughing up blood.

Avian Pneumovirus (**APV**)

It is very similar to MG - it is caused by a respiratory virus that is passed horizontally (directly and indirectly). Mortality is not associated with this disease although birds show symptoms similar to those of the human cold. It is often a secondary infection that can prove fatal. It cannot be passed vertically.

CHAPTER 5

GENETICS

Status as of 18/9/2000: in review. Fowl Play: Molecular evidence of interbreeding between wild and feral domestic Helmeted Guineafowl *Numida meleagris*. *Molecular Ecology*. Co-authors: A.L. Walker*, R.C.K. Bowie & T.M. Crowe. * First author.

SUMMARY

This study investigates the possibility that hybridisation between introduced domestic guineafowl, originating from West Africa's *Numida meleagris galeata*, and southern African guineafowl, *N. m. coronata,* might have contributed to the collapse in guineafowl populations within the KwaZulu-Natal Midlands. There is morphological evidence of such hybridisation in wild populations and it is known that domestic guineafowl do not survive well in the wild. Molecular analysis of the control region of mtDNA confirmed the occurrence of the domestic guineafowl haplotype in individuals present in wild populations of KwaZulu-Natal. The development of a simple diagnostic test based on restriction fragment-length polymorphisms allowed for a rapid and inexpensive assessment of hybridisation in wild guineafowl populations. The low level at which such hybridisation appears to occur at present suggests that genetic 'pollution' due to introgression from domestic guineafowl may not be a major factor negatively influencing wild guineafowl populations.

5.1. INTRODUCTION

Of the nine subspecies of Helmeted Guineafowl, only *N. m. coronata,* occurs in South Africa (Crowe, 1978a). Wolff and Milstein (1987) suggested that a potential threat to guineafowl populations is the introduction of domesticated guineafowl into wild populations. This is because interbreeding between domestic and wild birds might undermine the ability of their offspring to survive in the wild (Hastings Belshaw, 1985; Crowe, 2000a).

The guineafowl has been domesticated since the times of ancient Greece and Rome, with the most recently domesticated stock originating from West Africa (*N. m. galeata)* (Ghigi, 1936) and introduced repeatedly world-wide (Long, 1981; Hastings Belshaw, 1985; Donkin, 1991). Over the centuries, selective breeding has produced domestic guineafowl with anatomy, plumage and behaviour quite distinct from that of wild guineafowl. The distinguishing characteristics of domestic guineafowl include: rapid growth to greater body mass; shorter yellow legs (black in wild type); white claws (black in wild type); reduced inclination to care for downy offspring; increased egg production and differing plumage, often with varying amounts of white feathers (Hastings Belshaw, 1985; Wolff & Milstein, 1987). In addition to these novel features, domestic guineafowl possess attributes which characterise its wild ancestors (*N. m. galeata*), e.g. white faces (bluish in *N. m. coronata*) and rounded gape wattles (pennant-shaped in *N. m. coronata*) (Fig. 5.1).

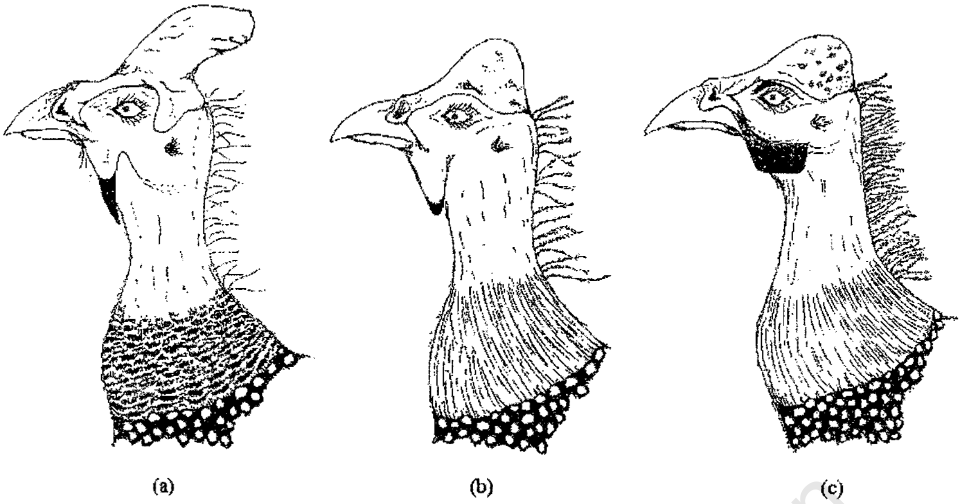

Fig 5.1. Diagnostic morphological features of wild, South African *N. meleagris coronata* (a), an intermediate, putative hybrid (b) and West African *N. m. galeata* (c).

The guineafowl of KwaZulu-Natal currently occur as small populations persisting in fragmented habitats, and are often in close contact with introduced domestic guineafowl (Crowe, 2000a; Horner et al., in review). These are conditions typical of other hybridised populations (Lehman et al., 1991; Ward et al., 1999). Hybrids and hybrid zones, particularly between subspecies are a natural occurrence (O'Brian & Mayr, 1991). However, the interbreeding between native and introduced taxa, even on a small-scale, has the potential to cause widespread genetic introgression, jeopardising a species' genetic integrity. Indeed, the characteristics produced by artificial selection that make the domesticated guineafowl commercially valuable could undermine the viability of wild populations if interbreeding did take place (Crowe, 2000a).

Reports of individuals that are morphologically intermediate between wild and domestic guineafowl within wild guineafowl populations from KwaZulu-Natal suggest that such hybridisation is occurring (Ratcliffe & Crowe, 1999b). Furthermore, across Africa, individuals within the Helmeted Guineafowl species-complex at hybrid zones (intergrades) do show characters intermediate to the often strikingly distinct parental subspecies (Ghigi, 1936; Crowe, 1978a). Similarly, hybrid offspring of guineafowl (as with other galliforms e.g. Junglefowl *Gallus gallus*) appear to be morphological intergrades of both the parental varieties (Ghigi, 1936; Hastings Belshaw, 1985).

Rossouw (1996) provided direct molecular evidence for the introgression of domestic guineafowl DNA into wild populations in South Africa when, as part of a study of the molecular phylogeography of Helmeted Guineafowl, she discovered diagnostic sequences (from D-loop - control region - of mtDNA) for domestic guineafowl in captive birds which appeared morphologically intermediate between wild and domestic birds. Much more disturbingly, she also found the domestic haplotype in one bird in the wild, which morphologically appeared to be a pure wild type guineafowl. Thus, there appeared to be morphologically undetectable genetic introgression from domestic guineafowl into wild populations.

Based on molecular data, there are many cases reporting introgression after contact with introduced species (Largiadèr & Scholl, 1996; Thulin et al., 1997; Ward et al., 1999). The non-recombinant inheritance and rapid substitution rate of mitochondrial DNA (mtDNA) provides an effective marker to screen for hybridisation at the individual and population level (Carr et al., 1986; Lehman et al., 1991; Thulin et al., 1997; Ward et al., 1999).

In this chapter, the occurrence and extent of genetic introgression in wild Helmeted Guineafowl from the provinces of KwaZulu-Natal and the Free State provinces of South Africa is assessed using a molecular analysis of sequence data. Sampling of areas was done in a more intensive manner than Rossouw's (1996) more broadscale study which examined fewer samples, but from more localities, on an Africa-wide scale. In addition to sequencing analysis, a simple, relatively inexpensive, diagnostic test using restriction fragment-length polymorphisms (RFLPs) was sought to help identify domestic guineafowl mtDNA in wild guineafowl. This method of genetic testing could allow a rapid and relatively inexpensive assessment of hybridisation in wild guineafowl populations, and prove to be invaluable in facilitating informed management and conservation decisions.

5.2. MATERIALS AND METHODS

5.2.1. Sample collection

Guineafowl (n = 36) were collected from populations in the Midlands of KwaZulu-Natal and were characterised morphologically as domestic, intermediate or wild, based on coloration of plumage, face and legs, and the shape of the wattles (Fig. 5.1). A further 20 samples were obtained from a farm in the vicinity of Petrus Steyn in the Free State (27° 39’ 1" S; 28° 7’ 40" E). These individuals were described as either wild or possibly introgressed in appearance. There were very few specimens from the

Free State described as putative hybrids (<20% of a total bird bag >1000), whose identification was based on the presence of white claws, some yellow scales on the legs, or very pale grey background plumage.

5.2.2. Laboratory procedures

DNA was obtained from liver and heart tissue preserved in saturated sodium chloride (NaCl) with 20 % dimethylsulphoxide (DMSO) (Amos & Hoelzel, 1991). Total genomic DNA was extracted from 56 samples using a standard proteinase K digestion followed by phenol chloroform extraction (Sambrook et al., 1989).

A 550 bp fragment was amplified from total genomic DNA using primers that targeted the 5 end of the control region (D-loop) modified according to published chicken sequences (Desjardins & Marais, 1990): L16746 5-ACC CCA AGG ACT ACG GCT TGA A- (Wenink, et al., 1994) and H522 5-GCC TGA CCG AGG AAC CAG AG-3 (Quinn & Wilson, 1993).

Amplification reactions were performed using 0.2 U of *Taq* (Bioline), 1x *Taq* buffer (10x Stock), 2.5 mM MgCl2, 3 mM dNTPs, 0.5 pmols of each primer and 1 L of DNA, in a total volume of 50 L. The thermal profile was 27 cycles at (94C for 45 s; 68C for 45 s; 71C for 60 s). The PCR products (10 L) were run on 1% agarose gels (Techcomp LTD) for 1 h at 80 V. Gels were stained with ethidium bromide and visualised using UV light. The PCR products were purified using a Concert rapid PCR purification system (Life Technologies), a cycle sequenced using a dye terminator cycle sequencing kit and an ABI 373 STRETCH automated DNA sequencer. For verification both strands were sequenced separately. The sequences were aligned using DAPSA (Harley, 1999).

5.2.3. Identification of RFLPs

A restriction enzyme analysis (DNAMAN 4.13, Lynnon BioSoft) using sequence data identified a potential diagnostic RFLP site between domestic and wild within the mtDNA PCR product. Restriction enzyme digestion was, therefore, performed on amplified DNA with *Msp*I in an attempt to identify wild and domestic haplotypes by RFLP analysis. The fragments were separated on a 2% agarose gel for 1-3 h, stained with ethidium bromide and photographed under UV light.

5.3. RESULTS

5.3.1. Morphology

Each of the guineafowl collected was characterised as wild, domestic or intermediate (Fig. 5.1; Table 5.1).

5.3.2. MtDNA Sequence data

Sequence data identified six diagnostic sites in the control region, which distinguish wild from domestic haplotypes (Table 5.2). Pure domestic guineafowl occurred only in the populations from KwaZulu-Natal, and had a haplotype distinct from that of wild birds from KwaZulu-Natal and the Free State. In KwaZulu-Natal, three of the

Table 5.1. Phenotypes and Restrictive Fragment-length Polymorphism (RFLP) haplotypes (in parentheses domestic vs wild) of mtDNA from Helmeted Guineafowl.

Locality	Phenotype		
	Wild	Intermediate	Domestic
Free State	8 (0 8)	12 (0 12)	0
KwaZulu-Natal	14 (1 13)	14 (3 11)	8 (8 0)
Number of Individuals	22	26	8

Table 5.2. Diagnostic sites for mtDNA haplotypes (i.e. wild and domestic) of Helmeted guineafowl from KwaZulu-Natal and the Free State, South Africa. The base-pair (bp) position indicates the locus of each site on the amplified mtDNA D-loop (control) region. A dash (-) indicates an indel site.

Haplotype	Diagnostic site (base-pair position)					
	1 (152 bp)	2 (173 bp)	3 (190 bp)	4 (225 bp)	5 (236 bp)	6(279bp)
Wild	C	A	A	A	T	C/T
Domestic	T	G	G	-	-	A

putative hybrids and one individual described as wild were identified as possessing the domestic haplotype (Table 5.1). In the Free State samples, all individuals described as wild and putatively hybridised possessed the wild bird haplotype (Table 5.1).

5.3.3. RFLPs

The *Msp*I restriction enzyme digestion of the 550 bp mtDNA D-loop region produced distinct patterns for domestic and wild individuals, depending upon the presence or absence of a single restriction site (Table 5.3). This single restriction site corresponded to the third diagnostic site at the 190 bp position based on sequence data (Table 5.2). This *Msp*I restriction site is absent in wild guineafowl. The validity of the diagnostic fragment pattern was confirmed with individuals whose haplotype had been identified by sequence data.

Table 5.3. RFLP patterns (and approximate sizes in kb) generated from *Msp*I digestion of 550 bp PCR-amplified D-loop (control) region of mtDNA for guineafowl.

Haplotype	Fragment sizes (in bp)		
Wild	336	197	
Domestic	257	197	159

5.4. DISCUSSION

5.4.1. Genetic evidence of hybridisation

The findings of this investigation confirm that there is intraspecific hybridisation between wild and domestic guineafowl populations in KwaZulu-Natal, and the identification of a diagnostic restriction fragment pattern provides a useful method for rapidly determining the mtDNA haplotype of any given individual. The presence of hybrids confirms the suspicion that morphologically intermediate individuals may indeed be hybrids. The lack of a 1 : 1 correspondence between morphological and molecular evidence of hybridisation may be a result of low levels of intraspecific mating or problems associated with using a maternally inherited molecular marker such as mtDNA. To assess the full extent to which hybridisation has taken place between domestic and wild guineafowl populations in KwaZulu-Natal, a biparentally inherited nuclear marker needs to be used (Compton, 1990).

It is also possible that the decline of wild guineafowl populations, as a result of habitat fragmentation and modern agricultural practices (Ratcliffe & Crowe, in press), facilitated hybridisation with introduced domestic guineafowl which were released in the hopes of resuscitating declining populations (Crowe, 2000a). There are several other well-documented examples where introduced domestic taxa have hybridised with native species under similar conditions (Hubbard et al., 1992; Butler, 1994; Ward et al., 1999).

The identification of only one morphologically 'wild' individual from KwaZulu-Natal with the domestic haplotype also confirms that birds with a wild-type morphology may have a hybrid ancestry. Thus, it is not always possible to identify hybrids morphologically, and both morphological and molecular analyses are necessary to confirm the occurrence of hybridisation conclusively (Rhymer & Simberloff, 1996; Wilson & Bernatchez, 1998). In addition, this result reiterates that, based upon this study, morphology may only be suggestive of hybridisation but not necessarily the lack thereof.

The apparent absence of introgressive hybridisation in populations from the Free State is, perhaps, not unexpected. This is because the morphological characteristics that defined individuals from this area as putative hybrids were much more subtle (i.e. traces of yellow on legs and one or more white claws on toes) than those apparent in intermediate birds from KwaZulu-Natal. In addition, no individuals described as pure domestic in morphology were identified in the populations from the Free State. The occurrence of discoloration on the feet may, therefore, be explained by natural local variation (e.g. private alleles, local inbreeding effects, etc.), and were not phenotypic expressions of introgression, rather a population-specific variation.

5.4.2. Conservation implications

Potential hybridisation in wild guineafowl populations should be taken into account in the implementation of effective management strategies. Feral guineafowl populations on the island of Nantucket, off the coast of Massachusetts, experienced low breeding success through poor incubation and rearing by a population of domesticated descent (Crowe, 1970). The identification of populations with hybrids is therefore essential if conservation efforts are to be targeted correctly, since the efficiency of reproduction in, and overall fitness of introgressed populations may be relatively low. The diagnostic restriction fragment pattern shown in this study is an easy and rapid genetic test in this identification process. The potential implications of hybridisation between wild and domestic for the conservation of guineafowl are multifaceted:

i) The mixing of gene pools and the loss of genotypically distinct populations, may jeopardise the genetic integrity of the species (Rhymer & Simberloff, 1996). An important role of biological conservation is to safeguard against the loss of genetic purity and locally adapted genetic variation, regardless of the taxonomic status, to maintain levels of biological diversity.

ii) Domesticated animals have been selected for survival and reproduction in captivity, and not under wild conditions. These individuals may have characteristics that are maladaptive in the wild and, in turn, may reduce the viability of populations in natural systems (Wolff & Milstein, 1987; Rhymer & Simberloff, 1996). Furthermore, reduced viability of hybrid individuals might exacerbate a decline in wild populations, or perhaps retard recovery.

iii) The maintenance of genetically wild populations is important for the sustainable management of this economically important species (Largiadèr & Scholl, 1996). The implications of translocating or re-stocking populations with individuals with a hybrid ancestry would be counter-productive to the long-term success of populations. Indeed, the objective of the study by Rossouw (1996) was to determine the genetically most suitable donor populations for a conservation programme to restore guineafowl populations in depleted areas within KwaZulu-Natal.

For the Helmeted Guineafowl, its economic value as a gamebird underpinning a local hunting industry might become undermined by the potentially detrimental impacts of hybridisation. However, the fact that only one ‘wild-type’ individual tested positive for DNA from domestic guineafowl suggests that the effects of morphologically undetectable introgression from domestic birds into wild populations may be relatively minor. Further research into this problem should focus on the breeding success of feral, genetically introgressed individuals that resemble wild birds to assess the viability of hybrids which have back-crossed with wild birds. In the meantime, mitigation measures which involve the removal of morphologically intermediate birds from wild populations seem to be justified.

Finally, it is essential that issues pertaining to the damaging effects of such hybridisation be addressed for the implementation of effective conservation and management strategies. Therefore, the identification of hybridisation in natural populations is an increasingly important issue in conservation biology. Moreover, it is fundamental for the sustainable utilisation of economically important species, such as the guineafowl, that the genetic integrity of wild populations is maintained (Largiadèr & Scholl 1996).

CHAPTER 6

HABITAT UTILISATION

Status as of 18/9/2000: in press. Habitat utilisation and home range size of Helmeted Guineafowl *Numida meleagris* in the Midlands of KwaZulu-Natal province, South Africa. *Biological Conservation*. Co-author: T.M. Crowe.

SUMMARY

Data were collected on home-range size and compositional habitat use by 15 radio-tracked helmeted guineafowl (*Numida meleagris*) representing stable, declining and near extinct populations during the non-breeding, winter months (April to September) of 1997. This was done to identify environmental factors that may be responsible for declines of populations of this species within the KwaZulu-Natal Midlands over the past two decades. Mean home range size increased as one moved from stable (11.4 ha) through to near extinct (252.7 ha) populations. Habitat-use by Helmeted Guineafowl appeared to be strongly influenced by diet, with small maize fields, waste grain and fallow lands forming important components. Grassland habitats were avoided. Thriving populations were associated with fragmented habitats providing a mosaic of resources in a relatively small area, whereas declining and near extinct populations were more dispersed within areas of extensive grazing or grassland habitats.

6.1. INTRODUCTION

The fragmentation of natural habitats through agricultural practices can negatively affect the viability of avian populations (Lauga and Joachim, 1992). Sub-division and loss of suitable habitat pushes populations into a range size where stochastic events are likely to lead to their extinction (Gilpin and Soule`, 1986). The consequences of habitat fragmentation have thus become a key issue in conservation biology (Soulé, 1986). There are, however, many species that have expanded their range by exploiting moderately fragmented habitats. The fragmentation of habitats has impacted positively on bird species that require a range of habitats, which are heterogenous in nature (Malan and Benn, 1999).

Gamebirds have been managed and harvested in these modified habitats for hundreds of years, yet recent changes in farming techniques and agricultural crops, have had severe negative impacts on numerous species (Potts, 1980; Rands et al., 1988; Jansen et al., in press). In South Africa, the increase in agriculture, especially low intensity crop farming, has promoted the range expansion of Helmeted Guineafowl *Numida meleagris* by providing key resources such as food, water, cover, and roosting sites in fragmented habitats (Crowe et al., 1986).

The decline in guineafowl within KwaZulu-Natal resulted in the fragmentation of populations, the status of which varies from stable, declining to near extinct as identified by Pero & Crowe (1996). This chapter investigates the home range size and habitat preferences of Helmeted Guineafowl at these different population levels, and thus gain insight into landscape changes that may have brought about their collapse.

6.2. METHODS

6.2.1. Study area

Four districts were identified as representing Helmeted Guineafowl populations of varying status - stable, declining and near extinct (Fig.6.1) (Pero & Crowe, 1996). Stable populations occurred in a Mixed Woodland biome and incorporated three flocks from a farm in the Ladysmith (29°48'E; 28°30'S) district, and two flocks from a farm in the neighbouring Elandslaagte (29°57'E; 28°18'S). The principal farming practices are stock farming, interspersed with a mosaic of crop (maize

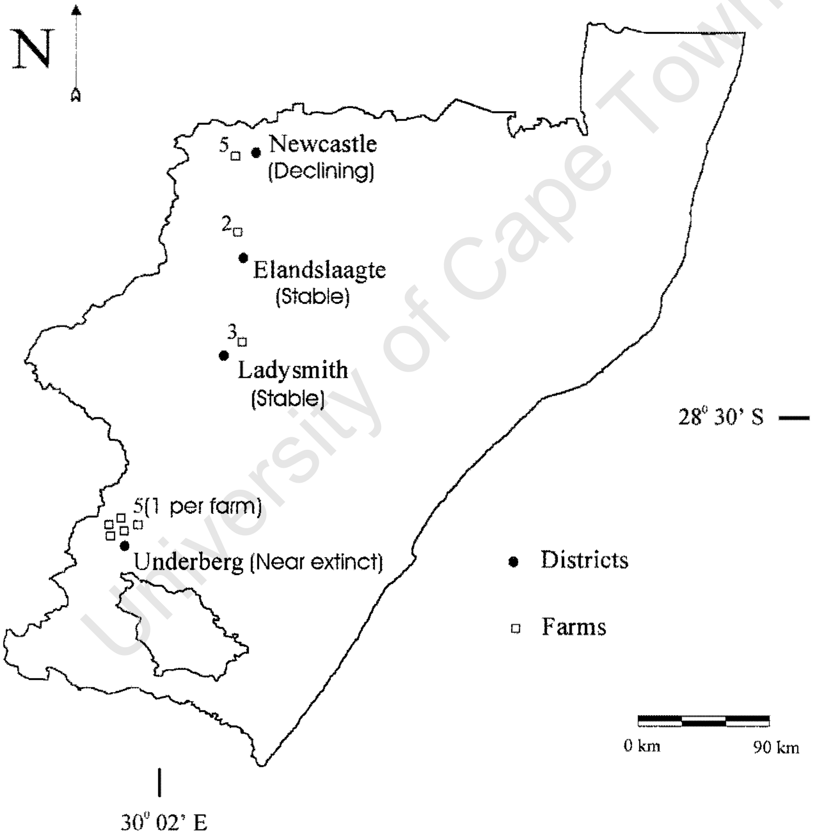

Fig. 6.1. Location of stable, declining and near extinct populations within the study area.

and sorghum) and fodder (lucern) lands, some of which are fallow. No pesticides are used on either of the two farms.

Declining populations consisted of five flocks located along the interface between the Chelmsford Nature Reserve, and a neighboring farm, in the northen district of Newcastle (29°51'E; 27°57'S). The reserve is characterised by tall, moist grassveld (mostly *Hyparrhenia hirta* and *Themeda triandra*) with scattered pockets of alien Black Wattle (*Acacia mearnsii*) trees. The neighbouring farm is predominantly crop agriculture (soya beans with small patches of maize), with intensive use of pesticides.

Near extinct populations were located in the sourveld grasslands of the Underberg (29°33'E; 29°42' S) district. Here, five flocks were radio-tracked from five bordering farms. Stock farming predominates along with commercial grasses (*Lolium perenne* and *Eragrostris* spp.) and cultivated crops such as maize and vegetables (Pero & Crowe, 1996). Pesticides are used extensively and pastures are burnt on a biennial basis.

6.2.2. Population size

During the non-breeding winter months, Helmeted Guineafowl form relatively stable flocks of 15-40 birds, with much larger aggregations being associated with numerous flocks converging on some superabundant resource (Crowe, 2000a.). This behaviour resulted in estimates being made, to the closest 10 individuals, for the various radio-tracked flocks. Counts of the 15 flocks were done in the late afternoon at respective roosting sites. This was extended over the three-month period from June to August.

6.2.3. Radio-telemetry

Fifteen adult Helmeted Guineafowl were trapped using walk-in funnel traps baited with commercial poultry food. Necklace radio-collar transmitters (Bootjack Co. Ltd, Kenward, 1987) were fitted, weighing 2.9 g, with a battery life of up to one month and signal transmission of up to 1200 m. Location fixes were taken every 2 hours, from before sunrise until after sunset, using a Yaesu FT-290R II all mode VHF multi-purpose transceiver and a five-element Yagi antenna, over a period of 8 days for each site. Directional radio fixes were obtained by three-point triangulation and plotted onto 1:10 000 orthophoto maps. The home-range study was conducted over the three winter months - June to August of 1997 - when birds had aggregated into flocks. Trapping during the breeding period was not considered due to it being a sensitive period involving paired individuals that are difficult to locate. In winter, the individual collared birds were representative of the movements and activities of the entire flock, and thus the data reflects a larger portion of the respective populations.

6.2.4. Home ranges

The Range IV software program (Kenward, 1990) was used to analyse the radio location data. Three types of home ranges were calculated for each population: (i) the minimum convex polygon (MCP), or so-called minimum area polygon (Mohr, 1947) enclosing 95% of radio locations, (ii) the harmonic-mean area using isopleths limited by the isoline which enclose 95% of radio locations (Dixon & Chapman, 1980), and, (iii) the Kernel analysis home range with the same 95% isoline (Worton, 1989). In addition, data on both the home range width and maximum distance from the roost was incorporated.

6.2.5. Habitat use

We followed Aebischer et al. (1993) to compare habitat use to habitat availability within the home range of the individual, as defined by the Minimum Convex Polygon (MCP). Assuming that use is non-random, habitats can be ranked according to relative use, and significant between-rank differences located. Proportional habitat use was calculated based on % radio-locations vs. home range composition as defined by the MCP. Non-utilised but available habitats were replaced by 0.1 % - a figure less than the smallest recorded non-zero percentage.

Available habitat within the MCP home range was calculated by superimposing a scaled 50 x 50 m grid on a 1:10 000 orthophoto, and counting the grid cells that fall within each of the habitat types of the home range. The proportions of radio-locations for each animal in each habitat type (y) and available habitat (yo = MCP home range) were transformed to log-ratios using the proportion of grassland (for declining and near extinction populations) and mixed woodland (for stable population) as the denominator, and then calculated the difference ($d = y - yo$). In this case, the log-ratio transformation of percentage radio locations would be:

$$yi = In\ (xi/xj)$$

where, xi/xj = percentage radio location of bird x in habitat i / percentage of grass (denominator) available.

Hypothesis testing relies on a generalised likelihood ratio statistic Λ that tests simultaneously over all habitat types for random habitat use (Jansen et al., in press). The residual matrix R2 is the matrix of raw sums of squares and cross products calculated from d; R1 is the matrix of mean-corrected sums of squares and cross-products calculated from d then $\Lambda = \text{R1} / \text{R2}$. If the quantity $-N\ ln\ \Lambda$ yields $P < 0.05$, habitat use is significantly non-random when compared to χ^2. The habitat types are then ranked in order of use through setting up a matrix for each bird of the kind described in Aebischer et al. (1993, Table 3). Then, at each position in the matrix, the mean and standard error of the elements were calculated over all five birds in all three study sites. For each element, the ratio mean/standard error gives a *t*-value measuring departure from random use, thereby pinpointing where non-random use occurs. Each mean element in the matrix is replaced by its sign and counts of positive values in each row achieves a habitat rank.

6.2.6. Mosaic index

A habitat mosaic index (*i*) was calculated for each population to determine the relationship between habitat diversity and guineafowl population status. The number of habitats, total area covered by the home ranges, as well as the total amount of edge habitat within the home ranges (edge was defined as the area 2 m. on either side of the interface between an agricultural land, and the surrounding vegetation), were assessed for each population, and a mosaic index determined as follows:

$$i = (e_{total}/MCP_{total})(\text{\# of habitats within MCPs}) \times 100$$

where, e_{total} = the total edge habitat (ha) within the home ranges of the respective populations

MCP_{total} = the total area (ha) covered by the home ranges (as defined by the MCPs) of each population.

6.2.7. Data analyses

All statistical procedures were carried out using Statgraphics statistical graphics package (Anon, 1986). Mann-Whitney *U*-test was used to compare home ranges within populations, as well as between populations in respect of home range size, width and maximum distance moved from the roost. A *t*-Test was conducted for a comparison between populations of the mean distance moved from the roost. Finally, a Mann-Whitney *U*-test was again applied to determine significance between population density estimates.

6.3. RESULTS

6.3.1. Population size

Population estimates (Table 6.1) showed a significant decline in population size with a change in population status. Stable populations were significantly larger than both declining ($Z = 1.964$, $P = 0.0495$) and near extinct ($Z = 1.964$, $P = 0.0495$) populations, while declining populations were in turn significantly ($Z = 1.964$, $P = 0.0495$) larger than near extinct populations.

Table 6.1. Comparative populations densities of Helmeted Guineafowl based on winter counts of radio-tracked flocks.

Population[a]	# of birds			Mean	density/1000 ha
	June	July	August		
stable	546	570	579	565	0.565
declining	207	196	203	202	0.202
near extinct	129	135	120	128	0.128

[a] Each population is represented by a total estimation of 5 radio-tracked flocks.

6.3.2. Home range

The definition of home range was defined as the smallest area containing 95 % of the utilisation distribution (Seaman & Powel, 1996). Mean home ranges among the 15 studied populations of helmeted guineafowl ranged from 11.43 to 252.74 ha (Table 6.2). Comparisons between different home range estimates within a population, and between populations, are summarised in Table 6.3.

Table 6.2. Home-range (ha) analysis utilising 95 % radio locations (excluding 5% outliers) for Helmeted Guineafowl with varying population status.

Population status	Flock #	MCP[a] home range	Harmonic mean	Kernel analysis	Range[b] width (m)	Metres from roost	
						Mean	Maximum
Stable	1	38.56	28.78	10.49	877	224	521
	2	27.92	18.62	7.13	968	313	563
	3	26.5	19.84	10.29	753	232	525
	4	31.35	30.66	18.37	1040	390	760
	5	17.99	14.08	10.86	840	435	840
	mean	28.464	22.396	11.428	895.6	318.8	641.8
declining	6	113.9	86.24	54.58	1490	802	1235
	7	161.3	133.2	64.14	2049	558	1365
	8	116.7	92.23	57.97	1643	578	1474
	9	89.32	88.37	35.42	2309	1053	2088
	10	77.82	77.2	22.27	1376	629	1158
	mean	111.808	95.448	46.876	1773.4	724	1464
near extinct	11	474.9	381.4	173	4107	798	3157
	12	523.3	428.3	127.6	3746	1341	3159
	13	204.2	192.5	62.97	2350	1147	1798
	14	41.99	40.83	18.83	1393	510	1096
	15	19.32	19.32	11.82	770	394	770
	mean	252.742	212.47	78.844	2473.2	838	1996

[a] MCP = Minimum Convex Polygon
[b] calculated from MCP home-range

Table 6.3. Significant ($P < 0.05$) comparisons between different home range estimates within (a) and between (b) populations.

(a) Within populations:

	Kernel vs MCP	Kernel vs Harmonic
stable	< ($Z = 2.402$, $P = 0.009$)	< ($Z = 2.402$, $P = 0.016$)
declining	< ($Z = 2.611$, $P = 0.009$)	< ($Z = 2.611$, $P = 0.009$)
near extinct	*	*

(b) Between populations:

	Stable vs declining	Stable vs near extinct
MCP	< ($Z = -2.611$, $P = 0.009$)	*
Harmonic	< ($Z = -2.611$, $P = 0.009$)	< ($Z = -1.98$, $P = 0.047$)
Kernel	< ($\mathrm{Z} = -2.611$, $\mathrm{P} = 0.009$)	< ($Z = -2.402$, $P = 0.016$)

< = significantly smaller .
* = did not differ significantly.

Home range width was significantly smaller in stable populations than declining populations ($Z = -2.611$, $P = 0.009$). Near extinct populations were not significant in this regard, possibly due to the comparatively narrow home ranges of flocks 14 and 15 (Table 6.2). The maximum and mean distance moved from the roost was also significantly larger in both declining (maximum: $Z = -2.611$, $P = 0.009$; mean: $t = -3.98$, $P = 0.004$) and near extinct (maximum: $Z = -2.402$, $P = 0.016$; mean: $t = -2.795$, $P = 0.023$) populations when compared to those distances in a stable population scenario. Finally, stable populations had a high degree of range overlap with large aggregations associated around a common food source (Fig. 6.2). This overlap in home ranges was reduced in declining populations and did not occur in near extinct populations (Fig. 6.3).

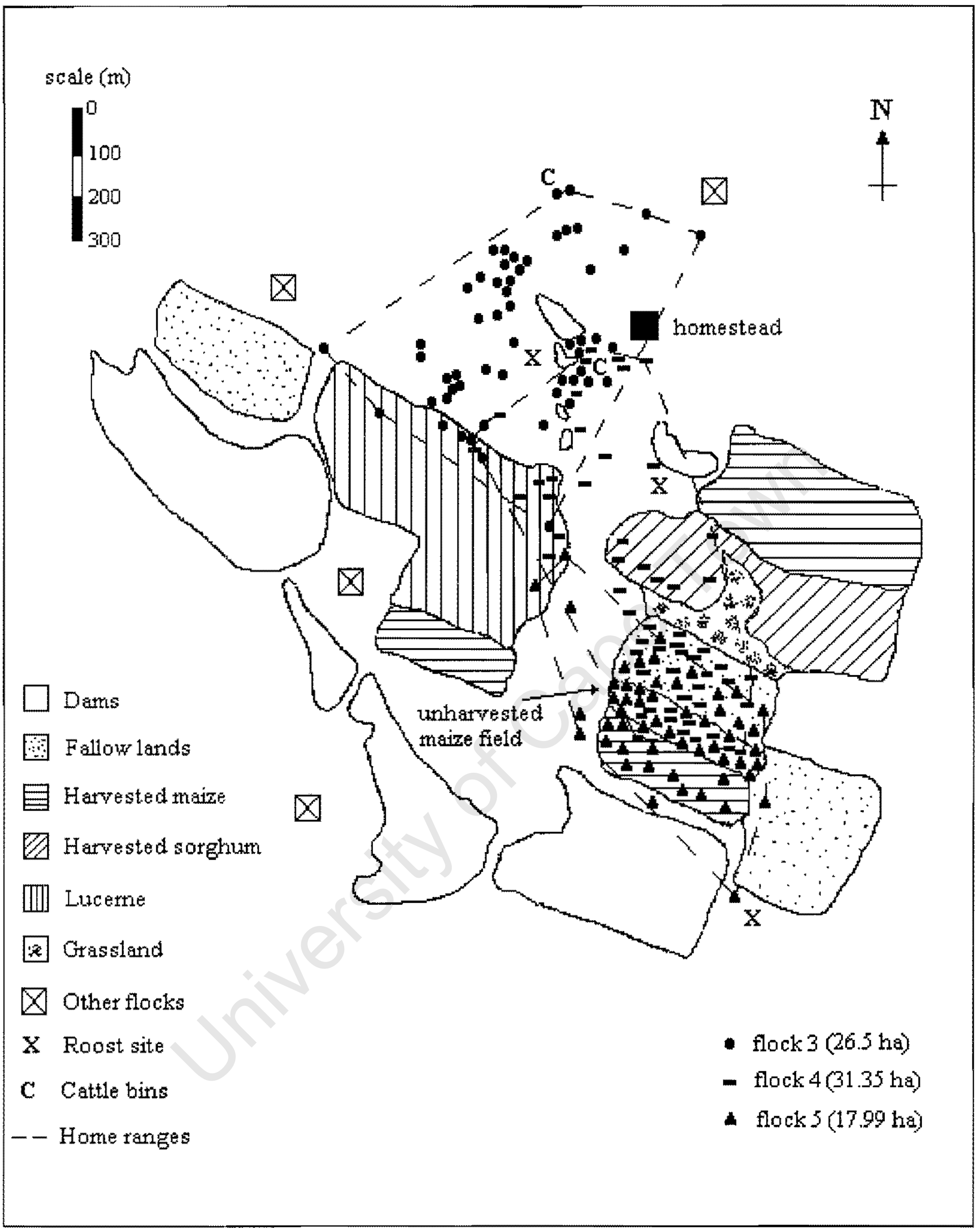

Fig. 6.2. Habitat map and location fixes of three flocks representing the home ranges of stable Helmeted Guineafowl populations. The clear areas are Mixed Woodland habitat.

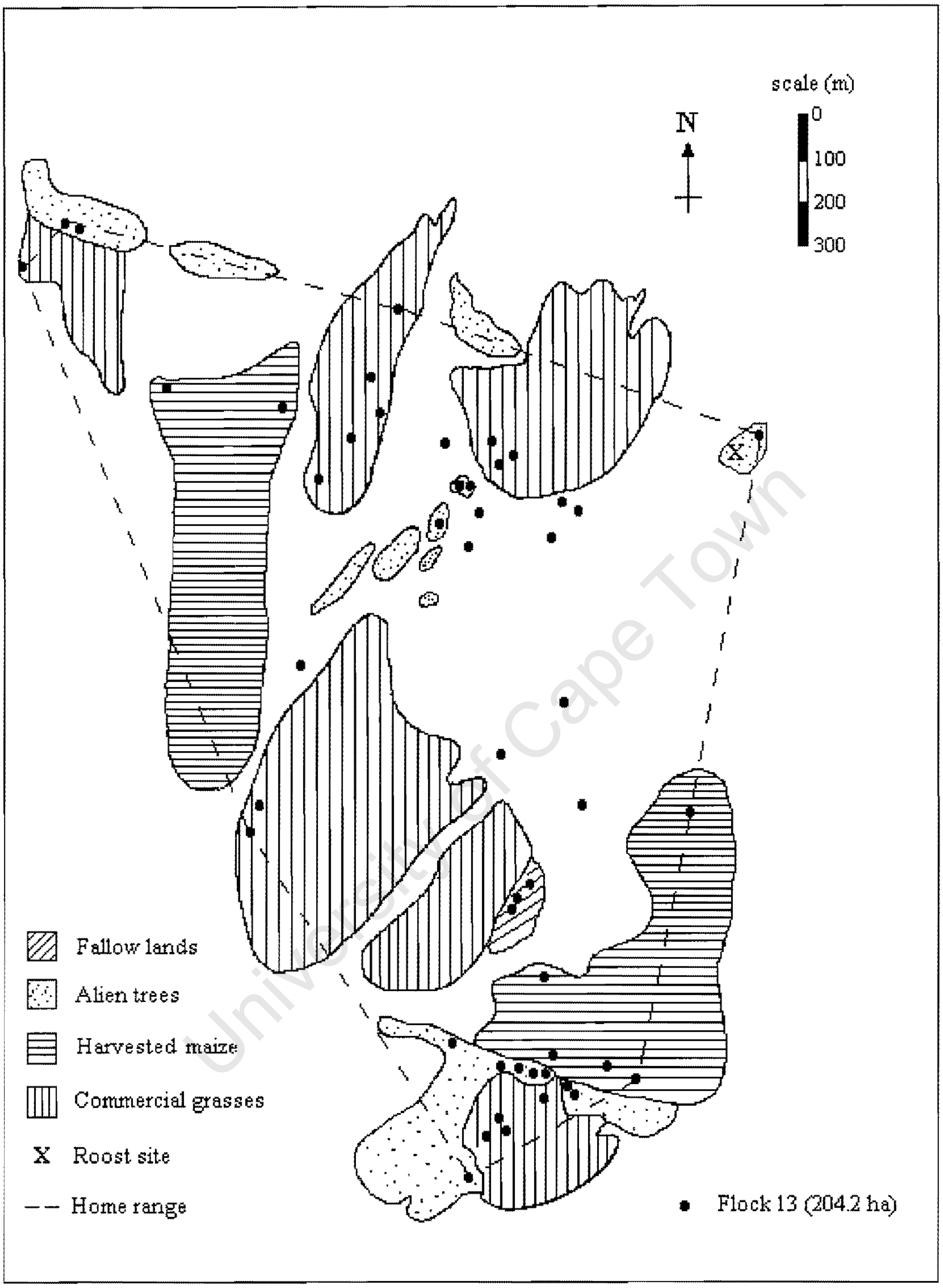

Fig. 6.3. Habitat map and location fixes of a flock representing the home range of near extinct Helmeted Guineafowl populations. The clear areas are grassland habitat.

6.3.3. Habitat use

The percentage radio locations and percentage habitat availability (Table 6.4) were used to calculate log-ratios (y & y_0) and difference in log-ratios (d) in Appendix 6.1. The difference in log-ratios was more significant in stable ($\Lambda = 0.0412$, $\chi^2 = 16$, $P < 0.01$) than declining ($\Lambda = 0.1602$, $\chi^2 = 9.157$, $P < 0.05$) and near extinct ($\Lambda = 0.0668$, $\chi^2 = 13.534$, $P < 0.025$) populations, suggesting non-random habitat use for all three scenarios.

The calculations presented in Appendix 6.2 (tables 1, 2 & 3) were used to construct a ranking matrix for habitat preferences of Helmeted Guineafowl for all three population scenarios. A simplified ranking matrix (Table 6.5) ranked habitat preference for stable populations as follows: grains > aliens > commercial grasses > fallow = other > mixed woodland > grassland. Declining populations were ranked: fallow > alien > grains > burnt grassland > grassland, whereas near extinct populations, the order was: fallow = other > aliens > commercial grasses > burnt grassland > grassland > grains.

6.3.4. Mosaic index

The habitat mosaic index declined with populations status. The highest index was that of stable populations ($i = 11.2$), followed by declining ($i = 3.15$) and near extinct populations ($i = 3.01$).

6.4. DISCUSSION

Both MCP and Harmonic mean home ranges were consistently larger than Kernel estimates, thus implying that Kernel home range estimates underestimate the true home range of the individual. The overall mean home range for Helmeted Guineafowl in the Midlands was 1.31 ± 1.6 km^2 (the high standard of deviation being attributable to the incorporation of three different population

Table 6.4. Percentage radio locations (a) and percentage habitat availability within MCP (b) of three Helmeted Guineafowl populations.

(a)

			% radio locations							
population status	bird number	number fixes	Mixed Woodland	Grassland	Burnt grassland	Commercial grasses	Grain crops	Fallow lands	Alien tree spp.	Other
stable	1	52	9.6	3.8				67.3	1.9	17.3
	2	55	3.6					96.4	0.1	
	3	55	63.6			10.9				25.5
	4	57	14	0.1		12.3	31.6	33.3		8.8
	5	55	9.1			3.6	61.8	25.5		0.1
declining	6	51		27.5	35.2		11.7	21.6	4	
	7	52		34.6	19.2		32.7	9.7	3.8	
	8	50		14	10		46		30	
	9	49		8.2	16.3		30.6	36.7	8.2	
	10	49		20.4	0.1		57.1	8.2	14.3	
near extinction	11	51		4	25.4	60.8	2	2	3.8	2
	12	51		2	37.3	49	0.1		7.8	3.9
	13	49		20.4		30.6	14.3	6.1	28.6	
	14	49		24.5		55.1	16.3		4.1	
	15	49		16.3		79.6			4.1	

(b)

		% habitat availability							
stable	1	13.3	27.2				57.6	1.3	0.6
	2	9.6					89.5	0.9	
	3	56			11.2				32.8
	4	39	11.4		9.8	22.8	14.6		2.4
	5	27.4			1.4	39.7	26		5.5
declining	6		66.7	21.9		1.4	7.4	2.6	
	7		67.6	10.9		14.4	4.2	2.9	
	8		71.9	3.3		22.3		2.5	
	9		72.3	9.9		13.3	3.2	1.3	
	10		48.7	9.2		37	3.5	1.6	
near extinction	11		13.7	47	23.7	10.7	1.2	3.4	0.3
	12		3	52	38.7	4		1.4	0.9
	13		54.2		26.5	14.9	1	3.4	
	14		39		34.9	18		8.1	
	15		26.9		69.3			3.8	

Table 6.5. Ranking matrices for stable (a), declining (b) and near extinct (c) Helmeted Guineafowl populations based on comparing percentage habitat availability within the MCP home range, with the percentage radio locations (data from table 6.4). Each mean element in the matrix was replaced by its sign; triple sign indicates significant deviation from random at $P < 0.05$ (from Appendix 6.2., tables 1c,2c & 3c).

(a) Stable population

	Mixed Woodland	Grassland	Commercial grasses	Grains	Fallow lands	Alien tree spp.	Other	Rank
Mix.W	*	+	---	---	-	---	-	1
Grass	-	*	-	-	-	---	-	0
Comm.gr.	+++	+	*	-	+++		-	3
Grains	+++	+	+	*	+++		+	5
Fallow	+	+	---	---	*			2
Alien	+++	+++			+	*	+++	4
Other	+	+	-	-	-	---	*	2

(b) Declining population

	Grassland	Burnt grassland	Grains	Fallow lands	Alien tree spp.	Rank
Grass	*	-	---	---	---	0
Burnt gr.	+	*	-	-	-	1
Grains	+++	+	*	-	-	2
Fallow	+++	+	+	*	+	4
Alien	+++	+	+	-	*	3

(c) Near extinct population

	Grassland	Burnt grassland	Commercial grasses	Grains	Fallow lands	Alien tree spp.	Other	Rank
Grass	*	-	-	+	---	-	---	1
Burnt.gr.	+	*	-	+	---	---	---	2
Comm.gr.	+	+	*	+	---	-	---	3
Grains	-	-	-	*	---	-	---	0
Fallow	+++	+++	+++	+++	*	+++		5
Alien	+	+++	+	+	---	*	---	4
Other	+++	+++	+++	+++		+++	*	5

scenarios). Previous home range studies of this species in South Africa show estimates of 8.8 ± 6.3 km^2 (Crowe, 1978) from the Northern Cape and 6.04 ± 1.68 km^2 (Winterbach, 1991) from the North West province, whereas in the Kainji Lake Basin area in Nigeria, the average home range was estimated at 8.75 ± 6.32 km^2 (Ayeni, 1983a; 1984). Helmeted Guineafowl thrive in moderately fragmented habitats (Malan & Benn, 1999), and thus these larger home ranges are possibly due to these studies occurring in more arid, less fragmented regions resulting in greater distances between certain key resources such as food, water and roosting sites.

Rolstad (1991) notes that habitat fragmentation not only reduces suitable habitat, but limits dispersal through increased distances between these habitats and subsequently triggering a range retraction. Furthermore, unsuitable bordering landscapes may in turn increase adverse factors, such as predation, thus further contributing to a range reduction. In gamebirds, this pattern has been recorded in both Bobwhite Quail (*Colinus virginianus*) (Rosene, 1969), as well as Ringnecked Pheasant (*Phasianus colchicus*) (Lachlan & Bray, 1976). Closer to home, home ranges in species such as Redwing Francolin (*Francolinus levaillantii*), are similarly affected (Jansen et al., in press) however, a reduction in home range is not always the case. Home ranges in the Cape Spurfowl (*Pternistis capensis*) were found to increase as habitat quality deteriorated in deciduous fruit farming areas of the south Western Cape (Little & Crowe, 1998).

In this study, home range size (Fig. 6.4) and width, as well as the mean and maximum distance from the roost (Table 6.2), increased along a declining population gradient. Furthermore, there is overlap of home ranges in stable populations, but this phenomenon decreases and eventually ceased as one moves through declining to near extinct populations (Figs 6.2 & 6.3).

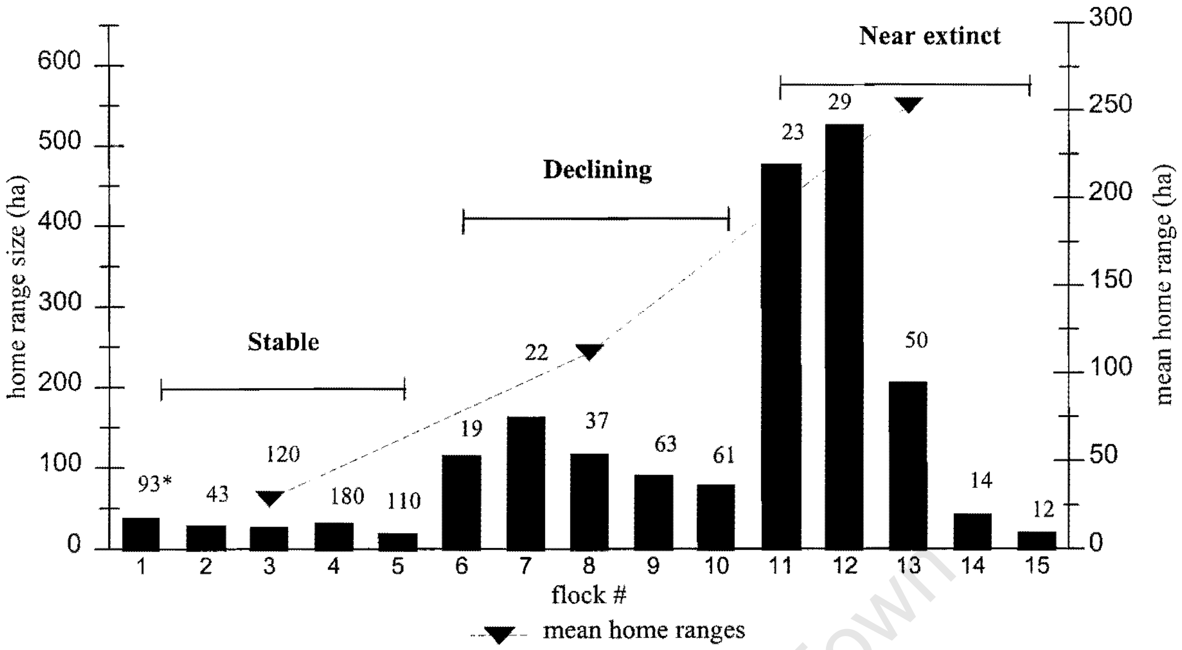

Fig. 6.4. Individual home ranges and mean home ranges of stable, declining and near extinct populations (* = flock size).

A situation in which there are small, well-spaced flocks holding large home ranges suggests that birds are having to travel great distances between key fragmented resources. In localised, high quality habitats with stable populations, large aggregations were often associated around a common food source resulting in small, overlapping home ranges (Fig. 6.2). Stable, resident flocks tolerate each other at such gatherings, reserving aggressive behaviour for when resources become limited, or when a 'floating' flock invade their territory (Crowe, 2000a). In declining populations, home ranges also overlapped, and there was greater aggression between flocks (unpubl. pers. obs.) suggesting that resources were limited. Flocks in near extinct populations were mostly too isolated for any interaction to take place (Fig. 6.3). Birds covered large distances between various resource foci, and consequently had larger home ranges with less overlap. The lack of strong territoriality between flocks in stable populations suggests that territorial boundaries are set more by the availability of important resources (food, water, cover, etc.), and less by the proximity of neighbouring flocks.

The increase in home range size with deterioration in habitat quality is supported by Ayeni (1984) who found that guineafowl home ranges in Nigeria varied from 0.79 - 1.77 km^2 in the Kainji Lake National Park, to 7.55 - 21.24 km^2 in adjacent farmlands. There was loss of habitat through extensive burning in the farmlands, while fire management in the reserve through block burning created greater heterogeneity of habitats for guineafowl, and thus greater population densities through smaller home ranges.

Flocks 14 and 15, with small home ranges of 41.99 and 19.32 ha respectively (Fig. 6.4), differ from other near extinct flocks in both habitat and cover. Their habitats are characterised by rye-grass and intensively grazed grassland pasture which is burnt extensively. Winter food may thus be limiting (little waste grain available) (unpubl. pers. obs.), whereas the intensive grazing and burning of grassland pasture may impact on cover for summer food and nesting sites. Both flocks having declined markedly over the last three seasons from c 30, to 14 and 12 individuals respectively (T. Turton, pers. comm.), and thus these flocks are possibly near extinction. Furthermore, with such a drastic decline in flock size, these flocks may well be socially dysfunctional and therefore unable to successfully defend a territory, thus further restricting their range.

Non-random habitat utilisation occurred in all populations and reflected important components in the winter diet of Helmeted Guineafowl. More than 80% of the guineafowl's diet can be attributed to agricultural crops (Grafton, 1971; Mentis et al., 1975) with the main components including arthropods, many of which are agricultural pests (Skead, 1962; Ayeni, 1983b; Little et al., 1995; Witt et al., 1995), weeds and tubers of cultivated areas, pasture greens and agricultural waste grain (Grafton, 1971; Mentis et al., 1975). During the winter months, grains, weeds and underground tubers form the most important components of their diet (Grafton, 1971; Mentis et al., 1975). In all three population categories, either grain crops, fallow lands or areas with waste maize grain from cattle bins and stock pens, were preferred habitats, thus reflecting the winter diet. This was especially highlighted in stable populations (Fig. 6.2) with large concentrations of birds occurring around cattle bins, fallow lands and partially harvested maize fields.

The habitat mosaic index suggests that thriving guineafowl populations are associated with extensive edge habitats with a variety of food sources - fallow lands, grains and winter fodder - in close proximity to one another. The preference for habitat compartmentalisation is supported by the utilisation of smaller croplands (Fig. 6.2) rather than large extensive crops, which are more dispersed (Fig. 6.3). There is little difference in the mosaic index between farms with declining and near extinct populations, but we recognise that the mosaic index utilised in this study does not take cognisance of the distribution of vital resources within the home range, as well as habitat structure (e.g. quality of edge habitats) and composition. By example, the presence of the neighbouring Chelmsford Reserve in declining populations may thus give the competitive edge (cover and nesting sites) over near extinct populations, a factor which is not accounted for in this index..

There is a trend to more random habitat selection with a decline in population status i.e. habitat preference becomes less significant. This trend may be a result of birds having to travel larger distances between resources, and thus being recorded in less suitable habitats while in transit (Fig. 6.3). This is illustrated by the low habitat ranking for grassland habitats. Birds were rarely observed utilising this habitat for any purpose other than in transit. The large movement between roosting sites in the Chelmsford Reserve (predominantly grassland) and neighbouring farmland in declining populations supports this hypothesis. The range expansion of Helmeted Guineafowl into the grassland biome has mostly come about through the advent of agriculture and forced introductions (Little, 1997), but traditionally, Helmeted Guineafowl is a bird of savannas (Crowe et al., 1986). Although guineafowl will utilise grasslands during summer months for food, cover and nesting sites, this habitat is avoided during winter as food is limiting, while summer grazing and winter burning programmes reduce cover extensively in many areas (Malan, 1998).

6.5. CONCLUSIONS AND CONSERVATION IMPLICATIONS

This study supports the concept that Helmeted Guineafowl thrive in moderately fragmented habitats. As this optimum habitat becomes increasingly fragmented, home ranges increase and populations decline. Habitat selection appears to be driven by dietary constraints, whereas the avoidance of grassland vegetation types underlies the importance of farming activities (e.g. traditional contouring, small croplands, agricultural waste grain, etc.) in supporting Helmeted Guineafowl populations in a mostly grassland biome. Habitat distribution, not just composition, appears to be a key to healthy populations. Stable populations are associated with a habitat mosaic (small-scale, highly diverse habitat), whereas near extinct populations are characterised by large expanses of uniform habitat in between optimally fragmented habitats. As populations become isolated, they become increasingly vulnerable to a range of mortality factors that may lead to local extinction.

Resuscitating guineafowl populations to viable levels requires re-creating a habitat mosaic of interconnected habitats on a landscape scale. Conservation measures suggested by this study include the preservation of edge habitats, the use of small (10 - 20 ha) croplands rather than large monocultures, and the availability of waste grain material during winter. It is also suggested that a minimum of three habitat types - ideally mixed woodland, crop lands (maize or lucern) and neighbouring fallow lands are required, along with suitable water and roosting sites, in order to sustain a flock.

Appendix 6.1. Log-ratios and difference in log-ratios calculated from the data in Table 6.4 for comparing habitat based on percentage radio location distribution and habitat availability within the MCP home range.

		Log-ratios of radio locations (y)						log-ratios of available habitat within MCP (y_0)					
Population	bird number	Grass/ Mix.W	Comm.gr./ Mix.W	Grain/ Mix.W	Fallow/ Mix.W	Alien/ Mix.W	Other/ Mix.W	Grass/ Mix.W	Comm.gr./ Mix.W	Grain/ Mix.W	Fallow/ Mix.W	Alien/ Mix.W	Other/ Mix.W
stable	1	-0.927	0	0	1.947	-1.62	0.589	-1.095	-1.853	-1.095	0.302	-4.05	-1.241
	2	0	0	0	3.288	-3.584	0						
	3	0	-1.764	0	0	0	-0.914						
	4	-4.942	-0.129	0.814	0.867	0	-0.464						
	5	0	-0.927	1.916	1.03	0	-4.511						

	Difference in log-ratios ($d = y - y_0$)[a]					
1	0.168	1.853	1.095	1.645	2.43	1.83
2	1.095	1.853	1.095	2.986	0.466	1.241
3	1.095	0.089	1.095	-0.302	4.05	0.327
4	-3.847	1.724	1.909	0.565	4.05	0.777
5	1.095	0.926	3.011	0.728	4.05	-3.27

		Log-ratios of radio locations (y)				log-ratios of available habitat within MCP (y_0)			
Population	bird number	Burnt.gr./ Grass	Grain/ Grass	Fallow/ Grass	Alien/ Grass	Burnt.gr./ Grass	Grain/ Grass	Fallow/ Grass	Alien/ Grass
declining	6	0.247	-0.855	-0.241	-1.928	-1.753	-1.429	-2.835	-3.363
	7	-0.589	-0.056	-1.272	-2.209				
	8	-0.336	1.19	0	0.762				
	9	0.687	1.317	1.499	0				
	10	-5.318	1.029	-0.911	-0.355				

	Difference in log-ratios ($d = y - y_0$)[a]			
6	2	0.574	2.594	1.435
7	1.164	1.373	1.563	1.154
8	1.417	2.619	2.835	4.125
9	2.44	2.746	4.334	3.363
10	-3.565	2.458	1.924	3.008

Appendix 6.1. (continued).

		Log-ratios of radio locations (y)						log-ratios of available habitat within MCP (y_0)					
Population	bird number	Burnt.G/ Grass	Comm.gr./ Grass	Grain/ Grass	Fallow/ Grass	Alien/ Grass	Other/ Grass	Burnt.G/ Grass	Comm.gr./ Grass	Grain/ Grass	Fallow/ Grass	Alien/ Grass	Other/ Grass
near extinct	11	1.848	2.721	-0.693	-0.693	-0.051	-0.693	0.794	0.587	-0.67	-3.367	-1.827	-3.55
	12	2.926	3.199	-2.996		1.361	0.668						
	13	0	0.405	-0.355	-1.207	0.338	0						
	14	0	0.81	-0.408	0	-1.788	0						
	15	0	1.586	0	0	-1.38	0						

	Difference in log-ratios ($d = y - y_0$)[a]					
11	1.054	2.134	-0.023	2.674	1.776	2.857
12	2.132	2.612	-2.326	3.367	3.188	4.218
13	-0.794	-0.182	0.315	2.16	2.165	3.55
14	-0.794	0.223	0.262	3.367	0.039	3.55
15	-0.794	0.999	0.67	3.367	0.447	3.55

[a] Significance test for nonrandom habitat use:
stable: $\Lambda = 0.0412$, $\chi^2 = 16$, $P < 0.01$
declining: $\Lambda = 0.1602$, $\chi^2 = 9.157$, $P < 0.05$
near extinct: $\Lambda = 0.0668$, $\chi^2 = 13.534$, $P < 0.025$

Appendix 6.2.

Table 1. Stable population ranking matrix (a) calculated from percentage radio locations & percentage habitat availability, mean ± SE calculated for all flocks (b), and t-values (c) calculated by dividing the mean by SE.

	Bird #		Mix.W	Grass	Comm.gr.	Grain	Fallow	Alien	Other
(a)	1	Mix.W	*	-0.168	-1.831	-1.095	-1.645	-2.43	-1.83
		Grass	0.168	*	-0.736	0	-1.477	-2.262	-1.662
		Comm.gr.	1.831	0.736	*	0.736	2.132	-2.219	0.59
		Grain	1.095	0	-0.736	*	1.397	-2.955	-0.146
		Fallow	1.645	1.477	-2.132	-1.397	*	-0.785	-0.184
		Alien	2.43	2.262	2.219	2.955	0.785	*	0.6
		Other	1.83	1.662	-0.59	0.146	0.184	-0.6	*
	2	Mix.W	*	-1.095	-1.831	-1.095	-2.986	-0.466	-1.241
		Grass	1.095	*	-0.736	0	1.397	-2.955	-0.146
		Comm.gr.	1.831	0.736	*	0.736	2.132	-2.219	0.59
		Grain	1.095	0	-0.736	*	1.397	-2.955	-0.146
		Fallow	2.986	1.397	-2.132	-1.397	*	2.519	-1.542
		Alien	0.466	2.955	2.219	2.955	-2.519	*	2.809
		Other	1.241	0.146	-0.59	0.146	1.542	-2.809	*
	3	Mix.W	*	-1.095	-0.067	-1.095	0.302	-4.05	-0.327
		Grass	1.095	*	-0.736	0	1.397	-2.955	-0.146
		Comm.gr.	0.067	0.736	*	0.736	2.132	-2.219	1.504
		Grain	1.095	0	-0.736	*	1.397	-2.955	-0.146
		Fallow	-0.302	-1.397	-2.132	-1.397	*	-4.352	-1.542
		Alien	4.05	2.955	2.219	2.955	4.352	*	2.809
		Other	0.327	0.146	-1.504	0.146	1.542	-2.809	*
	4	Mix.W	*	3.847	-1.702	-1.909	-1.169	-4.05	-0.777
		Grass	-3.847	*	-5.548	-5.756	-4.411	-2.955	-4.623
		Comm.gr.	1.702	5.548	*	-0.208	1.136	-2.219	0.925
		Grain	1.909	5.756	0.208	*	1.345	-2.955	1.132
		Fallow	1.169	4.411	-1.136	-1.345	*	-4.352	-0.211
		Alien	4.05	2.955	2.219	2.955	4.352	*	2.809
		Other	0.777	4.623	-0.925	-1.132	0.211	-2.809	*
	5	Mix.W	*	-1.095	-0.904	-3.012	-0.728	-4.05	3.27
		Grass	1.095	*	-0.736	0	1.397	-2.955	-0.146
		Comm.gr.	0.904	0.736	*	-2.107	0.174	-2.219	4.174
		Grain	3.012	0	2.107	*	2.282	-2.955	6.28
		Fallow	0.728	-1.397	-0.174	-2.282	*	-4.352	3.999
		Alien	4.05	2.955	2.219	2.955	4.352	*	2.809
		Other	-3.27	0.146	-4.174	-6.28	-3.999	-2.809	*
(b) Mean±SE									
		Mix.W	*	0.79±0.959	-1.267±0.346	-1.641±0.377	-1.245±0.541	-3.009±0.709	-0.181±0.898
		Grass	-0.79±0.959	*	-1.698±0.962	-1.151±1.151	-0.339±1.16	-2.816±0.139	-1.345±0.871
		Comm.gr.	1.267±0.346	1.698±0.962	*	-0.021±0.553	1.541±0.392	-2.219±0	1.557±0.675
		Grain	1.641±0.377	1.151±1.151	0.021±0.553	*	1.564±0.402	-2.955±0	1.395±2.786
		Fallow	1.245±0.541	0.339±1.16	-1.541±0.392	-1.564±0.402	*	-2.264±1.381	0.104±1.019
		Alien	3.009±0.709	2.816±0.139	2.219±0	2.955±0	2.264±1.381	*	2.367±0.442
		Other	0.181±0.898	1.345±0.871	-1.557±0.675	-1.395±2.786	-0.104±1.019	-2.367±0.442	*
(c) *t*-value									
		Mix.W	*	0.824	-3.662	-4.353	-2.301	-4.244	-0.202
		Grass	-0.824	*	-1.765	-1	-0.292	-20.259	-1.544
		Comm.gr.	3.662	1.765	*	-0.038	3.931	0	2.307
		Grain	4.353	1	0.038	*	3.891	0	0.501
		Fallow	2.31	0.292	-3.931	-3.891	*	-1.639	0.102
		Alien	4.244	20.259	0	0	1.639	*	5.355
		Other	0.202	1.544	-2.307	-0.501	-0.102	-5.355	*

t - value at 95% significance for 4df = 2.776

Appendix 6.2.

Table 2. Declining population ranking matrix (a) calculated from percentage radio locations and percentage habitat availability, mean ± SE calculated for all flocks (b), and t-values (c) calculated by dividing the mean by SE.

	Bird #		Grassland	Burnt gr.	Grain	Fallow	Alien
(a)	5	Grassland	*	-2	-0.574	-2.594	-1.435
		Burnt gr.	2	*	1.425	-0.593	0.566
		Grain	0.574	-1.425	*	-2.018	-0.86
		Fallow	2.594	0.593	2.018	*	1.158
		Alien	1.435	-0.566	0.86	-1.158	*
	6	Grassland	*	-1.164	-1.373	-1.563	-1.154
		Burnt gr.	1.164	*	-0.208	-0.398	0.011
		Grain	1.373	0.208	*	-0.19	1.219
		Fallow	1.563	0.398	0.19	*	0.409
		Alien	1.154	-0.011	-1.219	-0.409	*
	10	Grassland	*	-1.417	-2.619	-2.835	-4.125
		Burnt gr.	1.417	*	-1.202	-1.081	-2.708
		Grain	2.619	1.202	*	-1.405	-1.506
		Fallow	2.835	1.081	1.405	*	-0.528
		Alien	4.125	2.708	1.506	0.528	*
	11	Grassland	*	-2.44	-2.746	-4.334	-3.363
		Burnt gr.	2.44	*	-0.306	-1.893	-0.922
		Grain	2.746	0.306	*	-1.587	-0.616
		Fallow	4.334	1.893	1.587	*	0.971
		Alien	3.363	0.922	0.616	-0.971	*
	12	Grassland	*	3.565	-2.458	-1.924	-3.008
		Burnt gr.	-3.565	*	-6.023	-5.488	-6.572
		Grain	2.458	6.023	*	0.536	-0.548
		Fallow	1.924	5.488	-0.536	*	-1.084
		Alien	3.008	6.572	0.548	1.084	*
(b)	Means+-SE						
		Grassland	*	-1.753±1.807	-1.954±0.422	-2.65±0.479	-2.617±0.571
		Burnt gr.	1.753±1.807	*	-1.263±1.263	-1.891±0.936	-1.925±1.288
		Grain	1.954±0.422	1.263±1.263	*	-0.933±0.476	-0.462±0.453
		Fallow	2.65±0.479	1.891±0.936	0.933±0.476	*	0.185±0.432
		Alien	2.617±0.571	1.925±1.288	0.462±0.453	-0.185±0.432	*
(c)	*t*-value						
		Grassland	*	-0.97	-4.63	-5.532	-4.583
		Burnt gr.	0.97	*	-1	-2.02	-1.495
		Grain	4.63	1	*	-1.96	-1.02
		Fallow	5.532	2.02	1.96	*	0.428
		Alien	4.583	1.495	1.02	-0.428	*

t - value at 95% significance for 4df = 2.776

Appendix 6.2.

Table 3. Near extinct population ranking matrix (a) calculated from percentage radio locations & percentage habitat availability, mean ± SE calculated for all flocks (b), and t-values (c) calculated by dividing the mean by SE.

	Bird #		Grass	Burnt.gr.	Comm.gr.	Grain	Fallow	Alien	Other
(a)	11	Grass	*	-1.054	-2.134	0.023	-2.674	-1.776	-2.857
		Burnt.gr.	1.054	*	-1.08	1.077	-1.619	-0.721	-1.802
		Comm.gr.	2.134	1.08	*	2.156	-0.54	0.359	-0.723
		Grain	-0.023	-1.077	-2.156	*	-2.697	-1.798	-2.879
		Fallow	2.674	1.619	0.54	2.697	*	0.898	-0.182
		Alien	1.776	0.721	-0.359	1.798	-0.898	*	-1.081
		Other	2.857	1.802	0.723	2.879	0.182	1.081	*
	12	Grass	*	-2.132	-2.612	2.326	-3.367	-3.188	-4.218
		Burnt.gr.	2.132	*	-0.48	4.457	-4.161	-1.056	-2.086
		Comm.gr.	2.612	0.48	*	4.936	-3.954	-0.576	-1.606
		Grain	-2.326	-4.457	-4.936	*	-2.697	-5.513	-6.543
		Fallow	3.367	4.161	3.954	2.697	*	1.54	-0.182
		Alien	3.188	1.056	0.576	5.513	-1.54	*	-1.03
		Other	4.218	2.086	1.606	6.543	0.182	-1.03	*
	13	Grass	*	0.794	0.182	-0.315	-2.16	-2.165	-3.55
		Burnt.gr.	-0.794	*	-0.207	-1.465	-4.161	-2.621	-4.344
		Comm.gr.	-0.182	0.207	*	-0.497	-2.341	-2.346	-4.137
		Grain	0.315	1.465	0.497	*	-1.845	-1.849	-2.879
		Fallow	2.16	4.161	2.341	1.845	*	1.54	-0.182
		Alien	2.165	2.621	2.346	1.849	0.005	*	-1.723
		Other	3.55	4.344	4.137	2.879	0.182	1.723	*
	14	Grass	*	0.794	-0.223	-0.262	-3.367	-0.039	-3.55
		Burnt.gr.	-0.794	*	-0.207	-1.465	-4.161	-2.621	-4.344
		Comm.gr.	0.223	0.207	*	-0.04	-3.954	0.184	-4.137
		Grain	0.262	1.465	0.04	*	-2.697	0.224	-2.879
		Fallow	3.367	4.161	3.954	2.697	*	1.54	-0.182
		Alien	0.039	2.621	-0.184	-0.224	-1.54	*	-1.723
		Other	3.55	4.344	4.137	2.879	0.182	1.723	*
	15	Grass	*	0.794	1	-0.67	-3.367	-0.447	-3.55
		Burnt.gr.	-0.794	*	-0.207	-1.465	-4.161	-2.621	-4.344
		Comm.gr.	-1	0.207	*	-1.258	-3.954	0.552	-4.137
		Grain	0.67	1.465	1.258	*	-2.697	-1.156	-2.879
		Fallow	3.367	4.161	3.954	2.697	*	1.54	-0.182
		Alien	0.447	2.621	-0.552	1.156	-1.54	*	-1.723
		Other	3.55	4.344	4.137	2.879	0.182	1.723	*
(b)	Means±SE								
		Grass	*	-0.161±0.609	-0.757±0.692	0.22±0.538	-2.987±0.246	-1.523±0.575	-3.545±0.215
		Burnt.gr.	0.161±0.609	*	-0.436±0.169	0.228±1.166	-3.653±0.508	-1.928±0.428	-3.384±0.59
		Comm.gr.	0.757±0.692	0.436±0.169	*	1.059±1.123	-2.949±0.678	-0.365±0.531	-2.948±0.741
		Grain	-0.22±0.538	-0.228±1.166	-1.059±1.123	*	-2.527±0.17	-2.018±0.95	-3.612±0.733
		Fallow	2.987±0.246	3.653±0.508	2.949±0.678	2.527±0.17	*	1.412±0.128	-0.182±0
		Alien	1.523±0.575	1.928±0.428	0.365±0.531	2.018±0.95	-1.412±0.128	*	-1.456±0.164
		Other	3.545±0.215	3.384±0.59	2.948±0.741	3.612±0.733	0.182±0	1.456±0.164	*
(c)	*t*-value								
		Grass	*	-0.264	-1.094	0.409	-12.142	-2.649	-16.488
		Burnt.gr.	0.264	*	-2.58	0.196	-7.191	-4.505	-5.736
		Comm.gr.	1.094	2.58	*	0.943	-4.35	-0.687	-3.978
		Grain	-0.409	-0.196	-0.943	*	-14.865	-2.124	-4.928
		Fallow	12.142	7.191	4.35	14.865	*	11.031	0
		Alien	2.649	4.505	0.687	2.124	-11.031	*	-8.878
		Other	16.488	5.736	3.978	4.928	0	8.878	*

t - value at 95% significance for 4df = 2.776

CHAPTER 7

DIGESTIBILITY STUDY

Status as of 18/9/2000: in review. The digestibility of raw and processed soybeans by Helmeted Guineafowl *Numida meleagris*. *Ostrich*. Co-authors: R.M. Gous, H.K. Swatson & T.M. Crowe.

SUMMARY

Helmeted Guineafowl *Numida meleagris* populations have declined significantly within the Midlands of KwaZulu-Natal since the early 1980s. Because guineafowl have been observed feeding on harvested soybean lands, and because raw soybeans are known to contain anti-nutritional factors, research into the digestibility of raw soybean beans was investigated as a possible localised factor in suppressing populations of these birds. A digestibility study on the Apparent Metabolizable Energy (AME) and the amino acids of both raw and processed soybeans, as well as a number of other feed ingredients, using Helmeted Guineafowl and adult roosters as a control, was conducted. The results of the energy balance studies were similar for both the guineafowl and the roosters. Soybean, both raw and processed, was found to be comparable with the other feed ingredients in terms of the digestibility of gross energy, but the amino acid digestibility of raw soybeans was considerably lower than that of processed soybean oilcake meal. Further research needs to be conducted on the potential nutritional constraints that raw soybean may have on guineafowl and other farmland birds.

7.1. INTRODUCTION

The Midlands of KwaZulu-Natal is farmed intensively and is characterised by a combination of stock and crop agriculture - predominantly maize and wheat (Malan, 1998), as well as soybeans. More than 80% of the diet of the guineafowl can, in one way or another, be attributed to agriculture (Grafton, 1971; Mentis et al., 1975) with the main components including arthropods, many of which are agricultural pests (Skead, 1962; Ayeni, 1983a; Little et al., 1995; Witt et al., 1995), weeds and tubers of cultivated areas, pasture greens and disused agricultural grain (Grafton, 1971; Mentis et al., 1975). The consumption of large quantities of agricultural grains in winter (Grafton, 1971; Mentis et al., 1975) underlines the importance of this food source during this critical period. In the Midlands, this source is in the form of maize, wheat and soybean.

Soybean protein is generally regarded as a high quality protein in animal nutrition, implying that the composition of soybeans is similar to the composition of the body protein being formed. However, this is only true when soybeans are supplemented with the amino acid methionine, which is available in a synthetic form, as this amino acid is the first-limiting amino acid in soybean protein, being present in low concentrations compared with the body protein being formed. For this reason, diets based on soybeans must be supplemented with a source of methionine. However, raw or uncooked soybeans are of a lower nutritional value than those that have been heat-treated. Raw soybeans cause depression of growth, particularly in young birds, as they contain a number of inhibitory factors, some of which decrease the utilisation of sulphur-containing amino acids (methionine and cysteine) (Saxena et al., 1963). Furthermore, they contain at least four proteins that inhibit the digestive enzyme trypsin. These anti-trypsin factors decrease protein digestibility, and increase the excretion of nitrogen and sulphur (Kunitz, 1947). Heat treatment of soybean successfully inactivates these inhibitors. Soybeans may also contain goitrogenic substances, which may cause goitre in the long term, particularly where the level of dietary iodine is low.

The characteristics of raw soybean may thus have nutritional implications for birds utilising it as a food source. Helmeted Guineafowl have been observed feeding on post-harvest soybean lands (raw soybean), while crop samples from specimens collected within the Midlands confirm the ingestion of soybean (pers. obs., unpubl. data). The availability of this waste grain coincides with the critical winter period for guineafowl during which protein-rich food is scarce. Therefore, in areas of extensive cultivation, soybean may form an important component of their winter diet, yet the associated nutritional and energy constraints may result in poor condition and/or limitations in various metabolic functions.

Furthermore, being a legume, soybean is often rotated with maize - every three years in KwaZulu Natal (D. van Rooien, pers.comm.) - and thus although flocks are territorial over the winter period, this rotation exposes these sub-populations to soybean at some period during this cultivation. Indeed, in the late 1980s, many farmers within the Midlands switched from maize to soybean (Fig. 7.1) due to it being more drought resistant and resulting in less crop theft (D. van Rooien, pers.comm). Thus, the potential adverse effects of soybean may be increasing and thus contributing to suppression in the recovery of guineafowl populations in these areas.

This study investigates the digestibility of the gross energy in raw soybean and other feed ingredients by Helmeted Guineafowl, using domestic roosters as a control.

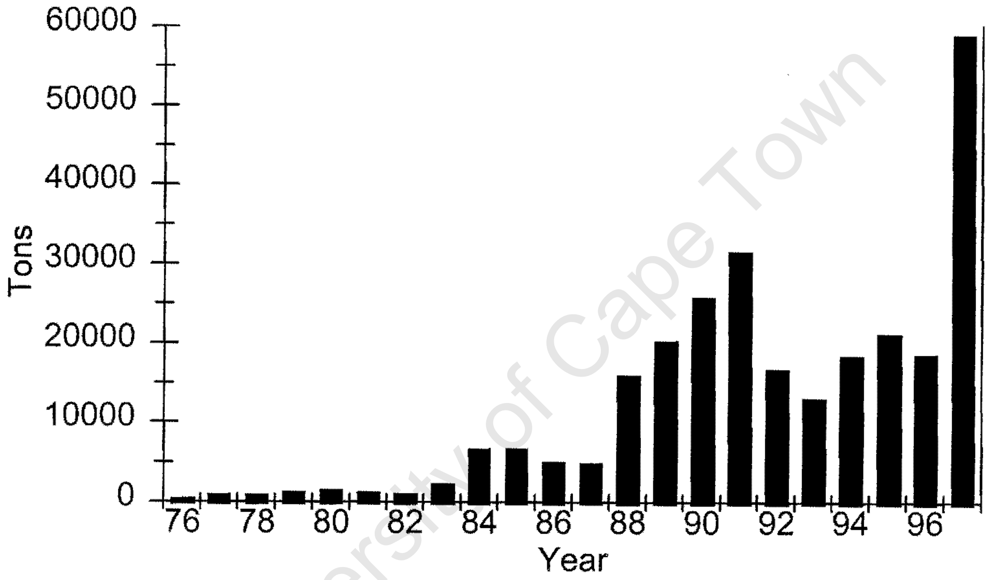

Fig. 7.1. Annual changes in soybean production from 1976 to 1997 (D. van Rooien, pers. comm.).

7.2. METHODS

The Apparent Metabolizable Energy (AME) content of a feed ingredient is a measure of the digestibility of its gross energy (GE). In this experiment, the method used to measure the AME content of soybean and other selected ingredients was the method of McNab & Blair (1988). The assay method is quick and accurate, and is used extensively for the measurement of the metabolizable energy content of feeds and feed ingredients in poultry nutrition. Essentially, birds are housed individually in cages, and a measured amount of feed is placed into the crop of each bird by intubation. The excreta voided by the birds is then collected over the following 48 hr period, after which it is dried, weighed, and its GE content measured in a bomb calorimeter. In order to reduce carry-over effects of the feed consumed prior to the assay period, the birds are fasted for 48 hours before the test ingredient is fed. During this two-day fasting period the birds are given water and glucose in order to ensure that they do not lose weight, and to encourage any feed residues to be flushed from the system. During the 48 hr collection period the birds are again given water by intubation. In the case of the guineafowl used here, 30g of each ingredient was fed to each bird, whereas 50g of the same ingredients were fed to adult roosters.

The calculation of AME is as follows:

$$[(GE_{food} \times \text{food intake}) - (GE_{excreta} \times \text{excreta weight})] / \text{food intake}$$

Seven guineafowl were used in the experiment. The ingredients evaluated were raw soybeans (either whole or cracked), soybean oilcake meal, fishmeal, sunflower oilcake meal and maize. The resultant AMEs, calculated for these ingredients, were compared with those calculated for adult roosters fed in the same manner. Each ingredient was fed to seven birds and the data were averaged. Each ingredient was evaluated over a one-week period, after which the birds were allowed *ad libitum* access to food for ten days before the next trial began. Whole soybean was not fed to the roosters. Excreta weights produced by the roosters fed 50g were adjusted to an intake of 30g/d in order that comparisons could be made between the two species.

The feed and excreta samples for the raw, full-fat soybeans and for the cooked soybean oilcake meal obtained from the roosters were analysed for amino acid content using a Beckman Amino Acid Analyser, from which the digestibility of each of the essential amino acids was calculated. Amino acid losses of endogenous origin were accounted for by measuring these in the excreta of six roosters who were fed 50g of glucose in place of the test ingredients.

7.3. RESULTS

The results of the energy digestibility study are summarized in Table 7.1, and of the amino acid digestibility study, in Table 7.2.

Table 7.1. A comparison of the $GE_{excreta}$, excreta weights, the digestibility of the GE, and AME of raw, full-fat soybeans, either whole or cracked, cooked soybean oilcake meal, fishmeal, sunflower oilcake meal, and maize, using Helmeted Guineafowl (GF) and adult roosters (R). Results are for an intake of 30g of food. Units are in MJ/kg, and weights are in g.

	Soybean whole	Soybean cracked		Soybean oilcake meal		Fishmeal		Sunflower oilcake		Maize	
	GF	GF	R	GF	R	GF	R	GF	R	GF	R
GE_{food}	21.3	21.3		18		19.6		18.3		16.7	
$GE_{excretion}$	20.4	19.5	14.9	13	13.7	11.4	12.4	13.9	15.4	14.6	15.7
Excreta wt	12.6	9.9	12.4	16.7	14.8	17.2	13.4	21.3	20.2	7.3	4.6
GE_{out}	257	194	15	217	202	195	162	298	311	107	71
Digestibility	0.6	0.7	0.71	0.6	0.63	0.67	0.72	0.46	0.43	0.77	0.86
AME	12.8	14.9	15.1	10.8	11.2	13.1	14.2	8.4	7.9	13.2	14.3

Table 7.2. Digestibility of amino acid of, raw full-fat soybeans and cooked soybean oilcake meal, measured using adult roosters.

Amino acid	Raw full-fat soybeans	Cooked soybean oilcake meal
Valine	0.653	0.829
Methionine	0.657	0.883
Isoleucine	0.675	0.838
Leucine	0.694	0.842
Tyrosine	0.732	0.874
Phenylalanine	0.705	0.851
Histidine	0.753	0.85
Lysine	0.724	0.824
Arginine	0.793	0.885
Mean	0.71	0.85

7.4. DISCUSSION

For both guineafowl and roosters, all forms of soybean appeared to be equally digested, with little improvement having been brought about by the cooking process. A greater improvement in digestibility resulted from the cracking of the seed than in removing the oil and heat-treating the extracted soybean oilcake, which was an unexpected result. The considerable improvement in digestibility of whole soybean bean when it was cracked, as opposed to its being fed whole was no doubt a function of a greater digestible surface area, and thus other feeds should similarly improve in digestibility in this form. Indeed, a similar observation has been made in our laboratory (University of Natal) with Canola (Rape) seed, which is very poorly digested unless it is cracked open, due to the thick husk over the endosperm.

The higher AME value for the whole and cracked soybean compared with the oilcake meal is the result of the higher oil content in the whole grain, as can be seen from the higher GE. The digestibility of the GE of maize was the highest measured in this trial, whilst that of sunflower oilcake meal was the lowest. The energy extracted from soybeans and from fishmeal, through digestion, was similar. There appeared to be little difference in the digestibilities measured by means of the two species, the differences that were observed being consistent across ingredients.

In general, roosters appeared to digest the feed ingredients slightly better than guineafowl, but this is due, in part, to the larger quantity of endogenous energy losses (metabolic faecal, and endogenous urinary energy) emanating from the roosters, whose body mass and hence intestinal surface area is considerably larger than those of guineafowl. These endogenous energy losses form part of the excreta output, and should be deducted from the total excreta output, to yield a true estimate of the excreta energy losses of dietary origin (Fisher & McNab, 1989).

The endogenous amino acid losses were taken into account when measuring the digestibility of the amino acids in raw and heat-treated soybean, evaluated with adult roosters. Differences in digestibility between these two forms of soybean are far more obvious in this case than in the case of GE digestibility. The improvement in digestibility of soybeans through the process of heat treatment is considerable, the average digestibility of eight of the essential amino acids increasing from 0.71 to 0.85 (Table 7.2). This improvement is the more remarkable, in that the measurement was made with adult roosters, where the digestibility is not as severely affected as it is in young growing birds (Saxena et al., 1963). Such differences in digestibility would be expected to be harmful to young guineafowl under conditions of limited protein.

Although this experiment indicates that guineafowl are able to obtain energy from soybean (i.e. comparable with maize), what is unknown are the long term effects that soybean may have on factors such as growth and additional nutritional loss. A high protein diet is essential for gamebird gonadal development and egg production (Potts, 1986), and insufficient food for laying hens has been found to reduce breeding success (Dobson et al., 1988). Furthermore, a high protein diet, in the form of insects, is essential for the growth in young chicks (Crowe, 1984). In the Midlands, healthy guineafowl populations are associated with extensive edge habitats which provide both food and cover (Malan & Benn, 1999; Ratcliffe & Crowe in press; Ratcliffe & Crowe in review). In areas of extensive soybean monoculture, these habitats are greatly reduced thus limiting the associated, protein-rich, insect life. Furthermore, data from Chapter 6 (section 6.2.1.) indicate that only declining populations encountered extensive soybean habitat, of which, only a small percentage formed part of their home range (average 17.68 %, Table 6.4.). Birds are known to have "nutritional wisdom" (G. Bradford, pers. comm.) when selecting food and will thus actively seek out items within their diet which are lacking. This may explain the relatively small percentage of soya within the home range of these populations. The availability of raw soybean as an alternative protein source may thus be inhibiting both adult, and especially chick, development. Further research is needed in this regard.

As a major factor resulting in population decline, soybean is confined mostly to the northern areas of the Midlands and thus may only be contributing to some dietary constraints on a local scale. Furthermore, the increases in soybean production (Fig. 7.1) in the late 1980s, are more likely a reflection of replacement and/or rotation of maize with soybean than an increase of land under agriculture, with subsequent habitat loss.

CHAPTER 8

ASSOCIATED SPECIES DIVERSITY

Status as of 18/9/2000: in review. The effects of agriculture and the availability of edge habitat on populations of Helmeted Guineafowl *Numida meleagris* and on the diversity and composition of associated bird assemblages in KwaZulu-Natal province, South Africa. *Biodiversity Conservation*. Co-author: T.M. Crowe.

SUMMARY

We investigated the effects of agriculture and the availability of edge habitat on populations of Helmeted Guineafowl *Numida meleagris* and associated avian diversity and species composition in woodland and grassland biomes in the Midlands of KwaZulu-Natal province, South Africa. Study sites within woodland biome had greater species diversity than those in grassland, whereas adjacent, high-quality, protected habitat in grassland sites, enhanced diversity within this biome. Both guineafowl populations and overall avian diversity declined with increasingly intensive agriculture and disappearance of edge habitat and associated, optimally fragmented habitat mosaic. Furthermore, traditional agriculture in the form of contouring in a pesticide-free environment, resulted in extensive edge habitat that appeared to provided additional food and cover for birds. This, in turn, caused an increase in bird diversity in general, and in guineafowl populations in particular. The widespread decline in Helmeted Guineafowl populations in the Midlands that started in the 1980s, and possibly the decline in species associated with this variegated landscape, was therefore caused by the loss of the habitat mosaic to intensive, modern, monoculture, crop agriculture. Maintaining species diversity and healthy guineafowl populations within these habitats requires the persistence or re-creation of a habitat mosaic and the resulting edge habitat on a landscape scale.

8.1. INTRODUCTION

8.1.1. Effects of agriculture on biodiversity

Bird populations on agricultural land have declined in many parts of the world (Pain & Pienkowski, 1997). In Europe in particular, agriculture is the dominant form of land-use in most countries, but there is widespread concern over the status of many characteristic bird species and assemblages associated with farmland environments (Hustings et al., 1990; Fuller et al., 1991; Bohning-Gaese, 1992; Zang, 1993; Tucker & Heath, 1994). In North America, attention has focused more on grassland bird species which have declined significantly since the 1960s (Johnson & Schwartz, 1993; Peterjohn & Sauer, 1993; Knopf, 1994; Warner, 1994).

These trends have not been confined to the Northern Hemisphere. In Africa, crop agriculture, livestock farming and forestry have been the most significant land-use practices that have lead to large-scale transformation of the structure and functioning of ecosystems (Downing, 1978; Happold, 1995; Allan et al., 1997). There is, however, little understanding as to the influence of these transformations on fauna and flora and various ecosystem processes outside of protected areas, thus making effective, broad scale conservation of avian diversity problematic (Macdonald, 1989).

In southern Africa, the changes in associated avifauna over the landscape from protected areas through to farmland have not been well documented. Relatively few studies highlight the conservation of biodiversity in agricultural landscapes (Macdonald, 1989; Little & Crowe, 1994; Allan et al., 1997; Malan, 1998; Jansen et al., 1999). Farming activities can create a heterogeneous and dynamic environment within which species must cope, and adapt to, markedly fluctuating conditions, one of the most disturbing of which is fragmentation of natural habitats. The habitat fragmentation has placed plant and animal populations in jeopardy, both at the species and community levels (Morrison, 1986; Hockey et al., 1988; Harrison et al., 1994; Allan et al.,1997). Thus, management practices within agricultural landscapes potentially hold the key to the persistence of biodiversity associated with rural landscapes.

8.1.2. Adaptable species

There are many bird species that have expanded their ranges in southern Africa by exploiting transformed habitats. Hockey et al. (1989) noted that, in the Western Cape province of South Africa alone, 85 species have benefited from some form of habitat transformation. Some of these species have been given a markedly enhanced competitive edge, because of their ability to exploit landscapes transformed by humans and thus have become more widespread and abundant. One such species is the Helmeted Guineafowl *Numida meleagris* - see Chapter 1.

8.1.3. Associated biodiversity

Birds are conspicuous in the wild and, therefore, are often used as indicators of habitat transformation and the status of other organisms (Morrison, 1986). This is based on the belief that, "if the factors determining the distribution of animals are known, then specific predictions can be made concerning the response of animals to some perturbation, and, in a more general sense, certain animals can then be used to monitor environmental quality"(Morrison, 1986).

The decline in Helmeted Guineafowl populations in KwaZulu-Natal is not only of concern for the status of this valuable and popular gamebird, but also of that of overall biodiversity associated with agricultural environments. This chapter investigates changes in overall avian diversity at different population levels of Helmeted Guineafowl in the Midlands of KwaZulu-Natal. Furthermore, the quality of edge habitat associated with this fragmented habitat is also examined because Malan & Benn (1999) found that healthy Helmeted Guineafowl populations frequented extensive edge habitat.

8.2. METHODS

8.2.1. Study area

The study area outlined in Chapter 6 (section 6.2.1) was similarly utilised for this aspect of research with the exception of the Elandslaagte site, as only one stable population site was selected. Stable populations occurred on a farm within in a Mixed Woodland biome in the district of Ladysmith (29°48'E; 28°30'S). The landscape is moderately fragmented; no pesticides are used; and there is extensive edge habitat. Declining populations were situated on a property in the Newcastle (29°51'E; 27°57'S) district bordering the Chelmsford reserve, and is predominantly under crop agriculture (soya beans with small patches of maize) resulting in pockets of fragmented habitats in amongst large croplands. Near extinct populations were located on a farm in the sourveld grasslands of the Underberg (29°33'E; 29°42' S) district. Extensive areas are under pasture, while commercial grasses contribute to a more grassland-like habitat. Pastures are burnt on a biennial basis.

8.2.2. Guineafowl populations

The same data, and thus methodology, from Chapter 6 (section 6.2.2.) was utilised to obtain population estimates.

8.2.3. Total counts

Overall bird species diversity and relative abundance were recorded in all habitats, at each study site, in early spring (September) over a period of two weeks. Survey sites were selected on the basis that they were homogeneous and typical of that respective habitat. A total of 1.5 hours was spent in each habitat each day, with observations being made in three half-hour periods over three days. Counts were conducted between 07h00 and 10h00 by walking along a transect through each habitat. Birds were divided into guilds based on a preference for 'farmland', 'grassland', 'arboreal' or 'other' habitats (Appendix 8.1). 'Farmland' species included Helmeted

Guineafowl, but did not incorporate birds associated closely with homesteads, only with agricultural lands. Species associated with homesteads, along with waterbirds and aerial feeders (e.g. African Hoepoe, *Upupa africana*; Brownthroated Martin, *Riparia paludicola*; African Fish Eagle, *Haliaeetus vocifer*), were termed 'other' and regarded as specialists and thus were not included in this study.

8.2.4. Edge counts

The relationship between edge habitat and overall avian diversity was assessed in cropland habitats. Edge was defined as the area 2 m. on either side of the interface between an agricultural land, and the surrounding vegetation. Commercial rye-grass and harvested maize crop habitats were selected in all major study sites and categorized according to the presence of extensive (width of vegetated edge > 1 m) or sparse (edge vegetation absent) grassland edges. Avian diversity and abundance were then recorded using the same methodology as in total counts, with three half-hour observations over three days. That is, 'edge counts' were done separately from total counts.

8.2.5. Data analysis

Multivariate analyses of data for bird species encountered within edge habitats were performed using cluster analysis (CLUSTER) and non-metric multi-dimensional scaling (MDS) programs in the PRIMER software package (Plymouth Marine Laboratory, U.K.) to identify assemblages of birds. The resulting similarities were expressed using the Bray-Curtis similarity co-efficient (Bray & Curtis, 1957) and displayed as a dendrogram, reflecting the hierarchical relationships of various habitats between different sites, and as a two-dimensional MDS ordination plot which represents these similarities in a non-hierarchical manner. PRIMER was also utilized to obtain a measure of diversity for each particular habitat, using the Shannon-Wiener diversity index:

$$H' = -\sum_i p_i (\log p_i)$$

where p_i is the proportion of the total count (or biomass, etc.) arising from the ith species.

Finally, a Mann-Whitney *U*-test was applied to determine significance between population density estimates using the Statgraphics statistical graphics package (Anon., 1986).

8.3. RESULTS

8.3.1. Guineafowl populations

The results are summarized in Chapter 6, section 6.3.1, Table 6.1.

8.3.2. Total counts

Of the 95 species recorded over two weeks, 65 species (68.4%) were associated with stable guineafowl populations, 53 species (55.8%) with declining and only 44 (46.3%) species with near extinct populations (Appendix 8.1). The 'stable' study site had 31 (47.7%) and 27 (41.5%) species in common with the other sites respectively, whereas declining and near extinct sites had 33 (62.3%) species in common. The species turnover (beta diversity) recorded a net loss of 12 and 20 species respectively moving from stable to both declining and near extinct sites, whereas a further loss of nine species was recorded from declining to near extinct sites (Table 8.1).

Table 8.1. Associated avian species turnover across a Helmeted Guineafowl population gradient.

Population gradient	Loss	Gain	Net
Stable to declining	34	22	-12
Stable to near extinct	38	18	-20
Declining to near extinct	20	11	-9

In terms of guild composition, grassland species increased from stable through to declining and near extinct sites, whereas farmland species remained relatively stable. Arboreal species, however, declined markedly across this front (Fig. 8.1). Species with a high diversity index (Appendix 8.1) were associated with all three study sites, whereas species with a low index tended to be specific to one site only.

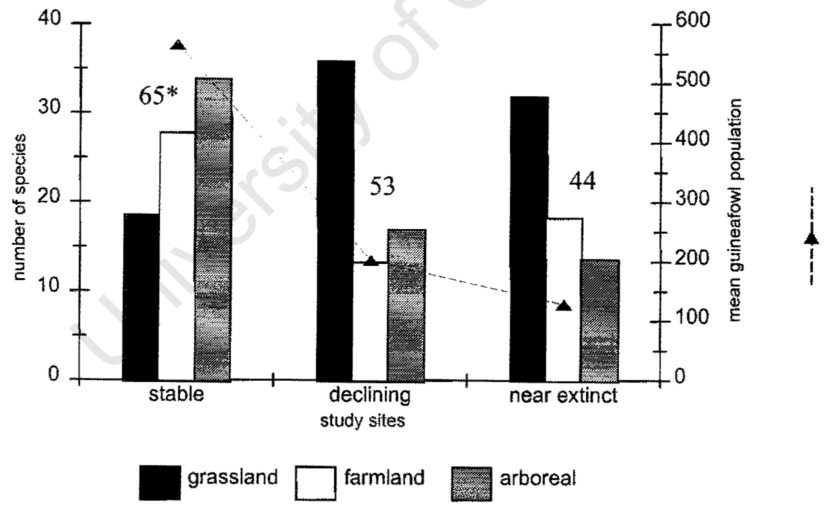

Fig. 8.1. Guild composition of birds associated with different levels of Helmeted Guineafowl populations (* = total associated species diversity).

8.3.3. Edge counts

Of the 45 species observed in maize and rye-grass edge habitats, only 17 (37.7%) occurred in sparse edged habitats whereas 42 (93.3%) (Appendix 8.1) occurred in extensive edge. Of these species, 14 (31.1%) were in common. Sparse and extensive edge habitats formed three distinct groups of bird assemblages (Fig. 8.2a & b). Group 1 included 26 (57.8%) species in extensive edge habitats. Group 2 consisted of 29 (64.4%) species incorporating both sparse and extensive edged habitats, whereas Group 3 totaled 11 (24.4%) species in sparse edge habitats.

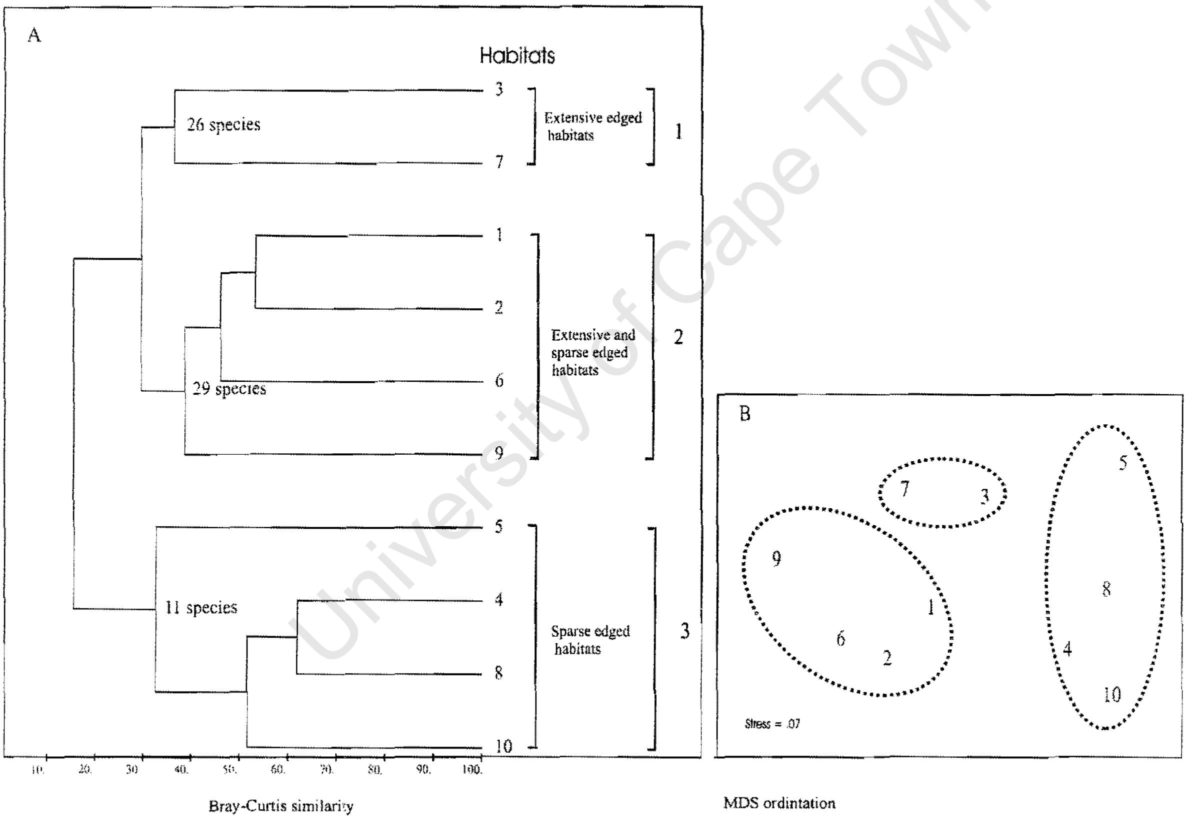

Fig. 8.2. Dendrogram of Bray-Curtis percentage similarities in bird assemblage structure between 10 maize and rye-grass edge habitats (A) and multidimensional scaling ordination (MDS) between these habitats (B).

8.4. DISCUSSION

Declines in both guineafowl populations and overall bird species diversity initially appear to be related to the associated biome. The range expansion of Helmeted Guineafowl into the grassland biome has mostly come about through the effects of agriculture and deliberate introductions (Little, 1997). However, traditionally, the Helmeted Guineafowl is a bird of savannas (Crowe et al., 1986), and thus prefers woodland to grassland habitats. Furthermore, within the grassland biome, the availability of protected roosting sites in a neighboring reserve, possibly provided declining populations a competitive edge over near extinct populations to the south. In terms of diversity, the Mixed Woodland biome has a more diverse vegetation structure than grasslands, and thus has a richer avifauna due to a greater niche' availability. The high turnover in species between this woodland and the grassland sites (Table 8.1) reflects this, whereas the high numbers of 'other' guild species in woodland (Fig. 8.1) further indicates the presence of many specialist species due to this diverse vegetation structure.

Similarly, the change in guild composition also reflects the respective biomes in which the study sites are situated (Fig. 8.1). Arboreal species such as Brubru *Nilaus afer*, Pied Barbet *Tricholaema leucomelas* and Scimitarbilled Woodhoopoe *Rhinopomastus cyanomelas* (Maclean, 1985) were not recorded in the declining and near extinct sites, whereas grassland species, including the endemic Pied Starling *Spreo bicolor* (Siegfried, 1992), are similarly absent from the stable site. The greater species diversity associated with declining in comparison to near extinct populations, is likely due to the influence of neighboring, high quality, moist grassveld (mostly *Hyparrhenia hirta* and *Themeda triandra*) habitat (Chelmsford reserve) increasing species richness at this site. In particular, grassland specialists such as Marsh Owl *Asio capensis*, Whitebellied Korhaan *Eupodotis cafra* and Pinkbilled Lark *Spizocorys conirostris* (Maclean, 1985) exemplify this condition. In addition, the arboreal species associated with these two grassland environments highlights the influence of alien tree species in boosting overall bird diversity in this biome.

The category farmland species (which includes Helmeted Guineafowl) however, appears to be less affected by conditions in indigenous habitat, since its component species are adapted to modified, rural landscapes. Indeed, data from radio-tracking Helmeted Guineafowl revealed a preference for modified habitats (maize and fallow lands) (Ratcliffe & Crowe, in press). Indigenous vegetation such as grassland and, to a lesser extent, woodland was avoided, or utilized only in transit. Although there is little discrepancy between sites in terms of diversity of 'farmland' species, the difference in abundance is more significant (Table 8.2). In particular, both Helmeted Guineafowl and Swainson's Spurfowl *Pternistis swainsonii*, both of which are associated with fragmented habitat (Crowe, 2000a; 2000b), decline markedly in abundance from stable through to the near extinct site. Thus, the decline in the abundance of farmland species from stable through to declining and near extinct populations is likely to be influenced by additional factors such as land use management (e.g. pesticide loads; contouring technique; double cropping).

All three study sites are located in intensive farming areas resulting in varying degrees of habitat fragmentation. Both the declining and near extinct sites are characterised by large areas under crops (declining) or pasture (near extinct), with the near extinct site being less fragmented due to the predomination of stock farming over crop agriculture. The stable study site was moderately fragmented, with a 'patchwork-quilt' of numerous, small, cultivated lands providing a mosaic of modified and unmodified habitats. The management of this landscape differs from the other two sites in that no pesticides are used on the farm concerned. This has resulted in extensive weed growth in cropland edge habitats. Furthermore, these lands mostly incorporate vegetated contours as opposed to the 'broad-based drains' of the other sites which have no vegetation cover at all (O. Geekie pers. comm., 1998). The growth of secondary species, such as successional plants, is known to provide insects and other food and shelter for a variety of vertebrates (Soule`, 1986). Thus, greater abundance of farmland species may be attributed to these well-vegetated edges and contours providing additional resources, such as food and cover.

Table 8.2. Abundance of 'farmland' species (Maclean 1985) occurring within each study site. Figures represent the average individuals observed over the study period.

Common name	Scientific name	Assemblage		
		stable	declining	near extinct
Black Crow	*Corvus capensis*	0	2	4.5
Blackheaded Heron	*Ardea melanocephala*	2	0.5	1
Blackshouldered Kite	*Elanus caeruleus*	1	2.5	1
Blackthroated Canary	*Serinus atrogularis*	13	5	0
Cape Turtle Dove	*Streptopelia capicola*	12.5	14.5	15
Cape Weaver	*Ploceus capensis*	5.5	7.5	11.5
Cattle Egret	*Bubulcus ibis*	0	4	1.5
Common Quail	*Coturnix coturnix*	0	1	0
Egyptian Goose	*Alopochen aegyptiacus*	6	0	1
Feral Pigeon	*Columba livia*	0.5	0	0
Fiscal Shrike	*Lanius collaris*	3	8	5.5
Helmeted Guineafowl	*Numida meleagris*	181	77.5	6
Laughing Dove	*Streptopelia senegalensis*	25.5	11	0
Pintailed Whydah	*Vidua macroura*	25	13.5	10
Redbilled Quelea	*Quelea quelea*	0	0	40
Rock Pigeon	*Columba guinea*	1	0.5	11
Spurwing Goose	*Plectropterus gambensis*	0	0	7.5
Stonechat	*Saxicola torquata*	7	12.5	12.5
Swainson's Francolin	*Francolinus swainsonii*	17	6	0
Whitenecked Raven	*Corvus albicollis*	1.5	0	0
Yelloweyed Canary	*Serinus mozambicus*	41.5	8.5	0
	Total individuals	343	174.5	128

The effects of these edge habitats is further illustrated in the Bray-Curtis similarity and associated MDS ordination in Fig. 8.2a & b. The associated overall bird assemblages formed three distinct groups with the quality of edge habitat distinguishing between associated numbers of species. Groups 1 and 2 incorporated extensive edged habitats which incorporated species associated with rank grass habitat, notably, Tawnyflanked Prinia *Prinia subflava*, Redshouldered Widow *Euplectes axillaris* and Redcollared Widow *Euplectes ardens* (Maclean, 1985). Significantly, habitat 9 occurred in the stable study site and thus the overall high diversity associated with this site may have contributed to the species richness of this sparse edge habitat. By contrast, these species did not occur in sparse edge habitats (Group 3), which had less diversity and was characterised by birds adapted to more open, short, grassland habitat such as Grassveld Pipit *Anthus cinnamomeus* and Ayres' Cisticola *Cisticola ayresii* (Maclean, 1985). Thus, extensive edge habitat can not only promote increases in the number of species, but also influences the species assemblages associated with these agricultural lands. Finally, Helmeted Guineafowl numbers were also affected by the quality of edge habitats. During counts in these habitats, guineafowl occurred in four out of five extensive edge habitats, and only in one out of five sparse edge habitats.

8.5. CONCLUSIONS

This study confirms that Helmeted Guineafowl thrive in a mosaic of moderately fragmented habitats (Malan & Benn, 1999) with population declines corresponding to a decline in habitat heterogeneity. Habitat fragmentation is synonymous with an increase in edge habitats (Laurance & Yensen, 1991), but the quality of these edge habitats appears to be of particular importance. Extensive edge habitat potentially supports larger food resources while providing greater cover and nesting sites for Helmeted Guineafowl. The findings by Malan & Benn (1999) support this, where a statistical correlation was found between extensive edge habitats and healthy guineafowl populations. Similarly, extensive edge habitats had greater species diversity than sparse edge, thus further underlying the importance of the quality of edge habitat.

However, the extreme fragmentation of natural habitats due to intensive agriculture, can negatively affect the viability of many avian populations (Lauga & Joachim, 1992). Indeed, many grassland species are under threat due to the large expansion of agriculture into the grassland biome (Jansen et al., 1999). The large increases in crop agriculture within the Midlands since the late 1970s (Ratcliffe & Crowe, 1999a) is thus of concern for avian diversity since even relatively adaptable species, such as Helmeted Guineafowl, are in decline. A decline in species diversity is often associated with increases in habitat fragmentation (Lauga & Joachim, 1992). In this study, however, this trend appears to have been ameliorated due to the effects of the associated biome, as well as the presence of neighboring, high quality habitat at sites such as Chelmsford reserve. Furthermore, land-use management practices can further enhance or restrict the creation of optimum habitats for individual species, and thus species diversity as a whole. The lack of pesticide use and the use of traditional contouring methods are both land-use practices that appear to have contributed to increasing species diversity in this regard.

If healthy Helmeted Guineafowl populations can be termed an indicator of an optimally fragmented habitat, then their decline is indicative of excessively fragmented habitats due to extensive, relatively weed-free modern crop agriculture. Many other bird species have also thrived due to the development of low-intensity agriculture in South Africa (Hockey et al., 1989). However, the recent shifts to high-intensity, weed/insect-free, monoculture crop farming have largely occurred without many serious attempts at assessing their impacts on biodiversity.

Appendix 8.1. Species list of birds (Maclean 1985) occurring in the study area. Birds are listed in order of declining diversity, according to their assemblage, guild and presence in associated edge habitat. Species classified as 'other' were considered specialists.

Common name	Scientific name	Assemblage	Guild	Edge habitat		Shannon-Wiener Diversity index
				Extensive	Sparse	
Forktailed Drongo	*Dicrurus adsimilis*	1,2,3	3			1.75
Stonechat	*Saxicola torquata*	1,2,3	2	*	*	1.74
Cape Turtle Dove	*Streptopelia capicola*	1,2,3	2	*		1.73
Fiscal Shrike	*Lanius collaris*	1,2,3	2	*	*	1.72
Orangethroated Longclaw	*Macronyx capensis*	1,2,3	1	*	*	1.61
Longtailed Widow	*Euplectes progne*	1,2,3	1	*	*	1.55
Levaillant's Cisticola	*Cisticola tinniens*	1,2,3	1	*	*	1.54
Greyheaded Sparrow	*Passer diffusus*	1,2,3	3	*		1.54
Grassveld Pipit	*Anthus cinnamomeus*	1,2,3	1	*	*	1.41
Cape Robin	*Cossypha caffra*	1,2,3	3			1.33
Cape Weaver	*Ploceus capensis*	1,2,3	2	*		1.31
Blackheaded Heron	*Ardea melanocephala*	1,2,3	2	*		1.28
Laughing Dove	*Streptopelia senegalensis*	1,2	2			1.28
Pintailed Whydah	*Vidua macroura*	1,2,3	2	*		1.28
Redeyed Dove	*Streptopelia semitorquata*	2,3	4			1.25
Cape Wagtail	*Motacilla capensis*	1,2,3	4	*	*	1.24
Blackeyed Bulbul	*Pycnonotus barbatus*	1,3	3			1.23
Cape Sparrow	*Passer melanurus*	1,2,3	4	*		1.21
Swainson's Spurfowl	*Pternistis swainsonii*	1,2	2	*		1.14
Helmeted Guineafowl	*Numida meleagris*	1,2,3	2	*	*	1.12
Yelloweyed Canary	*Serinus mozambicus*	1,2	2	*		1.11
Brownthroated Martin	*Riparia paludicola*	1,2,3	4	*		1.09
Common Waxbill	*Estrilda astrild*	2,3	4		*	1.08
Hadeda Ibis	*Bostrychia hagedash*	1,3	4	*	*	1.03
Blackshouldered Kite	*Elanus caeruleus*	1,2,3	2	*		0.995
Lesser Masked Weaver	*Ploceus intermedius*	1,2	3			0.943
Fantailed Cisticola	*Cisticola juncidis*	1,2,3	1	*	*	0.939
Ayres' Cisticola	*Cisticola ayresii*	1,2,3	1		*	0.937
Whitewinged Widow	*Euplectes albonotatus*	1,3	1	*		0.86
House Sparrow	*Passer domesticus*	1,3	4			0.846
European Swallow	*Hirundo rustica*	1,2	4	*		0.834
Tawnyflanked Prinia	*Prinia subflava*	1,2	3	*		0.831
Egyptian Goose	*Alopochen aegyptiacus*	1,3	2	*	*	0.796

Appendix 8.1. (continued)

Common name	Scientific name	Assemblage	Guild	Edge habitat		Shannon-Wiener Diversity index
				Extensive	Sparse	
Rock Pigeon	*Columba guinea*	1,2,3	2	*	*	0.767
Crested Barbet	*Trachyphonus vaillantii*	1,2	3			0.693
Titbabbler	*Parisoma subcaeruleum*	1	3			0.693
African Fish Eagle	Haliaeetus vocifer	2	4			0.693
Familiar Chat	*Cercomela familiaris*	1	4			0.693
Chinspot Batis	*Batis molitor*	1	3			0.693
African Hoopoe	*Upupa africana*	1,3	4			0.693
Cape Glossy Starling	*Lamprotornis nitens*	2	3			0.693
Pied Starling	*Spreo bicolor*	2,3	1	*		0.689
Quailfinch	*Ortygospiza atricollis*	2,3	1	*		0.689
Streakyheaded Canary	*Serinus gularis*	2,3	3			0.687
Anteating Chat	*Myrmecocichla formicivora*	2,3	1	*		0.683
Whitebellied Sunbird	*Nectarinia talatala*	1	3			0.673
Shelley's Francolin	*Francolinus shelleyi*	1	1			0.662
Whitenecked Raven	*Corvus albicollis*	1	2			0.637
Cape White-eye	*Zosterops pallidus*	3	3			0.637
Whitebellied Korhaan	*Eupodotis cafra*	2	1			0.637
White-browed Scrub Robin	*Cercotrichas leucophrys*	1	3			0.637
Black Crow	*Corvus capensis*	3	2	*	*	0.637
Redthroated Wryneck	*Jynx ruficollis*	2,3	3			0.637
Redbilled Woodhoopoe	*Phoeniculus purpureus*	1,2	3			0.637
Brubru	*Nilaus afer*	1	3			0.637
Cape Canary	*Serinus canicollis*	1,2	4	*		0.637
Plainbacked Pipit	*Anthus leucophrys*	2,3	1		*	0.637
Cattle Egret	*Bubulcus ibis*	2,3	2	*		0.562
Bokmakierie	*Telophorus zeylonus*	3	4	*		0.5
Redcollared Widow	*Euplectes ardens*	3	1	*	*	0.451
Blackthroated Canary	*Serinus atrogularis*	1,2	2	*		0.442
Blue Waxbill	*Uraeginthus angolensis*	1	3			0
Groundscraper Thrush	*Psophocichla litsitsirupa*	1	4			0
Pied Barbet	*Tricholaema leucomelas*	1	3			0
Orangebreasted Waxbill	*Sporaeginthus subflavus*	2	1			0
Black Swift	*Apus barbatus*	2	4			0
Blackbellied Korhaan	*Eupodotis melanogaster*	2	1			0
Pinkbilled Lark	*Spizocorys conirostris*	2	1			0
Marsh Owl	*Asio capensis*	2	1			0

Appendix 8.1. (continued)

Common name	Scientific name	Assemblage	Guild	Edge habitat		Shannon-Wiener Diversity index
				Extensive	Sparse	
Black Flycatcher	Melaenornis pammelaina	1	3			0
Longbilled Crombec	*Sylvietta rufescens*	1	3			0
Redfaced Mousebird	*Urocolius indicus*	1	3			0
Redshouldered Widow	*Euplectes axillaris*	2	1	*		0
Little Swift	*Apus affinis*	1	4			0
Redbilled Quelea	*Quelea quelea*	3	2	*		0
Goldenbreasted Bunting	*Emberiza flaviventris*	1	3			0
Rufousnaped Lark	*Mirafra africana*	1	1			0
Secretarybird	*Sagittarius serpentarius*	3	1			0
Scimitarbilled Woodhoopoe	*Rhinopomastus cyanomelas*	1	3			0
Blacksmith Plover	*Vanellus armatus*	1	4			0
Speckled Mousebird	*Colius striatus*	1	3			0
Spikeheeled Lark	*Chersomanes albofasciata*	2	1			0
Spurwing Goose	*Plectropterus gambensis*	3	2	*		0
Redwinged Starling	*Onychognathus morio*	1	4			0
Feral Pigeon	*Columba livia*	1	2	*		0
Fairy Flycatcher	*Stenostira scita*	3	3			0
Croaking Cisticola	*Cisticola natalensis*	1	1	*		0
Black Sparrowhawk	*Accipiter melanoleucus*	2	3			0
Wattled Plover	*Vanellus senegallus*	1	4	*		0
Black Sunbird	*Nectarinia amethystina*	1	3			0
Common Quail	*Coturnix coturnix*	2	2	*		0
Bleating Warbler	*Camaroptera brachyura*	2	3			0
Pallid Flycatcher	*Bradornis pallidus*	1	3			0
Namaqua Dove	*Oena capensis*	1	4	*		0
Neddicky	*Cisticola fulvicapillus*	1	1			0

Assemblages: 1 = stable, 2 = declining, 3 = near extinct.
Guilds: 1 = grassland , 2 = farmland, 3 = arboreal, 4 = other.

CHAPTER 9

SYNTHESIS AND MANAGEMENT POLICIES

Status as of 18/9/2000: in review. Declining populations of Helmeted Guineafowl *Numida meleagris* in the Midlands of KwaZulu-Natal, South Africa: causes and remedies. *South African Journal of Wildlife Research.* Co-author: T.M. Crowe.

SUMMARY

Populations of Helmeted Guineafowl *Numida meleagris* have declined significantly within the Midlands of KwaZulu-Natal province, South Africa, since the early 1980s. A three phase project was initiated in 1995 to assess the possible cause(s) of this decline. Initial research investigated socio-economic factors, such as illegal hunting, whereas subsequent statistical analyses based on questionnaires, revealed correlations between guineafowl population densities and certain land-use practices. Further research focused on other possible causal factors including agrochemicals, disease, genetic contamination and dietary constraints. Results indicate that, although all these factors might have negative effects on local populations, there is no evidence to suggest widespread action of any single factor. Radio-tracking of guineafowl at several sites revealed that healthy guineafowl populations thrive in moderately polarised landscapes, and that the loss of these habitats through increased, intensive crop agriculture, coupled with modern, 'clean' farming techniques, is largely responsible for observed declines in guineafowl populations due to population fragmentation and an undermining of meta-population structure. Resuscitating guineafowl populations to

sustainable levels involves creating suitable habitats on a landscape level through management strategies at the conservancy level, with the objective being to strive for habitat diversity and connectivity.

9.1. INTRODUCTION

The transformation of natural vegetation resulting from crop cultivation, stock farming, afforestation and urban development, presents the single most important threat to global biodiversity (Soule`, 1991; Dale et al., 1994). Macdonald (1989) estimated that up to 25% of South Africa has been converted to other forms of land use such as agriculture. "At the landscape scale, patterns of connectedness, which refer to the structural continuity between landscape elements, can affect the distribution of species" (Petit & Usher, 1998). Indeed, in southern Africa, the resulting polarisation of natural habitats through agricultural practices, has negatively affected the viability of many avian populations (Harrison et al., 1997).

There are, however, many bird species that have expanded their ranges by exploiting transformed habitats. Indeed, the polarisation of once continuous vegetation types has impacted positively on species that require a mosaic of habitats, which are heterogenous in nature (Malan & Benn, 1999). The adaptability of Helmeted Guineafowl *Numida meleagris* to these environments, and subsequent demise in the Midlands of KwaZulu-Natal, is outlined in Chapter 1. This chapter represents the final phase in a three-phase, five-year research project aimed at determining the causes of, and remedies to, these declines, and outlines management policies required to implement effective remedies.

9.2. METHODS

9.2.1. Study area

The study area is outlined in Chapter 2.

9.2.2. Phase 1

Phase 1 assessed the status of guineafowl populations in the Midlands - through questionnaires - as well as potential socio-economic factors that may have adversely affected it (Maphasa, 1996; Pero & Crowe, 1996). Data on various land use practices were analysed using both uni-variate and multi-variate analysis to ascertain statistical correlations between these practices, and associated guineafowl populations (Pero & Crowe, 1996).

9.2.3. Phase 2

Phase 2 initially investigated the effects of landscape transformation on guineafowl populations on 19 selected farms (Malan, 1998; Malan & Benn, 1999). Using a grid cell approach, the distribution of 11 land-uses were mapped on farms with different levels of guineafowl abundance. These attributes were then correlated with the presence or absence of guineafowl, as well as large (> 100 birds) or small (< 100 birds) flock size. Statistics, such as the Student's *t*-test and a correlation matrix, were then conducted through using the Statistica software package (Statsoft, 1995).

The remaining aspects of Phase 2 investigated potential factors in more detail and included:

1. the effects of agrochemicals through the analysis of liver samples (Ratcliffe et al., in

press);

2. analysis of genetic material to assess the extent of introgression from domesticated guineafowl (Walker et al., in review);
3. determining habitat utilisation of various populations through radio-tracking (Ratcliffe & Crowe, in press);
4. obtaining blood samples to test for the presence of various diseases (Horner et al., in review);
5. ascertaining the status of associated birdlife (Ratcliffe & Crowe, in review) and, finally,
6. experiments on captive birds to determine the digestibility of commercially grown soya by Helmeted Guineafowl (Ratcliffe et al., in review), were undertaken, as it was thought that the energy and nutrients in untreated soya might be unavailable to them.

9.3. RESULTS

9.3.1. Phase 1

The questionnaire survey indicated that illegal hunting occurs within the study area, but was confined largely to farms with poor labour relations (Maphasa, 1996). Deliberate poisonings (for meat or out of animosity) occur through the availability of agrochemicals, and may result in the decimation of local flocks, but these incidents are localised. Statistical analysis regarding the status of guineafowl populations, found correlations between extinct and/or declining guineafowl populations and intensive use of agrochemicals (Pero & Crowe, 1996).

9.3.2. Phase 2

A more detailed analysis on a landscape scale found correlations between healthy guineafowl populations on farms having high land-use diversity and extensive "edge" habitats (Malan & Benn, 1999).

Relatively few cases of deliberate poisoning were recorded (42 cases involving 145 birds since 1985) in the study area.

Very low concentrations (parts per billion) of DDT and Dieldrin were detected in the 36 samples analysed (Ratcliffe et al., in press).

Genetic contamination has occurred in only one of 36 birds sampled from eight wild populations (Walker et al., in review).

Analyses of blood samples detected no diseases capable of decimating populations of guineafowl (Horner et al., in review).

Habitat preferences included small maize fields, waste grain and fallow lands within a habitat mosaic (Ratcliffe & Crowe, in press). Of these grains, soya was found to be equal to maize in the energy obtained after digestion by guineafowl (Ratcliffe et al., in review).

Finally, associated bird diversity decreased with a decline in guineafowl population status (Ratcliffe & Crowe, in review).

9.4. DISCUSSION

In the initial stages of the project, many farmers, wingshooters and conservationists suspected the use of agrochemicals, illegal hunting, predation, genetic contamination and disease as potential causes of the decline of Helmeted Guineafowl populations. All of these factors have the potential to cause declines at the population level. However, the admittedly preliminary results presented here indicate that, although they might have serious effects on local populations, there is no evidence to suggest that these effects were or are acting on a more widespread scale (Ratcliffe & Crowe, 1999a).

We suggest that massive increases in maize production, and to a certain extent wheat and soya, during the late 1970s and 1980s (Berry & Whitehead, 1981; KwaZulu-Natal Department of Agriculture 1995a; 1995b) were responsible for the initial collapse of guineafowl populations in the Midlands and have prevented their subsequent recovery. Assuming that maize production depends on the amount of land under production, the negative correlation between pesticides and guineafowl numbers, reflects the increased crop agriculture, and therefore the subsequent indirect effects of agrochemicals, i.e. loss of food (weed seeds, leaves and insects) and cover (weeds in fallow areas or hedgerows). The statistical correlations between declining or near extinct guineafowl populations and extensive, crop monoculture agriculture, probably does not demonstrate a negative causal relationship between the use of herbicides and pesticides, but rather an indirect effect of habitat polarisation and a lack of a habitat mosaic and vital edge habitats.

The radio-tracking studies investigating use of habitat and assessments of associated overall avian diversity, showed that healthy guineafowl populations are associated with moderately polarised habitats, such as a matrix of small maize fields and fallow lands, which have extensive edge habitat (Ratcliffe & Crowe, in press). These edge habitats were further enhanced through the lack of pesticide and herbicide application resulting in optimal edge habitat that not only support healthier guineafowl populations, but also maintained high associated overall avian diversity (Ratcliffe & Crowe, in review).

It appears, therefore, that the large increases in crop agriculture from the mid-1970s - especially of maize - lead to a loss of this optimal guineafowl habitat and fragmentation of populations throughout the Midlands. In addition, the indirect effects of modern, more effective pesticides and herbicides probably exacerbated the situation by reducing the availability of food resources and edge habitats. Food sources and edge habitats have further been reduced through a whole host of modern farming equipment and techniques. These include the use of broad-based drains in contouring; centre pivots resulting in double cropping; combine harvesters reducing crop residues; and shorter growth and ripening periods of modern maize cultivars. This allows their early harvest which deprives guineafowl of an important winter food source (Pero & Crowe, 1996).

The result of these agricultural practices has been the fragmentation of populations that could have undermined the meta-population structure of Helmeted Guineafowl throughout the Midlands. Rolstad (1991) notes that habitat fragmentation causes 'distance-area effects, such as insularization and decreasing fragment size,' directly preventing dispersal and reducing population size. Furthermore, landscape effects, 'such as reduced fragment-matrix and interior-edge ratios, increase the pressure from surrounding predators, competitors, parasites and disease' (Rolstad, 1991). The isolation of guineafowl populations thus dampened or prevented immigration from neighbouring populations in the event of large localised mortality. They thus became susceptible to a variety of mortality factors such as disease, poisoning, illegal hunting, etc, all of which may result in localised extinctions. Guineafowl populations within the Midlands are thus probably being prevented from recovering due to the lack of optimal habitat continuity, on a landscape scale, between populations.

9.5. MANAGEMENT POLICIES AND RECOMMENDATIONS

Healthy guineafowl populations are associated with a mosaic of habitats on a landscape scale (Malan & Benn, 1999; Ratcliffe & Crowe, in press). Thus, creating suitable habitats involves combining management strategies at the conservancy level with the objective being to strive for landscape diversity and connectivity. Management strategies should include:

Habitat creation and management

1. Where possible, plant several small agricultural lands rather than one large monoculture. The idea here is to create a patchwork quilt of varying crops so as to increase edge habitats and habitat heterogeneity which are associated with healthy guineafowl populations (Ratcliffe & Crowe, in press).
2. Edge habitats can be enhanced further through implementing the use of traditional contour banks, as opposed to broad-based drains.
3. Field margins (headlands) should be preserved during mowing or the burning of fire breaks as this increases avian diversity while providing food and cover for guineafowl (Wolff & Milstein, 1987). When mowing pastures, leave a strip of uncut, weeded edge, while fire breaks should not incorporate these margins as part of the break. Furthermore, nesting guineafowl are known to be disturbed, injured or even killed during mowing of commercial pastures (pers. comm. Tony Porell, 1998), and thus the welding of an inexpensive ‘flushing bar’, as suggested by Wolff & Milstein (1987), can be implemented in mitigation.

4. Radio-tracking (Ratcliffe & Crowe, in press) revealed the importance of roosting sites in the Grassland Biome (sourveld) as a key missing habitat ingredient, and thus, in this habitat, even alien trees might be preserved or planted in the absence of indigenous species of similar form.

5. The extensive winter burning of, in particular, sourveld, results in large tracts of short grassland vegetation in late winter and early spring (Malan, 1998). This habitat is avoided by guineafowl (Ratcliffe & Crowe, in press). Thus, rotating burning so as to create a patchwork of burnt and unburnt areas within the bird's range, is preferable. Furthermore, it is desirable to avoid late (e.g. September and later) burns in winter as they fall close to the nesting period.

Food

1. Winter is a critical time for guineafowl to obtain preferred food sources/habitats including waste maize, weeds in fallow lands and some winter greenery (especially lucern). Again, the closer the proximity of this variety of food resources to one another, the better.

2. Radio-tracking confirmed that maize fields are preferred over those planted with soya as a food source although they are comparable in terms of energy (Ratcliffe et al., in review).

3. Early and efficient reaping of maize means that waste maize within these lands is unavailable to birds during winter. Leaving some patches of maize to be hand picked late in the season may ensure the availability of waste grain material through these critical periods. The remaining stalks also provide cover when birds are feeding on

this material. This may entail planting small sections of maize in the corners of lands which are left unattended (let the weeds grow!).

4. Radio-tracking data revealed that cattle feeding sites that were maize based - especially silage - were highly sought after by guineafowl (Ratcliffe & Crowe, in press). This reliance maybe as a result of the increased efficiency in maize harvest (combine harvesters) and subsequent reduction in waste grain. Any form of post-harvest waste should be left out for the birds if it is not to be used for silage.

5. If fields are to be ploughed, the earlier in winter the better, since guineafowl can benefit from the access to bulbs and tubers throughout the winter period.

Agrochemicals

1. Indirect effects of agrochemicals, especially insecticides and herbicides, appear to be more responsible for population declines in the Midlands than direct poisonings (Ratcliffe & Crowe, 1999a; Ratcliffe et al., in press). Use of these chemicals is an important component of modern, efficient farming. However, in order to reduce the potential direct and indirect effects they may have on wildlife in general and guineafowl in particular, three principles apply:

   i) identify the agricultural pest,
   ii) choose the correct chemical,
   iii) and most importantly, follow the manufacturers instructions regarding application.

2. In choosing a suitable agrochemical, Appendix 9.1 (Tables 1, 2 & 3) lists those associated with maize - in decreasing toxicity - and thus should provide some insight in this regard.

3. In addition, keep agrochemicals under lock and key, and educate users.

4. Spray early morning and evening as less insect life is airborne and thus the potential for chemicals to enter the food chain is reduced (Pero & Crowe, 1996).

Genetics

1. Do not introduce domesticated guineafowl in an attempt to resuscitate wild populations since interbreeding between domestic and wild birds may affect the viability of wild populations within the Midlands (Chapter 5).

2. Because domestic guineafowl are still assigned to the same species as wild forms, *Numida meleagris*, they are classed as game in the Natal Nature Conservation Ordinance, as though they were wild birds (Johnson, 1991). In terms of the ordinance permits are required to:-

- Keep guineafowl in captivity.
- Hunt guineafowl.
- Sell, dispose of or purchase guineafowl and eggs.
- Export and import, or transport live guineafowl and eggs.
- Remove eggs or live birds from the wild.
- Introduce guineafowl or eggs into any area.

The purpose of these laws is to prevent wild guineafowl stocks from being unnecessarily depleted or contaminated genetically.

3. During the wingshooting season, identify and, if necessary cull flocks that have birds which show signs of interbreeding with domestic guineafowl (see Ratcliffe & Crowe, 1999b).

Wingshooting

1. Monitor populations through yearly counts six to eight weeks before the

wingshooting season, and note the production of first year birds to get accurate assessments of the bag limit.

2. For grassland francolins *Francolinus* spp., 30 - 35 % of the available population is shot (Little & Crowe, 1993b). For guineafowl, due to the larger clutch sizes, shoot at least 35 up to 50 % of the available population.

3. Shoot each sub-population once, early in the season (June), so as to maximise the off-take of first-year birds which may be doomed to die or disperse anyway (Mentis, 1972; Johnson, 1984). Birds are more numerous, fatter and in better eating condition at this time than later in the season, while early harvest ensures greater survival for the remaining birds (various wingshooters, pers.comm.).

Socio-economics

1. Educate farm labourers and rural communities through involvement of communities in the sustainable utilisation of guineafowl (Maphasa, 1996). Create incentives in this regard through active involvement of farm labourers in the preservation, monitoring and utilisation of guineafowl populations.

It would be idealistic to think that landowners can implement all of these recommendations as economics often dictate otherwise. However, there have been some success stories. One conservation-oriented farmer had his guineafowl population reduced drastically by deliberate poisoning with maize kernels soaked in the insecticide Curaterr (I. Mitchell-Inness pers. comm., 1997). Since leaving patches of his less profitable land go to fallow, his populations have bounced back from less than 100 birds, to several hundred in less than two seasons. However, if his neighbours do not manage their properties similarly, and re-create the meta-population structure that existed up until the 1970s, sooner or later, his isolated guineafowl population could collapse again.

It is interesting to note that while research was being conducted within the KwaZulu-Natal Midlands, reports of similar declines in populations of Helmeted Guineafowl were noted in the neighbouring region of southeastern Mpumalanga (L. Kruger, pers. comm., 1998). Gamebirds have often been cited as environmental indicators of rural landscapes (Jansen et al., 1999), and thus the demise of this extremely resilient gamebird can be seen as a barometer of the state of biodiversity within these agricultural areas. Further studies need to highlight the conservation of biodiversity within rural landscapes, whereas viable, diversity-friendly, management practices must be sought as they potentially hold the key to the persistence of biodiversity associated with these areas.

Appendix 9.1.

Table 1. Insecticides used on maize crops within the study area, listed in order of decreasing toxicity. (Modified from Pero & Crowe, 1996).

Trade name	Active ingredient	Monogram	Form	Toxicity group
Nuvacron	Monocrotophos*	Organophosphate	SL	Ia
Temik	Aldicarb*	Carbamate	GR	Ia
Thimet	Phorate*	Organophosphate	GR	Ia
Counter	Terbufos	Organophosphate	GR	Ia
Curaterr	Carbofuran*	Carbamate	GR	Ib
Promet	Furathiocarb	Carbamate	CS	Ib
Oncol	Benfuracarb	Carbamate	EC,LS	Ib
Bestox	Alphamethrin	Pyrethroid	EC	Ib
Cybolt	Flucythrinate	Pyrethroid	EC	Ib
Decis	Deltamethrin	Pyrethroid	EC,UL	Ib
Dursban	Chlorpyrifos*	Organophosphate	EC,GR	Ib
Endosulfan	Endosulfan*	Organochlorine	EC	Ib
Karate	Lambda - cyhalothrin	Pyrethroids	EC	Ib
Lindane	Gamma-BHC	Organochlorine	EC,DP,DS	Ib
Lorsban	Chlorpyrifos*	Organophosphate	EC,WP	Ib
Semevin	Thiodicarb	Carbamate	FS	Ib
Thiodan	Endosulfan*	Organochlorine	UL,WP,EC	Ib
Tralate	Tralomethrin	Pyrethroid	EC	Ib
Baythroid	Cyfluthrin	Pyrethroid	WP,EC	II
Bulldock	Beta cyfluthrin	Pyrethroid	EC	II
Gaucho	Imidacloprid	Imidazolidine	WS	II
Lindstof	Gamma-BHC	Organochlorine	DS	II
Ripcord	Cypermethrin*	Pyrethroid	EC	II
Sumiciden	Fenvalerate	Pyrethroid	EC	II
Fenom	Cypermethrin*	Pyrethroid	EC	II
Bonus	Quinalphos	Organophosphate	RB	II
TMTD	Thiram	Dithiocarbamate	DS	II
Ambush	Pemethrin	Pyrethroid	EC	IV

* Active ingredients implicated in avian poisoning cases in KwaZulu-Natal

Appendix 9.1.

Table 2. Herbicides and fungicides used on maize crops within the study area, listed in order of decreasing toxicity. (Modified from Pero & Crowe, 1996).

Trade name	Active ingredient	Monogram	Form	Toxicity group
Gramoxone	Paraquat	Dipyridyl	SL	Ib
Basagran	Bendioxide	Organochlorine	SL	II
2,4-D Amine	2,4-D Amine	Chlorophenoxy	SL	II
Eptam	EPTC	Dithiocarbamate	EC	II
Gardimol	Terbuthylazine / atrazine	Organonitrogen	SC	II
Gesapax	Ametryn	Organonitrogen	SC	II
Guardian	Acetochlor	Organonitrogen	EC	II
Harness	Acetochlor	Organonitrogen	EC	II
Lasso	Alachlor	Organonitrogen	GR,EC	II
Preeglone	Diquat / paraquat	Dipyridyl	SL	II
Ratel	Acetochlor	Organonitrogen	SC	II
Relay	Acetochlor	Organonitrogen	EC	II
Spotaxe	2,4D	Chlorophenoxy	SL	II
Terbo	Terbuthylazine	Organonitrogen	SC	II
Topogard	Terbutryn / terbuthylazine	Organonitrogen	WP	II
Wenner	Acetochlor	Organonitrogen	EC	II
Punch - Xtra	Flusilazole	Triazole	SC	II
Impact	Flutriafol	Organonitrogen	SC	II
Galleon	Atrazine	Organonitrogen	SC	III
Gesaprim	Atrazine	Organonitrogen	SC	III
Sencor	Metribuzin	Organonitrogen	SC,WP	III
Suprazine	Atrazine / terbuthylazine	Organonitrogen	SC	III
Accent	Nicosulfuron	Organonitrogen	WG	IV
Dual	Metolachlor	Organonitrogen	EC,GR	IV
Falcon	Metolachlor	Organonitrogen	EC	IV
Roundup	Glyphosate	Glyphosate	SL	IV
Touchdown	Glyphosate	Glyphosate	SL	IV
Sting	Glyphosate	Glyphosate	SL	IV
Punch C	Carbendazim	Carbamate	SC	IV
Benlate	Benomyl	Carbamate	WP	IV
Derosal	Carbendazim	Carbamate	SC	IV

Appendix 9.1.

Table 3. Formulation types and their international codes (Agricultural and Veterinary Association of South Africa, 1993).

CODE	FORMULATION TYPE
DP	Dustable powder
DS	Dry seed powder
EC	Emulsifiable concentrate
ED	Electrochargable liquid
FS	Flowable concentrate for seed treatment
GE	Gas generated product
GR	Granule
OL	Oil miscible liquid
RB	Bait
SC	Suspension concentrate
SL	Soluble concentrate
SP	Water soluble powder
UL	Ultra-low volume liquid
WG	Water dispersible granules
WP	Wettable powder
WS	Water dispersible powder

The RSA classification code for agricultural and stock remedies (Agricultural and Veterinary Association of South Africa, 1993)

Group	Label	LD50 *Oral For The Rat Solids**	(mg/kg Body Mass) Liquids**
Ia	Extremely hazardous	5 or less	20 or less
Ib	Highly hazardous	5 - 50	20 - 200
II	Moderately hazardous	50 - 500	200 - 2000
III	Slightly hazardous	over 500	over 2000
IV	Acute hazard unlikely in normal use	over 2000	over 3000

*LD50 is a statistical estimate of a lethal dose of the chemical which will kill 50% of the test animal under stated conditions.

**The terms "solids" and "liquids" refer to the physical state of the product or formulation being classified.

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