Large mammal monitoring in Lambusango

Interim Progress Report January 2007

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Executive Summary

This report details work carried out thus far on three species of large mammal (anoa *Bubalus depressicornis*, Sulawesi wild pig *Sus celebensis* and Buton macaque *Macaca ochreata brunnescens*) in the Lambusango forests of Buton, South-east Sulawesi as part of the Lambusango Forest Conservation Programme.

Anoa have been surveyed by recording presence and absence of tracks along four 3km transects at each of six study sites and data have been analysed using patch occupancy analysis. The results from monitoring since 2004 suggest that the overall population in Lambusango is stable. However, significant declines were recorded in the Lawele area. Trends in the other study sites are currently unclear.

Hunting rates, surveyed by village questionnaires revealed hunting to be apparent at rates above those reckoned to be stable for the Lambusango anoa population. The highest hunting rates were also recorded from villages in the Lawele area.

Samples of faecal material have been taken for DNA analysis. Currently optimisation of methods is underway and results to date suggest that it will be possible to estimate population size based on the number and quality of samples taken.

Anoa track, hunting and faecal surveys are to continue in 2007.

Wild pig track surveys have been carried out at each of the camps for 2004. They will be repeated in 2007. The data have yet to be analysed.

Buton macaque line-transect surveys were carried out in 2005. Initial results suggest a healthy macaque population in the reserve, however, further data from a repeat survey in 2007 are required to estimate and detect trends in population size.

Purpose

The aim of this report is to outline data sets that have been collected during the project period to date and identify those that will be collected before the completion of the project in October 2008. It also includes preliminary analysis of some data sets as an indication of trends to date.

Scope

The report details information collected from the pre-project survey in July-August 2004 and from the beginning of monitoring for the project proper in July-August 2005 and subsequent survey periods in May-June 2006 and July-August 2006. Two species of large mammal are included: the Anoa (assumed to be Lowland Anoa *Bubalus depressicornis*) which is the primary focus of the monitoring programme and the Buton Macaque (*Macaca ochreata brunnescens*).

Outline

This report details progress to date of the Large Mammal Monitoring project operating as part of the Lambusango Forest Conservation Project (Proyek Konservasi Hutanan Lambusango - PKHL). The first chapter 'Introduction' gives a justification for techniques used. Chapter 2 'Methods' is a brief description of the methods used in these surveys. The third chapter 'Results' details a preliminary analysis of the presence/absence and hunting data for anoa and population estimates for the Buton macaque. The fourth chapter, 'Conclusions and Development', outlines some brief conclusions and details the future direction of the project up until its completion in October 2008. Appendix 2 contains a brief description of all data collected and planned data collection until the end of the project.

Contents

1: Introduction

1.1 Estimating population size

Ecological monitoring is a vital component of any conservation project so that the effect of management can be assessed. The foundation of any species-based conservation management programme is an estimate of population size or density of the species in question. Accurate estimates of population size as a function of environmental change and habitat disturbance are necessary to predict the long-term persistence of animal populations (Sutherland 1996). Unfortunately, an accurate estimate of population density is difficult to obtain for many species and requires considerable investment of resources and time (Witmer 2005). The most established survey method for estimating animal population size is the line transect. Line transects are conducted by travelling in a straight line, usually on a randomly allocated bearing so that the number of animals or group of animals seen from the transect can be recorded. The perpendicular distance from the transect line to any animal sighted is measured. An estimate of population density can be converted from these distances by modelling the probability of detection as a function of distance from the transect line (Buckland et al. 2001, Schwarz & Seber 1999). This technique has been used to estimate population density for a variety of mammal species in tropical forests, especially since the development of the computer programme DISTANCE by Buckland et al. (2001) which estimates densities from line transect and point sampling. As a rule of thumb, approximately 60 to 70 animal sightings are needed to generate robust density estimates using DISTANCE (Buckland et al. 2001). Where species are rare or elusive it may be difficult to

obtain sufficiently large sample sizes to estimate population size robustly. In rainforest habitats, elusive forest dwelling and nocturnal animals are often difficult to observe due to the poor visibility in these forests. Furthermore, when using line-transect methods researchers often make a lot of noise as they trace their line, causing animals to flee before they are observed (MIKE 2004). Distance sampling has been recommended as the most suitable survey technique for primates (Chiarello & de Melo, 2000, Fashing and Cords, 2000, Peres, 1999, Rosenbaum, *et al.* 1998) but has the additional problem that detection probability may vary with group size. Furthermore, for some species with fission-fusion dynamics, it is unclear what mean group size is.

Many animals leave a multitude of signs of their presence, such as dung, nests or tracks, which are often much easier to quantify than the animals themselves. Signs do not flee and they are often much more numerous than the animals themselves. For this reason researchers and managers often choose to count animal signs (MIKE 2004). However, indirect counts require conversion factors to be calculated to convert the count of animal signs (e.g. dung or nests) to the density of animals, which also take account of the production and decomposition rates of such signs (Plumptre 2000). However, gathering data on these rates is a time consuming and expensive task (MIKE 2004).

Another widely used method for obtaining information about animal populations is to use tagging or marking; this approach is known as the capture-mark-recapture (CMR) method. In CMR, one collects a series of samples. Animals captured in the first sample are tagged and then released back into the population. The second sample then has tagged (i.e. recaptured) and untagged animals. Population estimates are obtained by equating the proportion of tagged animals in the population with the proportion of tagged in the second sample to get the population size (Schwarz & Seber 1999). The accuracy of trapping-based methods depends on individuals being readily captured, but the handling of rare or endangered species may cause injury or even death of the

animals (Greenwood 1996). Besides that, animals may lose their tags, tags maybe overlooked or may not be returned when found by the general public (Schwarz & Seber 1999).

In recent years, the use of accumulation rates of species photographs derived from camera trapping as indices of population size for rare elusive large mammals has received a fair amount of attention. Individual animal identifications from these photographs have also been used as a new method of 'tagging', and population density can be estimated using mark-recapture analysis (Karanth 1995, O'Brien at al. 2003). Photo-identification methods for tagging individuals have none of the negative impact associated with direct animal tagging. However, the technique is best used for animals with distinctive morphological differences or natural markings that can be readily identified in photographs (Karanth et al. 2004). Consequently, non-invasive tagging methods that do not require handling of individuals, provide loss-resistant natural markings and can be applied to any species are required in order to study wildlife ecology more efficiently.

None of the above methods seem appropriate for surveying anoa. Line transect surveys detected only 2 anoas along 182.1 km of transects in Tanjung Peropa, 3 along 50 km in Tanjung Batikolo, and 3 along 202.7 km in Rawa Aopa Watumohai (Riley et al. 2001a, b). In a subsequent year, 20 sightings were detected along 372 km of transect in Tanjung Peropa and 8 sightings over 124.3 km in Tanjung Amolengo (Mustari 2003). Even automatic camera trapping generated very few anoa captures: 1 picture during a total camera-trapping time of 4930 hours in Tanjung Peropa (Riley et al. 2001b) and none were photographed during 3523 hours of camera-trapping in Rawa Aopa Watumohai (Riley et al. 2001a).

1.2 Patch occupancy analysis

An alternative to estimating population size or density in a wildlife monitoring programme is the proportion of total survey area occupied by the species. Determining whether a species is present at a sampling location may be much less costly and time consuming than collecting information required for

estimating density. Although abundance and occupancy address different aspects of population dynamics, these two variables should be positively correlated at an appropriate scale. Thus, occupancy should decrease as the species abundances decreases. Recent development of likelihood-based approaches that includes heterogeneity in probability of detecting animals in the field (e.g. MacKenzie et al. 2002) has provided a statistically robust framework for modelling occupancy data. This has enabled occupancy to be seriously considered as a surrogate for abundance in monitoring programmes. Moreover the production of a dedicated computer programme (PRESENCE; MacKenzie et al. 2006) for the analysis of presence-absence data to estimate patch occupancy has facilitated the use of the method for monitoring programmes. The technique involves repeated observations of a number of sample sites where the presence or absence of the study species is recorded. This allows the probability of detection (i.e. the probability that the species will be observed given it is there) to be estimated. It is then possible to estimate two parameters relevant to the distribution of the species: proportion of sites occupied and probability of detection. The former is analogous to distribution, the latter to relative abundance (Mackenzie et al. 2006). Typically observations of sites are repeated over time, but it is also possible to generate spatial replicates (e.g. sections along a transect). The method allows 'low-grade' observational data such as spoor to be used in a statistically robust way to estimate distribution and relative abundance.

1.3 Molecular methods

Molecular genetic data have become an essential tool in biodiversity conservation management. Conservation management is increasingly relying on molecular genetic approaches to obtain essential information such as measures of genetic variability in threatened populations, pattern of gene flow in fragmented populations, and paternity and kinship analysis. The adoption of non-invasive molecular methods has become a promising alternative for estimating wildlife abundance (Palsboll 1999).

It has been shown that important parameters in wildlife conservation such as population size have been virtually impossible to estimate in some cases using the 'traditional' mark recapture or transect methods, due to the high capture probabilities and/or the large numbers of animals required for reasonable estimates. These limitations are especially acute for rare elusive species with low densities (Mills et al. 2000).

Genetic capture-mark-recapture (CMR) techniques have been used to estimate population sizes of various mammal groups such as carnivores (Bellemain et al. 2005, Frantz et al. 2004, Wilson et al. 2003) and ungulates (Eggert et al. 2003) using techniques similar to the photo-identification method derived from camera trapping surveys (Karanth 1995, O'Brien at al. 2003). These studies showed that genetic CMR techniques have generated reliable population sizes that were well within the range estimated from concurrent independent field studies.

Individual-specific DNA fingerprints are obtained non-invasively from faeces, shed hair or skin without the necessity to sample the animal itself. Each fingerprint is treated as a 'mark' and a 'recapture' is recorded whenever an identical genotype is found in two separate DNA samples collected from the field. Population size then can be estimated using a capture-mark-recapture (CMR) analysis appropriate to the sampling design, based on the probability that a population of a given size and structure would yield the observed recapture rate.

The use of non-invasive approaches offers advantages over conventional CMR techniques, including increased capture probability, decreased tag loss, and the potential to minimize the impacts of capture and marking (Mills et al. 2000). Individual identification using traditional tags or natural markings has the disadvantage that the tag itself contains little or no information beyond the identification of the individual (Palsboll 1999). On the other hand, genetic marks contain information that can be used to describe various aspects of the species' natural history, population structure, and its sex, besides providing individual tagging for CMR density estimates. The common

genetic markers used in such studies include microsatellites, the SRY gene on the Y chromosomes and Mitochondrial DNA (Beebee & Rowe 2004).

1.4 Project summary and aims

This project will adopt non-invasive molecular methods to estimate anoa density and investigate their distribution within the Lambusango forest. Mitochondrial DNA will be extracted from faecal samples to re-determine which species occur in Lambusango (i.e. lowland or upland anoa). Furthermore, individual anoa will be genetically tagged using DNA recovered from faecal samples and microsatellite molecular genetic markers. The data will then be analysed using capture-mark-recapture (CMR) methods to estimate their population size and distribution.

As well as estimating population size using non-invasive molecular methods, site occupancy of the anoa and potential factors influencing it (i.e. human trails) will also be examined as an alternative rapid monitoring measure. Using this technique, the proportion of area occupied by anoa will be estimated by recording the presence and absence of tracks in the study sites. We will also estimate occupancy rates for Sulawesi wild pigs (*Sus celebensis*) and estimate abundance of Buton Macaque (*Macaca ochreata brunnescens*) at the start and end of the project.

The monitoring programme is conducted in six sampling nodes, established in 2004, that cover a wide range of altitudes and topography in the study area. Four sampling nodes are situated within the Lambusango Forest Reserve, and two are within the adjacent limited production forests. Each sampling node consists of four parallel 3 km-transects, each is 1 km from another and marked every 50 meters (Figure 1.1). Faecal samples are collected on the transects and from areas walked between transects whereas anoa and pig tracks are recorded along the transects. Encounters with Macaques are recorded along transects.

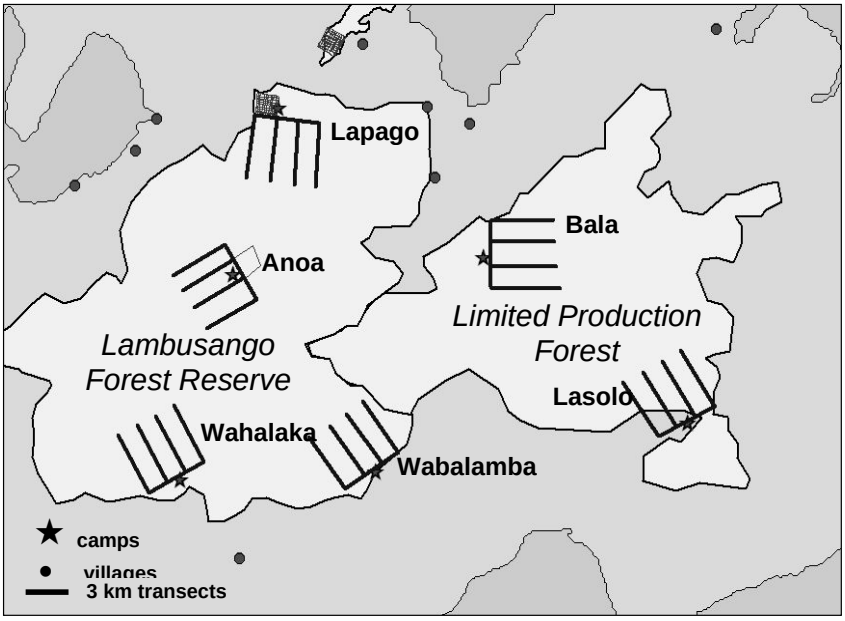

Figure 1.1. Locations of sampling nodes in the Lambusango forests (Seymour 2005).

The role of communities that directly and indirectly use forest resources is highly significant in determining the success of any wildlife conservation programme. Hence, in addition to the techniques detailed above used to examine the population structure, abundance and distribution of the anoa, village surveys will also be carried out to examine the level of hunting on the anoa population. Both approaches will eventually be combined and lead to conservation and management recommendations for the anoa.

2: Methods

2.1 Patch occupancy analysis

The distribution and relative abundance of anoa was determined from the presence of their tracks across the study area. Anoa tracks can be readily distinguished form other ungulates (feral cows and wild pigs) that exist in the study area on the basis of their size and shape. Anoa tracks were recorded every 50 metres along the transect lines during the months of July and August 2004, 2005 and 2006. Every year, each transect line was walked once at a pace of approximately 800 m per hour to look for anoa tracks. A set of tracks was recorded as a single count when moving across the transect or clearly following the transect line. Where several tracks were seen in close proximity but crossing the transect in different directions, they were regarded as separate sets of tracks.

For analysis, 3km transects were divided into 500m sections. To avoid spatial autocorrelation, only alternate 500m sections were used in the analysis. Within each section alternate 50m sections were used as independent replicate sampling points since anoa were never observed to follow the transect for more than 50m. The presence (1) or absence (0) of anoa tracks was recorded within each 50 m sampling point. Thus each year, a maximum of 72 sampling sites from 6 node camps each with 5 replicate sampling points were surveyed. A failure to survey some parts of the transect lines was regarded as missing data. Data were analysed using program PRESENCE V.2 (MacKenzie et al. 2006). Changes in both proportion of area occupied (ψ) and probability of detecting anoa (p) across the study sites will be a reflection of changes in anoa abundance. Royle and Nichols (2003) suggest that variations in abundance at different times and places are probably the most important reason for variation in detection probability. Lower number of animals usually result in lower detection probability during surveys. Bayle et al. (2004) found that density of salamanders in their study area affected the probability of detection for most species. They reported that as density increased, the probability of detection of individual salamanders also increased. Conversely differences in proportion of area occupied relate to changes in the distribution of the species.

In order to evaluate the effect of human disturbance on anoa distribution, the number and size of human trails within the study area were also recorded. Human trails were found in the forests crossing the transect lines. We scored human trails with values 1 to 3 based on their size and evidence of frequency of use. The following are descriptions of each score:

Trail Score	Details
1	Less than 50 cm wide covered with leaf litter along the trails showing infrequent use by humans
2	Between 50 cm to 1 m wide covered with thin leaf litter along the trails showing more frequent use by humans
3	One metre wide or more. Trails covered with thin leaf litter or none, showing frequent use by humans

'TRAILS' were included as covariates in the analysis to study their effect on anoa occupancy and probability of detection. The best model was chosen from various models generated from PRESENCE analysis using Akaike's Information Criterion (AIC) (Mackenzie et al. 2006). The proportion of area occupied (ψ) by the target species and the probability of detecting them (p) are functions of environmental covariates. Using PRESENCE, the effect of those covariates on ψ and p is assessed using a logistic regression approach (using the logit link) and described using covariates' coefficient values. Positive values indicate covariates that are positively associated with ψ and p while negative values indicate that the covariates are negatively associated with the relevant probability. In these analyses, the number of human trails of different sizes (size one, two or three), that exist in each 500m sampling site were used as site covariates.

In order to be able to elucidate the relationship between human trails and both ψ and p, two different sets of models were built at the same time. In the first, ψ was held constant while p was run as a function of human trails, ψ(.)p(trails). In the second, p was held constant while ψ was run as functions of

human trails, ψ(trails)p(.). From these various models, models with the highest AIC weight were chosen as the best estimators of ψ and p. Best fit coefficient values of the covariates human trails were noted only when these covariates were included in the best models.

2.2 Hunting Surveys

Data on anoa hunting in the study areas were collected from May to July 2006 by interviewing villagers living adjacent to the Lambusango forests. Each of four surveyors attempted to interview two villagers each week during a three month period using semi structured questionnaires. The villagers were asked when the last time they have eaten, bought or caught anoa was, or if they ever heard of any anoa hunting incidents.

Data generated from the questionnaires was used to count the number of hunting incidents in 2004, 2005 and 2006. However, since the nature of questions in the interview asked when the last time respondents have eaten, bought or caught anoa or if they ever heard of anyone catching anoa, these could only record the minimum incidents in each year.

2.3 Non-invasive DNA sampling

Faecal samples were collected from the six 9 km^2 sampling nodes in the Lambusango forests from 3 seasons over two years: dry season (July to August) 2005 and 2006 and wet season (May to June) 2006. A team of 3 persons walked along and outside the node camp transect lines covering as much area as possible to opportunistically search for faecal samples. Approximately 5 ml of each faecal sample were collected using a plastic scraper and put in 20 ml tubes. Where possible, two tubes of sample were collected for each faeces, one from the outer layer and another from the inside part of the faeces. Different scrapers and tubes were used for each sample to avoid cross contamination. ‘Fresh’, ‘recent’ and ‘dry’ samples were collected following Beyers et al. (2001) with some modifications. ‘Fresh’ faeces were defined as “sometimes still warm, with fatty acid sheen glistening on exterior and strong smell” and ‘recent’ faeces were defined as “odour present, there may be flies,

the fatty acid sheen has disappeared, but in general faeces were still moist". 'Dry' samples were also collected, where faeces had not yet disappeared but had generally lost their wetness.

In the field, samples were preserved in 95% ethanol at the point of collection and later were stored in the refrigerator. The preserved samples were subsequently stored in the freezer until the DNA was extracted. For each faecal sample, the collection date, collector's name and geographic location (using GPS) were recorded. Photographs were also taken of each sample for later reference.

Since feral cows *Bos taurus* also occur in the study area and their droppings look fairly similar to the anoa's, species identification of the faeces was conducted using the mitochondrial cytochrome b gene. Cow DNA were extracted from minced beef using the Phenol Chloroform method (Taggart et al. 1992). Primers were designed using aligned sequences of cow (GenBank accession number: AY526085), and lowland and upland anoa (Schreiber *et al.* 1999) to PCR amplify 3 fragments of the cytochrome b gene. Primers U12 and L235 were used to amplify 246 bp (PCR1), U108 and L235 for 150 bp (PCR2) and U12 and L155 for 164 bp (PCR3) respectively. Inter-specific sequence variation in the cytochrome b gene should allow the anoa faeces to be separated from that of the wild cattle species (Schreiber et al. 1999).

For DNA-based capture-mark-recapture (CMR), Lukacs & Burnham (2005) suggested sampling a set of quadrats to better define sampling occasion in faecal sampling. Using this method, quadrats can be considered as sampling occasions. In order to implement this on the difficult terrain of Lambusango, a set of 250 m by 250m virtual grids was overlain on maps of the node camps in order to build a clearly defined capture history for the anoa. Samples with the same genotype collected in different 250m square grids were thus considered as a recapture.

Various combinations of cycling temperature, $MgCl_2$ concentration and two types of Taq Polymerase (AmpliTaq Gold, ABI and BioTaq, Bioline) were trialled to optimise PCR conditions. PCR reactions totalling 10 µl, containing 20 ng of target DNA, 0.25 pmol of each primers, 0.2 mM dNTP, 1.5 to 2.5 mM

$MgCl_2$, Taq Polymerase and 1x PCR buffer, were prepared and thermocycled for 35 rounds at the following temperature conditions: 94^{o}C, 30s; 48 to 54^{o}C, 30s; 72^{o}C, 45s. PCR products were then separated on an agarose gel using electrophoresis, stained with ethidium bromide and viewed under ultraviolet (UV) light. Once PCR conditions were optimised, further tests were conducted to see if the conditions also worked for DNA concentrations as low as 0.025 ng/µl.

2.4 Macaque Surveys

Macaques were surveyed on line-transects. Transects were walked at a speed of around 1km per hour, starting at 6.30am. Primates are most active in the morning, and surveys were completed by 11.00am. When a group was encountered and it was possible to watch a group, the maximum number of individuals present was recorded over a 10 minute observation period. Distances and bearings from the transect for the individual first identified were recorded. The relatively low number of encounters precluded analysis with program DISTANCE in this case, but it is anticipated that when two seasons' data have been collected, sufficient sightings will have been made to allow a full DISTANCE analysis.

3: Results

3.1. Patch Occupancy Analysis

Program PRESENCE was used to generate a range of models for each study site and for the whole Lambusango area, ordered by their AIC weight. (Appendix 1). During the surveys, anoa signs were difficult to detect resulting in a much lower naïve estimate of proportion of area occupied (PAO) than if the probability of detections were incorporated in the analysis using program PRESENCE.

Figure 3.1 shows the naïve estimates of PAO from year to year as well as estimates of proportion of area occupied (ψ) and probability of detection (p) generated from best fitting models produced with PRESENCE. For the whole Lambusango area, from all six sites combined, there was no substantial or significant change in ψ or p over the three years, though the decline in p between 2005 and 2006 (from around 0.55 to 0.4) may indicate a small decline in population size.

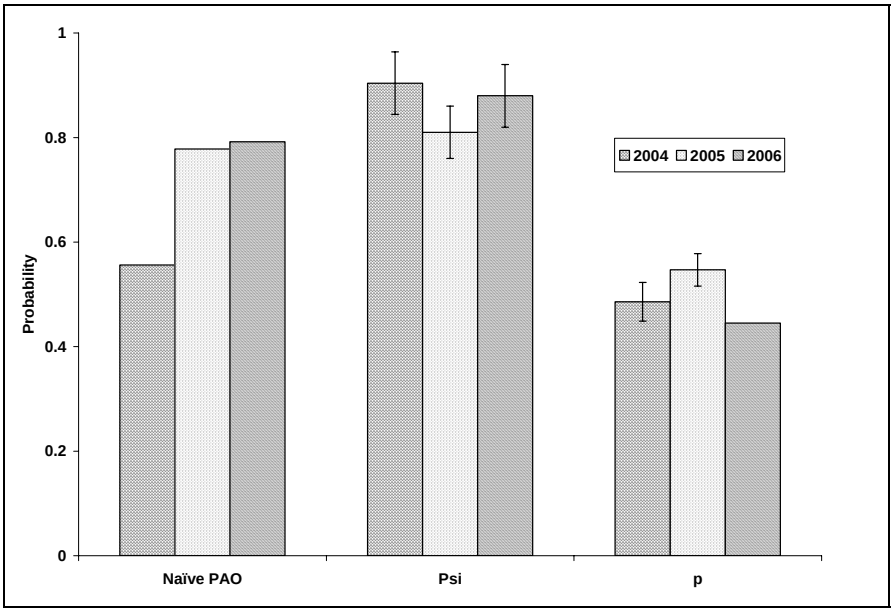

Figure 3.1. Naïve and PRESENCE estimates of the proportion of Lambusango forest area occupied by anoa (ψ) and the probability of detecting anoa (p) in the study area in 2004, 2005 and 2006.

Results of best-fit models for ψ and p are shown in figures 3.2 and 3.3. Since sample sizes for the camps are much smaller than for the overall area, error bounds are larger. The only camp that showed a significant decline in ψ was Lapago. No camps showed significant increases in ψ.

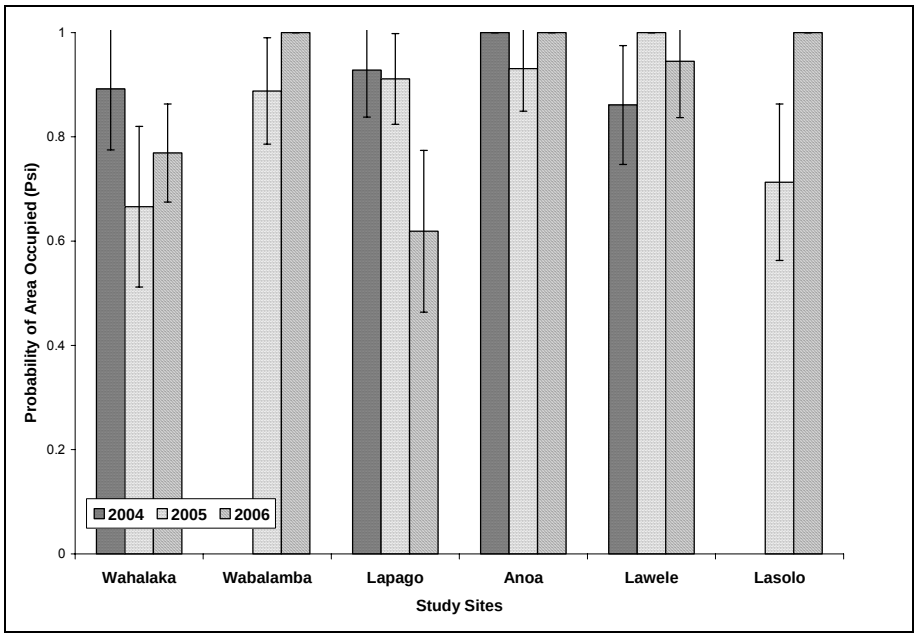

Figure 3.2. Proportion of area occupied (ψ) by anoa over years 2004, 2005 and 2006 in the six Lambusango forest study sites.

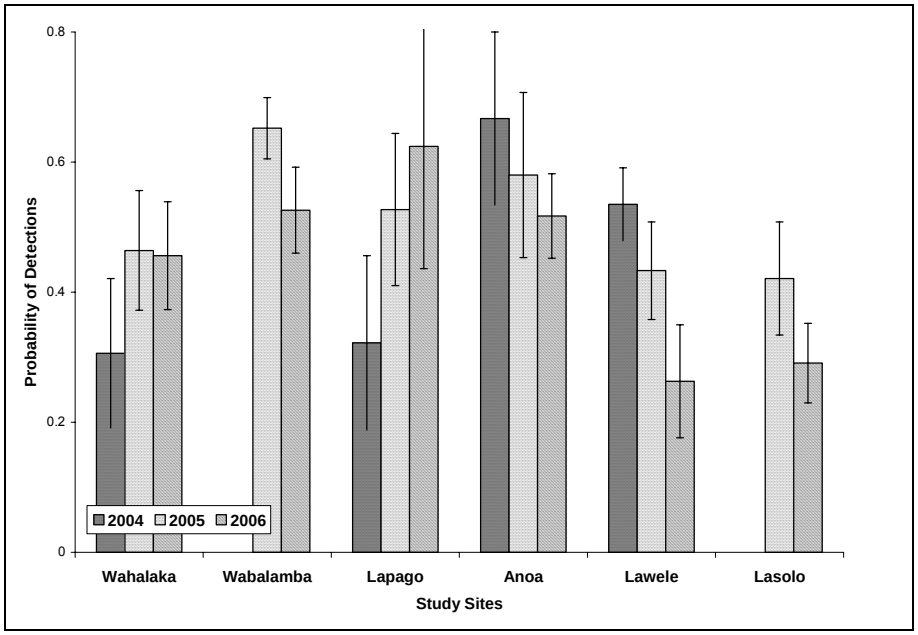

Figure 3.3. Probability of detecting anoa (p) over years 2004, 2005 and 2006 in the six Lambusango forest study sites.

Four of six study sites (Wabalamba, Anoa, Laweli and Lasolo) exhibited consistent declines in p over two or three years. Again, error bounds are large, but the consistency of the trend suggests that these are real declines in population size, particularly in the Lawele site. Lapago demonstrated a consistent increase in p, though, again error bounds are too large to draw firm conclusions.

The number of human trails counted on transects varied considerably from year to year and from camp to camp (Figure 3.4)

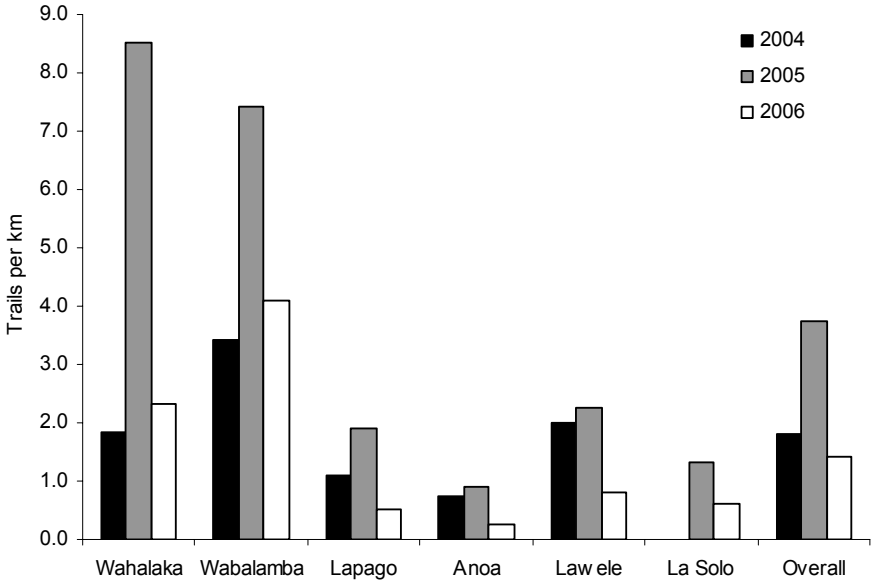

Figure 3.4. No. trails per km at each camp and overall in Lambusango

While all camps showed an increase in trails from 2004 to 2005 that might indicate a change in counting method or observer ability, these increases were not consistently large across camps. Moreover, the two sites which showed the largest increases in 2005 were also the only ones where 2006 trail density was higher than 2004. At the very least, this suggests that Wahalaka and Wabalamba have more human trails than any other camp and the very large increases in trails recorded at these two camps in 2005 represent qualitative increases in trail number.

The value of ψ and p at the study sites may be functions of the effects of human trails. The effects of trails on anoa distribution and abundance was investigated by including them as covariates in a PRESENCE analysis (Appendix 1). The relationships between trail number, size and occupancy is complicated and not consistent. Over the three years of surveys, trails of size 2 and 3 generally had negative correlations to the value of ψ and p, while trails size 1 had both negative and positive ones (Appendix 1).

3.2 Anoa hunting

During the three month survey period of May, June and July 2006, 173 respondents from 43 villages were interviewed. In these interviews, respondents reported anoa meat consumption covering the last 20 years from 1986 to 2006. Among them, 70 (41%) reported eating anoa in at least one year while the rest (59%) claimed never to have eaten anoa. Various ways of obtainig anoa meat were reported by the respondents. Three respondents said they were given anoa meat, 43 claimed to have bought it and 24 had hunted the animal themselves. This suggests that 60% of people who have eaten anoa (20% of respondents) paid for the meat and is tentative evidence that there may be a significant market in anoa meat.

During the survey, 11 hunters from various villages were reported to be active in the period 2004 to 2006. The survey also recorded a minimum of 24 anoa hunted during this 3 year period: two in 2004, 14 in 2005 and 8 in 2006. It is possible that the small number recorded from 2004 and higher number from 2005 reflect are simply due to inaccurate memory of respondents and should not be taken to indicate a change in hunting effort, rather the average annual take (8 individuals) should be regarded as representative of hunting effort. The highest hunting level occurred in the Lawele area with six anoa killed in 2005 and four in 2006. This was followed by Lasolo with one anoa in 2004, five in 2005 and two in 2006. In the Wahalaka-Wabalamba area four anoa were reported killed from 2005 to 2006 and in the Lapago area two anoa were reported killed between 2004 and 2006 (Table 3.1).

Table 3.1. The minimum number of anoa reported hunted in Lambusango from 2004 to 2006.

Adjacent study sites	Village	2004	2005	2006	Total
Lapago	Watambo	1			1
Lapago	Labundo Bundo			1	1
		1		**1**	**2**
Lasolo	Walompo	1	2		3
Lasolo	Karya Jaya		2		2
Lasolo	Lasembangi		1	1	2
Lasolo	Wasuamba			1	1
		1	**5**	**2**	**8**
Lawele	Sri Batara		1		1
Lawele	Lawele		5		5
Lawele	Lawele			1	1
Lawele	Suandala			2	2
Lawele	Togo Mangura			1	1
		0	**6**	**4**	**10**
Wahalaka	Wakaoleili		1		1
Wahalaka/Wabalamba	Kabongka		1		1
		0	**2**	**0**	**2**
Wabalamba	Wolowa		1		1
Wabalamba	PT Yuman			1	1
		0	**1**	**1**	**2**
Total		**2**	**14**	**8**	**24**

It is important to note that these levels of hunting (average 8 individuals per year) are above what Wheeler (2005) estimated to be sustainable for Lambusango (Figure 3.5).

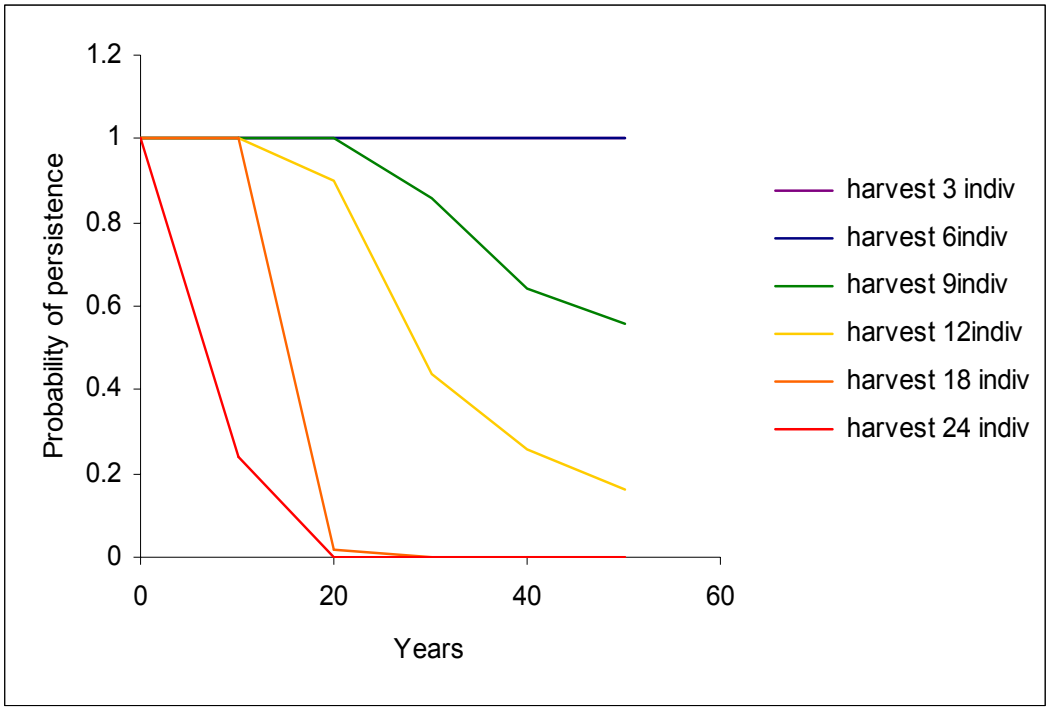

Figure 3.5 Probability of persistence to 50 years of Lambusango Anoa population under different hunting regimes. From Wheeler (2005)

3.3. Non-invasive DNA sampling

Anoa faecal samples were collected during three seasons over two years (2005-2006). During this time, 113 faecal samples were collected from the six node camps (Table 3.2). Kakenauwe Nature Reserve was additionally surveyed in the 2006 wet season as because it was thought that anoa from Lapago might have entered the Kakenauwe area. We collected five dung samples from Kakenauwe, thus a total of 118 samples were collected in two years.

Table 3.2. Number of anoa faecal samples collected in six node camps.

Camps	Number of faecal samples		
	dry season 2005	wet season 2006	dry season 2006
Wabalamba	8	6	25
Anoa	7	8	16
Wahalaka	6	12	10
Lawele	0	2	4
Lasolo	0	3	4
Lapago	0	-	2
TOTAL	21	31	61

The number of samples collected increased from year to year as the researchers gained more skills and knowledge of where and how to find anoa faeces. During the sampling it became apparent that anoa prefer forest areas with clear understorey and good canopy cover to rest and defecate. These places usually occur on relatively large, flat areas in valleys or on the top of hills. However, thorough searches along and off all transects were still conducted to avoid bias in sampling design.

Analysis of these samples is at an early stage, but optimisation carried out to date suggests that it will be possible to identify individual animals from the small amounts of DNA found in anoa faeces.

Macaque Surveys

In total 42 encounters with Macaques were made in the 2005 survey (Table 3.3). Wabalamba had most encounters, while nearby Wahalaka had least. There was no relationship between number of encounters and mean group size at a camp.

Table 3.3. Distribution of Macaque sightings

Camp	No. Encounters	Mean group size
Anoa	7	6
Bala	6	11.7
La Solo	9	4.1
Lapago	6	6.7
Wabalamba	12	9.3
Wahalaka	2	8.0
Total	42	7.5

Mean overall group size was 7.5 individuals but there was substantial skew in the distribution of group size and the modal group size was 4 individuals (Figure 3.6)

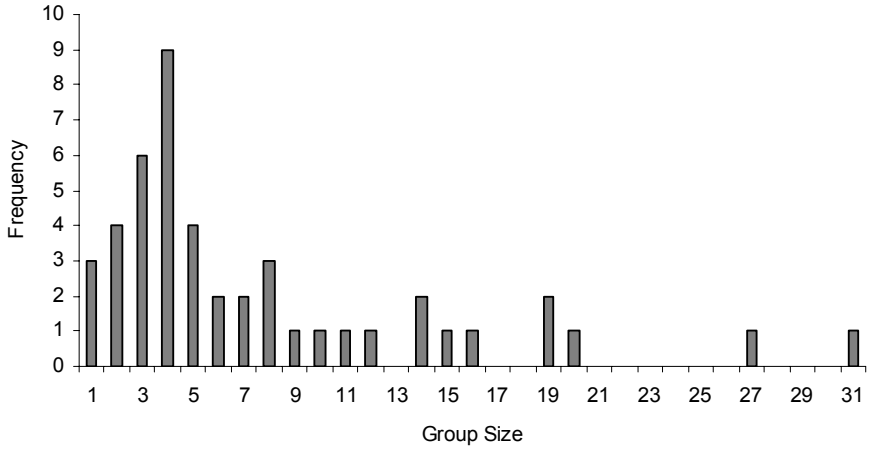

Figure 3.6 Frequency distribution of Macaque group sizes

There were some extremely large groups recorded, and it is likely that these are representative of true group size while the smaller groups that are more frequently observed are sub-groups that break away from the large group for foraging.

Because of the relatively small number of sightings, it is difficult to generate robust estimates of number of groups in Lambusango from Distance

sampling. Nevertheless, assuming that the maximum distance at which groups were encountered (70m) reflects the maximum possible survey area and that the mean distance of encounters (24m) is a reliable indicator of minimum area sampled, it is possible to produce ball-park estimates for population size. Using this method, the density of groups ranges from 2.6-7.7 per km^2 and of individuals, assuming modal group size of 4 from 10.4 to 30.7. The resulting overall population estimate for Lambusango (600km^2) ranges from 1555 to 4609 groups and from 6221 to 18435 individuals. Clearly the range of these figures is too great to be informative for monitoring purposes, but we would recommend that density estimates of groups per km^2 using either method be used as the indicator for monitoring, and that results be interpreted with the caution that the quality of data demand. We are currently seeking to develop an analysis method that will allow us to estimate population size robustly for the 2005 survey and the anticipated 2007 survey.

4: Conclusions and Development

4.1 Anoa

We have demonstrated that patch occupancy analysis using track counts is a useful monitoring tool for the anoa. Over the whole Lambusango area it provides robust estimates of the two key parameters, proportion of area occupied and probability of detection. Error bars for individual camps are large (to be expected given the relatively small number of sampling sites per camp), but some consistent trends are evident. We will maintain track monitoring for the final survey period and produce a multi-season analysis that will allow us to estimate local extinction and re-colonisation parameters. The multi-season analysis will also reduce the error bars on parameter estimates for individual camps and give a better indication of population trends.

It has been possible to collect sufficient faecal samples that, if most produce analysable DNA, we ought to be able to estimate population size and change over the survey period to a good degree of precision. Being able to collect and analyse samples of meat from hunted individuals in the study period would give us proof of where those individuals had been hunted (given that they had been sampled by faecal analysis).

Clearly hunting is still practiced by villagers around Lambusango, and modelling suggests that it is at an unsustainable level. Data for Lambusango overall suggest no consistent population trend in the two years since monitoring began, however results from some of the individual camps are disturbing, especially the Lawele camp which is the area where most hunting has been reported and the only camp showing a clear, consistent decline over the three study years. We would urge that enforcement activity be concentrated in this area, at least until the main hunters have been dealt with. The area is also close to what has been identified as a potential stronghold for the anoa, which makes local enforcement all the more important. Village surveys will continue as part of the work of the Forest Crime Unit. These surveys will inform spatial models of hunting and population size and threat.

4.2 Wild pig

Analysis of pig tracks using the same methods as for anoa will allow us to detect changes in the distribution and abundance of the species. Since pigs are both more widely distributed and abundant than anoa, parameter estimates ought to be more robust.

4.3 Macaques

It has not yet been possible to produce robust estimates of macaque population size. However, subsequent surveys will increase sample size and improve the accuracy of our estimates. Without detailed multi-site surveys of group size it will be difficult to estimate population size *per se* and instead a more reliable index of population change may be number of groups, supported by data on mean observed group sizes in each survey area.

References

Bayle, L. L., T.R. Simons, & K.H. Pollock. 2004. Estimating site occupancy and species detection probability parameters for terrestrial salamanders. Ecological Applications 14: 692-702.

Beebee, T.J.C. & G. Rowe. 2004. An introduction to molecular ecology. Oxford University Press, Oxford.

Bellemain, E., J.E. Swenson, D. Tallmon, S. Brunberg, & P. Taberlet. 2005. Estimating population size of elusive animals with DNA from hunter-collected feces: Four methods for brown bears. Conservation Biology 19: 150-161.

Beyers, R., L. Thomas, J. Hart, & S. Buckland. 2001. Recommendations for ground-based survey methods for elephants in the Central African forest region. Technical Report No 2. Monitoring Illegal Killing of elephants (MIKE).

Buckland, S.T., D.R. Andersen, K.P. Burnham, J.L. Laake, D.L. Borchers, & L. Thomas. 2001. Introduction to Distance Sampling: Estimating abundance of biological populations. Oxford University Press, Oxford.

Chiarello, A.G., & de Melo, F.R. 2001 Primate population densities and sizes in Atlantic forest remnants in northern Espirito Santo, Brazil. International Journal of Primatology 22: 379-396.

Eggert, L.S., J.A. Eggert, & D.S. Woodruff. 2003. Estimating population sizes for elusive animals: the forest elephants of Kakum National Park, Ghana. Molecular Ecology 12: 1389-1402.

Fashing, P.J., & Cords, M. (2000) Diurnal primate densities and biomass in the Kakamega forest: an evaluation of census methods and a comparison with other forests. American Journal of Primatology 50: 139-152.

Frantz, A.C., L.C. Pope, P.J. Carpenter, T.J. Roper, G.J. Wilson, R.J. Delahay, & T. Burke. 2004. Reliable microsatellite genotyping of the Eurasian badger (Meles meles) using faecal DNA. Molecular Ecology 12: 1649-1661.

Greenwood, J.D.J. 1996. Basic techniques. In. Sutherland, W.J. (ed.). 1996. Ecological census techniques. A handbook. Cambridge University Press, Cambridge: 11-110.

Karanth, K.U. 1995. Estimating tiger Panthera tigris populations from camera trap data using capture-recapture models. Biological Conservation 71: 333-338.

Karanth, K.U., J.D. Nichols & S. Kumar. 2004. Photographic sampling of elusive mammals in tropical forests. In. Thompson, W.L. (ed.). Sampling rare or elusive species. Concepts, designs, and techniques for estimating population parameters. Island Press, Washington, USA: 229-247.

Lukacs, P.M. & K.P. Burnham. 2005. Review of capture-recapture methods applicable to noninvasive genetic sampling. Molecular Ecology 14: 3909-3919.

MacKenzie, D. I., J. D. Nichols, G. B. Lachman, S. Droege, J. A. Royle and C. A. Langtimm. 2002. Estimating site occupancy rates when detection probabilities are less than one. Ecology 83: 2248-2255

MacKenzie, D. I., J. D. Nichols, J. A. Royle, J.A., K. Pollock, L. Bailey and J. E. Hines. 2006. Occupancy Estimation and Modeling - Inferring Patterns and Dynamics of Species Occurrence. Elsevier Publishing.

Mills, L.S., J.J. Citta, K.P. Lair, M.K. Schwartz, D.A. Tallmon. 2000. Estimating animal abundance using non-invasive DNA sampling: Promise and pitfalls. Ecological Applications 10: 283-294.

Monitoring the Illegal Killing of Elephants (MIKE) in Southeast Asia. 2004. Level 1 Law Enforcement Monitoring (LEM) training.

Mustari, A.H. 2003. Ecology and conservation of lowland anoa Bubalus depressicornis, in Sulawesi, Indonesia. D.Phil Thesis. University of New England.

O'Brien, T.G., M.F. Kinnaird, & H.T. Wibisono. 2003. Crouching tigers, hidden prey: Sumatran tiger and prey populations in a tropical forest landscape. Animal Conservation 6: 131-139.

Palsboll, P.J. 1999. Genetic tagging: Contemporary molecular ecology. Biological Journal of The Linnean Society 68: 3-22.

Peres, C.A. 1999 General guidelines for standardising line-transect surveys of tropical forest primates. Neotropical Primates 7: 11-16.

Plumptre, A.J. 2000. Monitoring mammal populations with line transect techniques in African forests. Journal of Applied Ecology 37: 356-368.

Riley, J., Y. Hunowu & M.T. Soleman. 2001a. Rawa Aopa Watumohai National Park, Sulawesi Tenggara, Indonesia: Biological surveys and management

recommendations. Wildlife Conservation Society for Indonesian Department of Forestry, Manado, Indonesia.
Riley, J., E. Maneasa, I. Hunowu, & Y. Hunowu. 2001b. Tanjung Peropa and Tanjung Batikolo Nature Reserves, Sulawesi Tenggara, Indonesia: Biological surveys and management recommendations. Wildlife Conservation Society for Indonesian Department of Forestry, Manado, Indonesia.
Rosenbaum, B., O'Brien, T.G., Kinnaird, M., & Suprinata, J. (1998) Population densities of Sulawesi crested black macaques (Macaca nigra) on Becan and Sulawesi, Indonesia: effects of habitat disturbance and hunting. American Journal of Primatology 44: 89-106.
Royle, J. A. & J. D. Nichols. 2003. Estimating abundance from repeated presence-absence data or point counts. Ecology 84: 777-790.
Schreiber, A., I. Seibold, G. Notzold, & M. Wink. 1999. Cytochrome b gene haplotypes characterize chromosomal lineages of anoa, the Sulawesi dwarf buffalo (Bovida: Bubalus sp.). Journal of Heredity 90: 165-176.
Schwarz, C.J. & G.A.F. Seber. 1999. Estimating animal abundance: review III. Statistical Science 14: 427-456.
Seymour, A. (ed.). 2005. Summary of biological research carried out by Operation Wallacea in the forest of Central Buton. Operation Wallacea.
Sutherland, W.J. (ed.). 1996. Ecological census techniques. A handbook. Cambridge University Press, Cambridge.
Taggart, J.B., R.A. Haynes, P.A. Prodohl, & A. Ferguson. 1992. A simplified protocol for routine total DNA isolation from salmonid fishes. Journal of Fish Biology 40: 963-965.
Thomas, L., Laake, J.L., Strindberg, S., Marques, F.F.C., Buckland, S.T., Borchers, D.L., Anderson, D.R., Burnham, K.P., Hedley, S.L., Pollard, J.H. and Bishop, J.R.B. 2003. Distance 4.1. Release "x"1. Research Unit for Wildlife Population Assessment, University of St. Andrews, UK. http://www.ruwpa.stand.ac.uk/distance/
Wheeler, P. 2005 The Anoa in Lambusango: umbrella, flagship or millstone? The Ecology of Lambusango: Five years of research, University of Hull, Scarborough Campus.

Wilson, G.J., A.C. Frantz, L.C. Pope, T.J. Roper, T.A. Burke, C.L. Cheeseman, & R.J. Delahay. 2003. Estimation of badger abundance using faecal DNA typing. Journal of Applied Ecology 40: 658-666.
Witmer, G. W. 2005. Wildlife population monitoring: Some practical considerations. Wildlife Research 32(3): 259-263.

Appendix 1

Akaike weights and best-fit parameter estimates for models considered for anoa track data from 2004 to 2006. Maximum AIC weight indicated in bold.

		AIC weight for Model ψ(.)p(cov)								AIC weight for Model ψ(cov)p(.)							Best fit parameters		
Study area	Year	No cov	Trail 1	Trail 2	Trail 3	T1+T2	T1+T3	T2+T3	T1+T2+T3	Trail 1	Trail 2	Trail 3	T1+T2	T1+T3	T2+T3	T1+T2+T3	β_1 (T1)	β_2 (T2)	β_3 (T3)
Wahalaka	2004	0.025	0.0095	0.156	0.031	0.059	0.012	**0.341**	0.128	0.022	0.025	0.031	0.015	0.022	0.052	0.025		-2.77	-15.67
	2005	**0.148**	0.139	0.085	0.078	0.054	0.053	0.037	0.021	0.067	0.066	0.066	0.026	0.042	0.048	0.02			
	2006	0.131	0.071	0.053	0.048	0.028	0.026	0.02	0.01	**0.152**	0.098	0.048	0.096	0.056	0.036	0.035	29.161		
Wabalamba	2005	0.08	**0.24**	0.036	0.041	0.089	0.088	0.016	0.033	0.0295	0.061	0.049	0.025	0.02	0.029	0.015	1.073		
	2006	**0.22**	0.084	0.106	0.081	0.042	0.031	0.039	0.015	0.081	0.081	0.081	0.03	0.03	0.03	0.011			
Lapago	2004	0.052	0.021	0.058	0.149	0.022	0.061	**0.167**	0.064	0.022	0.022	0.149	0.01	0.059	0.059	0.024		-1.56	-28.835
	2005	0.045	0.041	0.048	0.131	0.021	0.115	**0.139**	0.06	0.017	0.025	0.131	0.01	0.076	0.059	0.033		-0.663	-27.436
	2006	0.047	**0.329**	0.017	0.017	0.121	0.121	0.006	0.045	0.029	0.017	0.017	0.011	0.011	0.006	0.004	26.914		
Anoa	2004	0.125	**0.255**	0.047	0.046	0.094	0.094	0.017	0.034	0.046	0.046	0.046	0.017	0.017	0.017	0.006	-1.792		
	2005	0.031	0.225	0.011	0.058	0.083	**0.294**	0.021	0.108	0.012	0.011	0.012	0.005	0.005	0.005	0.002	26.443		-1.656
	2006	**0.232**	0.085	0.092	0.085	0.034	0.031	0.034	0.012	0.085	0.085	0.085	0.031	0.031	0.031	0.012			
Lawele	2004	0.145	0.088	0.053	0.121	0.032	**0.156**	0.045	0.057	0.055	0.053	0.057	0.02	0.024	0.021	0.009	-1.069		-0.899
	2005	0.07	0.026	**0.296**	0.038	0.112	0.014	0.124	0.052	0.032	0.038	0.032	0.017	0.017	0.014	0.008		1.417	
	2006	0.04	0.105	0.043	0.105	**0.112**	0.038	**0.112**	0.041	0.105	0.019	0.105	0.041	0.038	0.041	0.015	-28.421	-1.212	-28.381
Lasolo	2005	**0.173**	0.099	0.064	0.064	0.036	0.036	0.023	0.013	0.139	0.064	0.064	0.051	0.051	0.023	0.019			
	2006	**0.235**	0.087	0.087	0.087	0.032	0.032	0.032	0.012	0.087	0.087	0.087	0.032	0.032	0.032	0.012			
All Sites	2004	0.01	0.009	0.036	0.032	0.048	0.035	0.185	**0.335**	0.004	0.009	0.03	0.004	0.011	0.045	0.017	-0.563	-1.637	-1.287
	2005	**0.159**	0.075	0.079	0.15	0.055	0.082	0.058	0.039	0.059	0.061	0.06	0.023	0.022	0.024	0.009			
	2006	0.049	0.019	0.018	0.111	0.007	0.046	0.041	0.017	0.022	0.079	0.111	0.031	0.114	**0.152**	0.126		24.369	-40.231

Appendix 2. Datasets

Data to be collected are in italics

Anoa

Track counts from transects

Dry Season 2004: Anoa, Lapago, Lawele, Wabalamba, Wahalaka.

Dry Season 2005: Anoa, Lapago, Lasolo, Lawele, Wabalamba, Wahalaka.

Dry Season 2006: Anoa, Lapago, Lasolo, Lawele, Wabalamba, Wahalaka.

Dry Season 2007: Anoa, Lapago, Lasolo, Lawele, Wabalamba, Wahalaka.

Faecal DNA

2004: No samples

Dry Season 2005: Anoa, Lapago, Lasolo, Lawele, Wabalamba, Wahalaka.

Wet Season 2006: Anoa, Lasolo, Lawele, Wabalamba, Wahalaka.

Dry Season 2006: Anoa, Lapago, Lasolo, Lawele, Wabalamba, Wahalaka.

Wet Season 2007: Anoa, Lapago, Lasolo, Lawele, Wabalamba, Wahalaka.

Dry Season 2007: Anoa, Lapago, Lasolo, Lawele, Wabalamba, Wahalaka.

Hunting Surveys

2004: No surveys

2005: No surveys

2006: 173 surveys

2007: continued surveys through FCU

Wild Pig

Track counts from transects

Dry Season 2004: Anoa, Lapago, Lawele, Wabalamba, Wahalaka.

Dry Season 2007: Anoa, Lapago, Lasolo, Lawele, Wabalamba, Wahalaka.

Buton Macaque

Transect line surveys

Dry Season 2005: Anoa, Lapago, Lasolo, Lawele, Wabalamba, Wahalaka.

Dry Season 2007: Anoa, Lapago, Lasolo, Lawele, Wabalamba, Wahalaka.