
Frontier Madagascar Environmental Research

REPORT 8

Forest research methodology training manual



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The Society for Environmental Exploration (SEE)

The society is a non-profit making company limited by guarantee and formed in 1989. The society's objectives are to advance field research into environmental issues and implement practical projects contributing to the conservation of natural resources. Projects organised by The Society are joint initiatives developed in collaboration with national research agencies in co-operating countries.

The University of Toliara/IHSM

The University of Toliara was established in 1976 as a centre for learning and research in the biological sciences. The Institut Halieutique et des Sciences Marines (IHSM) is the marine department of the University of Toliara. The University is studying the flora and fauna of Madagascar and is conducting research into the maintenance and improvement of the environment and the sustainable use of the natural resources in the region.

The WWF Madagascar Dry Forest Programme

The WWF Dry Forest Programme was initiated in 1998. It is a 50-year programme with the aim of conserving key habitats throughout southwest Madagascar. This eco-region has suffered greater habitat loss than any other region in Madagascar. The project aims to implement management plans for 15-25% of the region, incorporating all major habitat types and including areas of high biodiversity.

The Frontier-Madagascar Forest Research Programme

The society for Environmental Research, the University of Toliara and the WWF Madagascar Dry Forest Programme have been conducting collaborative research into environmental conservation issues since July 2001 under the title of the Frontier-Madagascar Forest Research Programme. From July 2001 until July 2002, the Project has been working in the forests of the Sept Lacs region. This activity was aimed at identifying core areas of biodiversity and establishing baseline biodiversity and resource-use information in those areas. In addition, tourism feasibility surveys were undertaken with socio-economic support from the Association Nationale pour la Gestion des Aires Protégées (ANGAP).

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TABLE OF CONTENTS

	Page
General Introduction	3
1.0 Animal Collection techniques	6
1.1 Pitfall traps	6
Tips	8
1.2 Sherman and mesh trapping	8
Introduction	8
Materials and methods for trapping small mammals	9
Grid trapping	10
Capture-mark-recapture	12
Mammal stuffing	12
Parasites	13
Tips for small mammal trapping	13
1.3 Owl pellet collection	14
Introduction	14
1.4 Mist netting and harp traps	15
Mist netting	15
Harp trapping	17
Bat dung collection	18
1.5 Invertebrate collection	18
Butterfly collection	18
Canopy trapping	19
Sweep netting	20
Butterfly pinning	21
Litter sifting methods for a 20 sample transect	22
Malaise trapping	24
Spider and scorpion collection	26
1.6 Casual collection	26
2.0 Preserving and storing specimens	27
2.1 Specimen identification	27
2.2 Equipment	27
2.3 Procedures	28
2.4 DNA samples	30
3.0 Methods based on field observations	31
3.1 A species inventory for birds	31
3.2 Tortoise behaviour	32
3.3 Lemur observations	36
4.0 Resource-use surveys	37
4.1 Disturbance transects	37
4.2 Mapping	38
5.0 Taxonomic verification	41
6.0 Suggested readings	42

7.0 Bibliography	42
Appendix 1: Assessment lines and density estimates (small mammal surveys)	43
Appendix 2: Marking, measuring and sexing small mammals	45
Appendix 3: Small mammal data sheet	48
Appendix 4: Bat data sheet	49
Appendix 5: Reptile data sheet	50
Appendix 6: Amphibian data sheet	51
Appendix 7: Butterfly data sheet	52
Appendix 8: Butterfly specimen summary sheet	53
Appendix 9: GPS data recording for GS use	54

GENERAL INTRODUCTION

The Frontier-Madagascar Forest Research Programme

The Toliara region in southwest Madagascar is contained within the Spiny Forest Eco-region. This region has been divided into different zones, each with characteristic flora and fauna. To the north of Tulear is the Mikea zone. Along the coast from Morondava to Fort Dauphin is the littoral zone. The central southern zone contains the internal and transition zones, and inland from Toliara is the calcareous plateau zone.

The area around Toliara, from the littoral zone to the calcareous plateau, consists for the most part of dry spiny forest with fragmented gallery forest along the rivers. The climate is hot and dry. The spiny forest eco-region is the least studied region of Madagascar. It contains the highest percentage of plant endemism, yet has suffered the highest percentage of deforestation of any region in Madagascar.

WWF and ANGAP have highlighted the eco-region for conservation management. At present, the region contains 2,829,000ha of forest, at least 750,000ha of which is degraded. The percentage of forest under protection by ANGAP is just 68,850ha. This amounts to less than 2.5% of the entire forested area. At present, there is a real need to identify core areas of biodiversity and to assess the level of threat to these areas, and for these sites to become incorporated into the long-term management plans for the eco-region. The Frontier-Madagascar Forest Research Programme is working in collaboration with WWF and in association with ANGAP to survey sites in the Toliara region and to identify core areas of biodiversity.

This project has provided standardised and repeatable survey methods to assess the biodiversity and resource-use of specific survey areas to enable their importance and vulnerability to be determined and permit biodiversity and resource-use to be monitored over time.

When the Project was initiated in July 2001, it adopted the format and methods of the Frontier-Tanzania Forest Research Programme. After the first three months, the methodologies were re-assessed and amended. The updated methodologies are described in this report.

Introduction to methodologies and data collection

In order for a project to function with a sound scientific basis, methodologies must be developed that are suited to the habitat(s) and species to be surveyed. These methods should also be honed to allow comparisons with previous work that has been carried out in the country.

The reputation and usefulness of a project within the scientific community is strongly report and publication-based, and methodologies are often the basic stumbling block with regards to publications. Data collected using sub-standard methods will be imprecise and incomplete, and will reduce the use and publishing potential of the results.

Collected data should be recorded in a reliable, systematic manner that allows accessibility of results and utilisation of these data in further analyses. Efficient and accurate data sheets are an essential prerequisite to all data analysis, which is in itself a prerequisite to report-writing and publication production.

Identification of Methodologies

Methodologies described in this technical training manual were identified and compiled through the following process:

- 1) A review of existing methodologies through a library search of references relating to past biodiversity, ecological and behavioural surveys in the region or country.
- 2) The identification of methodologies that may be used, considering the environmental, logistical, personnel and financial constraints of the Project.
- 3) The identification, through field trial phases, of methodologies that would be suitable.
- 4) The identification of an efficient system for data collection, storage and use.
- 5) The production of a series of accurate, simple data sheets and databases that will enable efficient data collection and utilisation.

Based on the process described above, a series of methodologies were identified and tested. They fall loosely into the following categories:

- Biological diversity surveys
- Behavioural and ecological studies
- Resource-use studies

Behavioural and ecological studies are carried out when the opportunity arises to study a particular, usually threatened, species or habitat. The methodologies for these studies are often species-specific. The Frontier-Madagascar Forest Research Programme has identified methodologies for studying the distribution, abundance and behaviour of the Radiated tortoise (*Geochelone radiata*). Methodologies have also been identified to study the distribution, abundance and social structure of populations of Ring-tailed lemurs (*Lemur catta*) and Verreaux's sifaka (*Propithecus verreauxi verreauxi*). These methodologies will be discussed in sections 3.2 and 3.3 respectively.

Disturbance transects and mapping methodologies, and the associated data collection systems utilised in these surveys, will be discussed in Section 4.

The biological diversity surveys incorporate several methodologies, which will be discussed in Section 1. Specimen identification and preservation techniques will be discussed in Section 2.

Baseline biodiversity surveys

Protecting the biological diversity of an area is difficult unless the species in that area are identified and new species described. In order to propose management and monitoring plans for the area, a comprehensive biodiversity survey must first be carried out.

The biodiversity surveys focus on mammals, birds, reptiles and amphibians, and selected invertebrates. These groups are studied for several reasons. Firstly, they are relatively easy to observe or collect. Secondly, they cover aspects of all terrestrial habitats and their presence and relative abundance have ecological significance. Thirdly, they contain 'charismatic' species that will form the basis upon which conservation organisations can focus their attention (and funding). Many people value charismatic species such as lemurs, and will fund organisations that work to protect them. Lastly, there are taxonomists and institutions that are willing to accept and identify specimens from these selected groups.

Where a long-term intensive biodiversity study is not possible, indicator species may be a useful substitute when characterising ecosystems and defining the degree of degradation or recovery from disturbance. In this context, indicators are described as organisms “whose characteristics (e.g. presence or absence, population density, dispersion, reproductive success) are used as an index of attributes too difficult, inconvenient, or expensive to measure for other species or environmental conditions of interest” (Landres *et al.* 1988).

Reasons for taking specimens

Data on the surveyed fauna will be combined into species lists. In order to produce an accurate list, specimen identifications need to be formally verified by taxonomists. Many of these taxonomists work in Malagasy institutions. However, a significant number are based in institutions worldwide, such as the British Museum of Natural History, or the Chicago Field Museum of Natural History. To confirm species identifications, or to name new species, specimens need to be preserved, labelled and sent to the relevant institution.

Specimen collections and the ecological information that comes with them are used as a reference for students and lecturers, and as a specimen library for visiting scientists, and form a basis for all research in the country or area of origin.

The collection must contain a male and female of all known species, including colour variants. In cases where only very few specimens of rare or new species are collected, it is more productive to export them for formal identification than to have them remain unidentified in-country.

Institutions within Madagascar must have comprehensive specimen collections from their own country. The Frontier-Madagascar Forest Research Programme aims to provide the University of Tulear with a set of well-preserved, accurately labelled voucher specimens collected from the region. It is also important that a definitive reference collection of all species from all regions of Madagascar is retained in-country, not scattered throughout small institutions in different regions. Therefore, it is necessary to provide at least one specimen for the University of Antananarivo whenever rare or new species are collected.

The collections and species lists made by Frontier- Madagascar will contribute greatly to the overall knowledge of the range, behaviour and ecology of many species from Southwest Madagascar.

Specimen Deposition

Specimens are stored at the University of Toliara and the University of Antananarivo. For identification purposes, selected specimens are exported to the following institutions:

- Small mammals – sent to the Chicago Field Museum of Natural History.
- Reptiles and amphibians – sent to the American Museum of Natural History, New York.
- Butterflies – sent to the entomological Department of the Californian Academy of Sciences for identification, then deposited at the British Museum of Natural History, London, and the African Butterfly Institute, Nairobi.
- Other invertebrates – sent to the entomological Department of the Californian Academy of Sciences for identification.
- Primates and birds are not collected as specimens.

For more information regarding taxonomic verification, see Section 5.

1.0 ANIMAL COLLECTION TECHNIQUES

There are many methods for assessing zoological biodiversity. The Frontier-Madagascar Forest Research Programme has combined the methodologies from comparative surveys in Madagascar and has produced the animal collection techniques described below.

The herpetological methodologies follow those of Raxworthy and Nussbaum (1994) and the small mammal surveys follow those of Goodman (1999). The basic butterfly collection methodologies follow those of Kremen (1994) and the general invertebrate collection methods follow those of Fisher (1999).

When conducting a baseline biodiversity survey in an area, all collection methods are located in a similar habitat or area for a set period of time. This is called a 'trapsite'. The trapsite lasts for 8 days and consists of a combination of pitfall trapping, sherman and mesh trapping, bat collection using mist nets and harp traps, butterfly collection using sweepnets and canopy traps, invertebrate collection, and casual collections. (See below for full methodologies.)

1.1 Pitfall traps

Introduction

Pitfall traps are a cheap and effective method to sample reptile, amphibian and small mammal populations in an area. This allows an assessment to be made of the biodiversity of an area with minimum effort and collects large numbers of live specimens. The pitfall traps are 11 buckets sunk into the ground at 10m intervals along a 100m-long drift fence (commonly called bucket lines). Three bucket lines are normally used for each trapsite.

Pitfall traps that are used in conjunction with a drift fence are particularly effective. The drift fence channels animals that are moving through the forest into the buckets. This method favours the capture of small terrestrial animals that cannot climb out of buckets. It is particularly successful with fossorial species such as burrowing skinks and blind snakes, but will also collect small terrestrial reptiles, some ground dwelling frogs and small rodents and tenrecs. The species caught will depend on a number of factors including habitat, altitude, association to water, and season.

Equipment

For one bucketline:

- 11 Buckets 295 mm deep, 290 mm top internal diameter, 220 mm bottom internal diameter
- 3 x plastic Fairly thin, 100m X approx. 70cm, black or brown if possible
- Sticks About 70cm high, approx. 50
Enough to hold a 100m line of plastic sheeting
These can be collected from the forest where the habitat is suitable (do not cut live saplings)
- Shovels To dig holes to sink the buckets
- String To tie plastic to sticks (or use a stapler and staple gun)
- Tags To number the buckets and the bucket lines
- Permanent marker
- Gloves
- Specimen pots in a range of sizes

Procedure

Site selection will depend on the objectives of the study, but the area of habitat to be studied must be continuous for at least 100m and be relatively free of ground vegetation to minimise environmental disturbance.

Before starting the bucket line, cut the plastic to the correct size. Remove the handles from the buckets and place holes in the buckets to prevent water collecting. These should be no more than 3mm in diameter to prevent the escape of small specimens.

To assemble the bucket line, dig the buckets into the ground at 10m intervals along the chosen site. Make sure the lip of the bucket is level or below ground level to allow animals to fall in. Fill in any gaps around the bucket with earth. Unroll the plastic sheeting and lay it along the ground next to the buckets. Starting from one end, secure the top 60cm of the plastic onto sticks fixed vertically into the ground, and bury the bottom 10cm into the ground so that the plastic runs vertically along the bucket line for 100m. The drift fence should run vertically through the middle of the buckets with no opportunity for animals to crawl underneath. Slits should be made in the plastic at each side of the bucket. This allows the plastic to be buried to make a seamless finish. Finally, remove any debris that has fallen into the buckets and make the area as natural looking as possible, so as not to scare the animals away. Place a numbered tag near the bucket for accurate data recording.

Check the bucket line for the presence of animals early in the morning and frequently during the day to reduce predation of trapped animals. Buckets should be checked more frequently if they are located in strong sunlight or a wet area, to prevent unnecessary fatalities. Remove any debris in the bucket. All animals targeted by the study found in the buckets should be carefully collected in a specimen pot and taken to camp for identification.

Recording and analysing the data will again depend on the specific aims of the project, but the following data recording protocol should always be observed:

In the field

For each trap site, label each bucket line and each bucket so that when an animal is collected, the location of the animal can be easily recorded.

In the ‘trapsite protocol’

Per trapsite	The trapsite number (unique to each trapsite) Dates of trapsite Location of bucket lines (Region, Latitude/Longitude) Altitude General habitat description Personnel
Per Bucket	Microhabitat notes needed for the specimen data sheets Date and time Weather Presence of an animal Specimen named to genus, and species if possible (to be confirmed through formal identification) Specimen number if species is not known and a specimen is taken Number of each species

When dis-assembling the drift fence, Untie the string, collect the sticks, remove the tags and roll up the plastic. Pull the buckets out and fill in the holes.

Tips

- Collect sticks that are already dead. The sticks can be used for many trapsites.
- Pay particular attention to soil and plastic around the buckets to make a continuous seam from which animals cannot escape.
- Tie the plastic with a tight shoelace knot; it will ease the drift fence dis-assembly.
- Tie the plastic with no sagging, otherwise specimens could climb over the sheeting.
- During dis-assembly, roll the plastic from one end only. This way, you will end up with the plastic sheeting in one single neat, tight roll.
- The plastic can be re-used. If the plastic has been used before, line the buckets up with the slits in the plastic made from the previous buckets. Always keep the previous slits in the plastic facing the ground; they can be buried, keeping the top of the plastic sheeting undamaged.
- Make sure every animal is taken back to camp and its identification confirmed before returning it to the place of capture. Different species may look very similar, and will often require identification using appropriate field-guides.
- When checking the buckets, beware of dangerous animals such as scorpions.
- Where possible, large snakes that are seen around the bucket lines should be carefully collected and released some distance from the trapsite to reduce predation (record the observation).
- When leaving the site, try to leave the area in as undisturbed a state as possible by filling in the holes made by buckets, removing litter and redistributing leaf litter.

1.2 Sherman and mesh trapping

Introduction

Mammals occur in a wide variety of terrestrial and aquatic habitats throughout the world. The mammal fauna of Madagascar is remarkable for the variety of species not found anywhere else in the world.

Mammals constitute an essential part of the Malagasy fauna and are found in most habitats. Bats are one of the most diverse groups of small mammals, and the least studied group in Madagascar. Small mammals are relatively easy to catch, handle, and identify. Furthermore, small mammals have short life spans and changes in species diversity, density, and community composition over time and are therefore relatively easy to detect.

Small mammals are important parts of all ecosystems they are found in because they are central to the food-chain, feeding on vegetation and invertebrates and being preyed upon by larger mammals, reptiles and birds. Furthermore, mammals often function as seed dispersers and are therefore important for plant regeneration.

Small mammals could be a possible ecological indicator group. Data from repeated surveys could indicate the general state of an area and thereby contribute to the management of these areas. Larger species such as lemurs and tenrecs are of tourist interest and form an important part of tourism-oriented biodiversity surveys.

Mammals are threatened by many different factors. Habitats and features important to many mammals are being cleared or degraded or fragmented, leading to total loss of populations, or resulting in small non-viable populations. Humans often heavily hunt mammals for food, skins and for the pet-trade, which further threaten the existence of some species and populations.

Exotic species deliberately or accidentally introduced to Madagascar by humans can cause severe threats to native species. Introduced species often adapt very well to new surroundings and are very competitive due to their generalist and opportunistic feeding behaviour. Furthermore, these species may carry diseases and parasites that the native species are not resistant to, and populations may suffer from this. Studies of introduced species such as *Mus yemenensis* or *Rattus rattus* are necessary to assess the effect of these species on native species populations.

Materials and Methods for trapping small mammals

Baseline information from an area is an estimate of the diversity (inventory) of species in the surveyed area. Mammal species occurring in the same area or ecosystem are often of variable sizes, occupy different niches and show different behaviour. To carry out a biodiversity survey resulting in precise and reliable data, different methods for catching and observing animals need to be applied.

Pitfall traps

Pitfall traps (with or without bait) with drift fences are efficient for catching the smaller mammal species. *See section on Pitfall trapping for description.*

Sherman Traps

Sherman traps (H.B. Sherman traps, Inc. USA) are aluminium box traps designed for live trapping of a range of small mammals. The traps can be baited with various types of food (e.g. coconut with peanut butter, banana, or fish), depending on the target species. As Sherman traps are relatively small (23 x 9 x 7.5 cm) they will only catch animals of a limited size and should optimally be applied in conjunction with larger traps.

For the purpose of assessing the diversity in an area, the traps available should either be placed on transect lines traversing as many different habitats as possible, or be spaced out in an unstructured manner at suitable spots.

Suitable spots for placing the traps are where there is a high chance of catching the target species. Terrestrial mammals often nest and forage in places with a dense understorey, along fallen and rotting logs and near obvious food sources such as fruiting trees.

If Sherman traps are used in conjunction with pitfall traps they should not be placed too near the pitfall bucket line.

For the capture of arboreal species, the traps can be fastened with sellotape to a suitable stem or branch at the required height.

The traps contain a trigger plate on which the bait is placed. The trap must be set carefully to ensure that the trigger is sensitive. When an animal enters the trap, the body weight on the trigger plate releases the door, which closes behind it.

The trap should be placed as stable and horizontal as possible (no more than a 30 degree angle), to prevent the trap wobbling, which may keep the animal from entering. The entrance should be either level or pointing slightly downwards, as animals prefer to run up into a trap rather than down into one.

Most target species are nocturnal and the traps are baited and set at dusk. Depending on the season and weather, the traps should be checked early in the morning. As the trap material is metal they heat up quickly and animals may overheat if they are left for too long. Furthermore, as small mammals have a high metabolism they may starve if left in the traps too long without sufficient food.

If the door to the trap is closed, and there is an animal in the trap, open the door carefully inside a clear plastic zip-lock bag. Gently shake the animal into the bag. Remove the trap and quickly close the bag. With the animal now in the bag it is possible to identify it, keep it as a specimen, mark it and release it, or release it untouched.

If the bait is missing, but no animal has been caught, test the trigger. It may be that ants removed the bait, or it may be that the trigger has stuck. If the trigger has stuck, it will be necessary to re-sensitise the trigger (gently bend the trigger lever).

It is advisable to wash the traps regularly to prevent old bait, animal droppings or plant material falling underneath the trigger plate and blocking the moving parts. Furthermore, the scent of earlier trapped animals remains in the trap and may prevent other animals from being attracted and trapped.

If the bait is untouched it should be removed and replaced with fresh bait for the next trapping session.

After checking the traps they should all be closed. If the traps are left open during the day, animals may enter and suffer from overheating during the day. If the traps are left open for trapping of diurnal species they should be checked regularly according to the weather.

If the animal is not to be kept, always release it at the point of capture.

Mesh traps

Mesh Traps, either locally or commercially made, e.g. Tomahawk traps (Tomahawk Live Trap Co. USA) are large wire-mesh traps. They consist of a box containing a hook-trigger upon which chosen bait is placed. The traps can be used either for terrestrial or arboreal species as they easily can be tied or nailed to a branch. The larger size of these traps allows trapping of large insectivore and small carnivore species.

An animal entering the trap will move the baited hook, triggering the door to spring shut. A trapped animal can be easily seen inside the trap. It can be removed by hand (while wearing thick gloves) and marked, released or kept as a specimen.

When setting the trap, make sure the entrance is clear and that the trigger and door can move freely.

The trapping procedure is the same as for Sherman trapping.

Grid trapping

For more advanced ecological surveys, such as estimating the abundance (total number of animals), density (number per unit area), and/or home range and community structure, a different trap set-up is required.

At least one hundred Sherman and/or mesh traps are essential for such a survey. The traps should be set up in a quadratic grid configuration, *see figure 1*.

The spacing of the traps depends on the size of the traps (and hence the target species) and the complexity of the habitat. If Sherman traps are used for trapping of small species, the distance should be 5-10 metres between the traps. If larger mesh traps are used, the distance should be 15-20 metres.

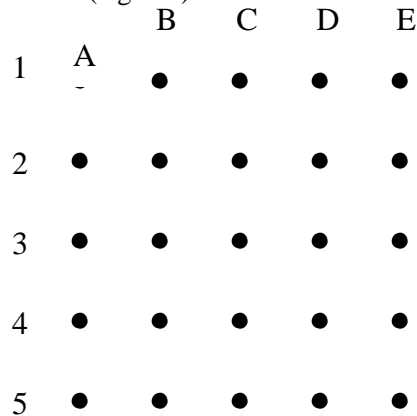
For the production of reliable and comparable data the distances in the grid should carefully be measured out with the use of compass and tape measure.

A grid consisting of at least 10x10 trap arrays is recommended as this covers a relatively large area and will retrieve the most results.

It is recommended to place two traps at every trapping station, which will avoid the saturation of traps with “trap-happy” individuals or species. The traps should be placed within two metres from the exact point, partly to produce precise data and partly to ease locating the traps when checking them.

If trapping of arboreal species is essential to the survey, traps should be placed in the trees (at varying heights) at all or every second trapping station.

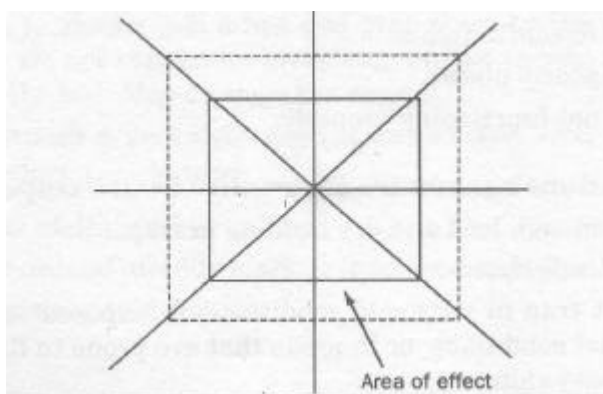
Figure 1. Grid configuration. Each dot represents a trapping station. Labelling the rows with letters and numbers gives each trapping station a unique reference code (e.g. C2).



Trapping should continue until less than 10% of the captures are unmarked (see section capture-mark-recapture) individuals (normally 10-14 days), this ensures that the majority of individuals are identified.

After grid trapping, all traps should be closed and the site should be left undisturbed for a recovery period of minimum 4 days. Then traps can be re-arranged and opened to form 8 “assessment lines” radiating from the centre of the former grid and extending beyond its border to estimate the area of effect, *see figure 2*.

Figure 2. Trapping grid with area of effect.



Area bordered by a permanent line is the former grid-trapping area. Eight assessment lines transverse the area.

Animals marked on the grid and then later captured on varying distances along the assessment lines provide information on the effective sample area of the grid. This is the area used for density estimates.

See Wilson et. al (1996) and Rabinowitz (1997) for detailed descriptions of grid trapping and assessment lines and Appendix 1 for example of analysis of area of effect.

Capture-mark-recapture

In order to assess the abundance, density and home range a capture-mark-recapture study is necessary. Marking the animals allows recognition of individuals and records their movements. There are several ways of marking animals, both permanently and temporary. *See Wilson (1996) for listing of methods.*

An easy and effective temporary method for marking animals is a fur clip marking method with various combinations for individual recognition, *see appendix 2 for example of fur clip method.* All captured animals are given a unique mark, which enable the identification of individuals. Sometimes animals have natural markings (e.g. scars or parts missing), that can be used for recognition.

If an individual is caught a number of times at different trap locations, its home range and core area can be measured. Measuring and weighing individuals can record changes over a period of time (e.g. for pregnant females or sub-adults).

If aspects like community dynamics, life cycles and breeding seasons over time are the aim of the survey, permanent-marking techniques should be applied.

Mammal stuffing

Stuffed mammal specimens are very useful for students to work with as they can be easily handled and measured. Stuffing takes a little bit of practice, but once it has been tried it is fairly easy done. It may be a good idea to practise on an individual of a common species, e.g. *Rattus*, not to damage valuable specimens of more rare species.

Equipment

Dissection kit
Thin metal wire
Wire cutters
Cotton wool
Thread
Needle

Procedure

Kill the animal with chloroform or ether.

Slit a two-cm. hole in the skin on the ventral side of the lower abdomen with a sharp scalpel, being careful not to cut through to the stomach.

Use fingers or a blunt tool to separate the skin from the body. Work towards the hind legs first. Loosen the skin all around the thighs, the 'push' the legs back out of the skin and cut the bone with sharp scissors below the knee. Repeat for other hind leg. Carefully separate the skin around the anus and beginning of tail (there may be sexual glands that complicates this, just be patient and do it slowly). Use a scalpel to separate the intestine from the skin. Pull the tail gently and slowly out of the skin, if done too hard the skin may break.

Free the front legs in same way as hind legs. Move towards the head. When necessary use scalpel to cut muscles and tendons. Be very careful when working around the ears, eyes and muzzle. It is only necessary to cut a little bit around each, if cut too much the holes will get too large and will not look natural. Cut the last bit of the muzzle off, just after the skull has ended, where the nasal cartilage is attached. This will leave the skin with nose and whiskers.

Clean the skin of remaining bits of fat and tissue. Use scissors and scalpel to clean all flesh off the legs. Start stuffing the skin with cotton wool immediately, if left for a while the skin will start to dry and make the stuffing difficult.

Roll up a bit of cotton wool, roughly the size of the animal. While the skin is inside out, hold the end of the cotton wool and the nose with forceps and pull the skin over the roll of cotton wool. Adjust the amount of cotton wool and shape the head. Pull a little bit of cotton wool out through the eye and mouth openings.

Cut pieces of wire of the length of legs and tail, make them a little bit longer to enable the ends to be stabilised in the cotton wool in the body. Cover the wire in a thin layer of cotton wool, resembling the thickness of the legs and tail (if the tail is thin do not use any cotton wool but just wire). Put the wire in the legs and pull the skin over. Arrange the front legs to be stretched out in front of the animal and the hind legs behind. Fill the body up with the necessary amount of cotton wool. Sew the hole in the abdomen up with needle and thread. Leave the specimen on a tray in the sun for a day to allow the skin to dry out completely.

Store in an insect-proof container with silica gel and naphthalene bags.

Parasites

Some taxonomists and other experts are studying the parasites of mammals and other animals. Parasites will have to be taken off freshly killed animals and stored separately.

Equipment

White cloth

Pointed forceps

Toothbrush or other brush

Eppendorf tubes

Ethanol

Indian ink pen

Procedure

Kill the animal and immediately place it on the white cloth, which makes parasites more visible. Brush the animal all over with the brush. Pick up parasites from the cloth with the forceps and put in an Eppendorf tube filled with ethanol. Look through the fur as not all the parasites will have fallen off. Pick the parasites off the animal with the forceps. If the animal have been kept in a bag or pot prior to and during killing check this for 'escaped' parasites. Many of the parasites are very small and difficult to spot. Look especially around the mouth, ears, neck and lower abdomen. On bats have a good look on the wings. Put all the collected parasites from one individual in the same sample tube. Write the MGF collection number on the tube and note on the specimen data sheet that parasites have been collected.

Tips for small mammal trapping

- Before starting the survey, decide what kind of data you want and design the survey after that.
- Decide the period of the survey. Some species go into hibernation and are then not likely to be caught.
- Most small mammals are less active during bright, moonlit nights, and are then not likely to be caught.
- Disturb the trapping area as little as possible, the target species might be scared and be less frequently trapped.
- Do not trap in exposed areas during hot weather conditions, and in areas prone to flooding during heavy rains.
- Choose the traps and bait according to the target species. The ideal might be a combination.

- Be careful when setting the traps, small mistakes may result in no captures.
- Handle the animals with care as stressed animals might die.
- Always record all possible data, it is better to have too much than too little.

1.3 Owl pellet collection

Introduction

Owls feed mainly on small mammals, but will also take nocturnal reptiles, amphibians and large insects. Owls usually swallow their prey whole, and after digesting the flesh, the indigestible matrix is regurgitated as compact pellets. These pellets contain bones, fur and insect parts. Because small mammals can be readily identified from their skulls, and in the pellets these will be relatively intact, the analysis of owl pellets can show which small mammal species are present within the locality. Owl pellets are thus particularly useful for detecting the presence of arboreal or rare species that are unlikely to be found during small-scale trapping studies. Some owl species feed on bats. Often, the presence of high-flying bat species in an area can only be confirmed through the analysis of owl pellets. Many species of reptile and amphibian are arboreal and nocturnal. This includes all tree frogs, and many geckos. Owls collect large numbers of frogs during the breeding season, often collecting hundreds of males at a time from breeding sites (leks).

Owl species

Useful owl species include the (Madagascan) long-eared owl and the Barn Owl. Both species roost regularly in a specific location and regurgitate pellets at that roost site, so regular collections can be made.

Methods

Owl roosts are commonly found along cliffs and in caves. A systematic search of these areas will be necessary to identify current roosts. Look for pellets on the ground. When pellets have been found, ascertain whether they have been produced recently, or whether they are old and crumbling.

When an active roost site has been found, the site should be marked with GPS and this information should be recorded for all samples collected from this roost. Locality data should be recorded, and should include the country, region and specific area or designated reserve. Habitat data should not be recorded solely for the roost site, as owls will have a range of several sq km. Instead, record the general habitat for the entire surrounding area. Record the date that each specimen was collected. This is essential if seasonal variation is to be studied.

Try not to scare the owl away each time you visit the roost site. It should be possible to visit the site regularly (every three or four days would be ideal) and build up a collection over time.

Samples should be dried in the sun if possible. Store each collection separately, and include data within the bag. Keep all samples from the same roost together, but in separate bags according to the date the samples were collected. At the end of the collection period, you should have several large bags, each pertaining to a specific roost. These large bags should hold a number of smaller bags containing pellet samples with associated data.

Data should also be entered into a simple excel database.

Pellets should be sent to an expert for examination. If it becomes necessary to dissect the pellets in the field, refer to the references listed below.

- 1) Goodman, S. M., Langrand, O. & Raxworthy, C. J. 1993. Food habits of the Barn Owl *Tyto alba* at three sites on Madagascar. *Ostrich* 64: 160-171.
- 2) Goodman, S. M., Langrand, O. & Raxworthy, C. J. 1993. Food habits of the Madagascar Long-eared Owl *Asio madagascariensis* in two habitats in southern Madagascar. *Ostrich* 64: 79-85.
- 3) Goodman, S. M. & Langrand, O. 1993. Foodhabits of the Barn owl *Tyto alba* and the Madagascar Long-eared Owl *Asio madagascariensis* on Madagascar: Adaptation to a changing environment. *Proc. VIII Pan-Afr. Orn. Congr.* 147-154.
- 4) Yalden, D. W & Morris, P. A. The Analysis of Owl Pellets. An occasional publication of the Mammal Society: NO. 13.

Results

Collections of owl pellets should supplement species inventories of small mammals, reptiles and amphibians. They should also allow the verification of species that cannot be collected without specific permissions, including primates such as bush-babies and mouse-lemurs.

If collections are made regularly over time, the data can be used to identify seasonal variation in predation of small mammal populations, and can provide the basis of a simple publishable study.

1.4 Mist netting and Harp traps

Mist netting

Equipment

- Mist nets
- Sisal or other string
- 3 metre-long wooden or metal poles (two per mist net)
- Machete
- Gloves
- Bat bags (at least 10)
- Specimen preservation equipment (see section 2)

Procedure

Mist nets are efficient and ideal for ground-level trapping of microchiroptera and small megachiroptera.

Mist nets are constructed from a mesh of fine, synthetic, supported by a framework of braided nylon and a number of horizontal shelf-cords. When the net is set it forms a capture area perpendicular to the ground with four or five horizontal pockets made from the netting. *See figure 3.* To erect the nets, the loops in the ends are fitted in sequential order on long wooden or metal poles that are tied to vegetation or stabilised in other ways. The net should be stretched fairly tightly, but still be elastic. It takes a minimum of two people to set up a mist net, preferably more if the net is long.

See Wilson et. al (1996) for detailed description of set-up and dismantling.

Mist nets are available in different lengths, ranging from 3 to 18 m (the most commonly used are the 6-12 m nets). The netting material is very fine and the bats can barely detect them. The bats are captured when they fly into the net and fall into a pocket, from where they rarely escape.

Figure 3. Mist net, with four capture pockets.

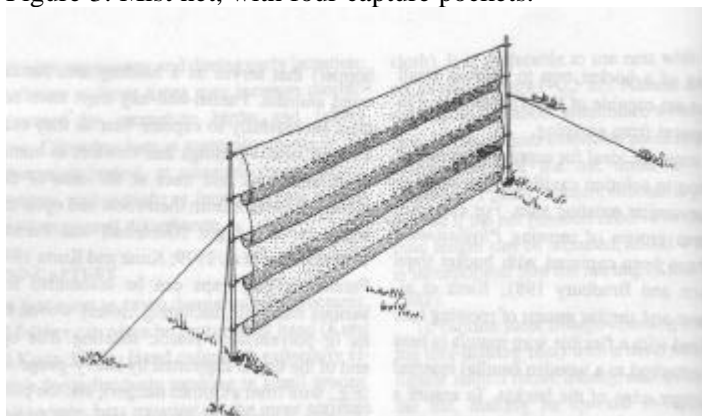


Figure from Wilson et al (1996).

Mist nets should be set in suitable locations such as along flight-paths and around roost and feeding sites. Ideal places are forest clearings, forest edges, paths, roads, streams and ponds. Placing the nets in enclosed areas with overhanging and surrounding vegetation helps to channel the bats into the nets and makes it difficult for them to escape.

Do not trap very close to roosting-sites, as too many individuals might be caught at one time and make it difficult to remove them all from the net in a timely fashion.

The lengths of the nets used will depend on the location. Short nets are suitable in dense forest and on narrow paths and roads, whereas long nets are suitable for open spaces and over water. It may be beneficial to place several nets (often of different lengths) in formation, to increase the probability of capture.

All bats are nocturnal and mist nets are only kept open from dusk to dawn. The most productive time to catch bats is often around dusk, when the bats emerge from the roosts, and at dawn when they return to the roosts. Different species may have different activity patterns and trapping sessions throughout the night will increase the chance of catching all possible species.

Some bats may quickly learn about the location of the mist nets and avoid them, and it is recommended not to trap for more than 2-3 days at each location. If nets are used at the same site for consecutive nights, either fold the net over the top-shelf during the day (so nothing can get caught in it) or take the net down and set it up again the next night. If people or large animals (e.g. cattle) are known to be in the area during the day always take the net down, otherwise it may get severely damaged.

When the nets are open they should be checked frequently, or, preferably, be manned at all times.

In rainy weather, the rain drops hangs in the net and make it easily detectable for the bats, which in turn are less likely to be caught. Furthermore, most bats are fairly inactive in heavy rain.

When a bat gets caught in the net, immediately untangle it; if left, it may get severely entangled and injure itself. When removing an individual from the net first find out from which side it flew in, then remove it from the same side. Hold the body gently but firmly with one hand (thumb under the chin to prevent it from biting, but be careful as small species are easily strangled) while gently untangling it from the net (normally feet and wings first) with the other hand. A fine crochet hook is sometimes helpful for untangling.

If the individual is to be kept, place it in a breathable cloth bag (individually numbered and firmly tied up) and leave it hanging in the bag until there is time to examine the specimen. Try

and keep all individuals in separate bags as they may injure each other if kept together. Do not leave specimens overnight. Small species easily die from starvation and stress, so handle them quickly and gently. Always wear gloves when handling bats; they are vicious biters and may carry rabies.

If an individual is to be released, either hang it from a branch (fruit bats) or gently throw them into the air.

Harp Trapping

Equipment

Harp traps

Strong nylon or cotton rope, at least 2 x 30 metres

Machete

Gloves

Bat bags

Specimen preservation equipment (see section 2)

Procedure

Harp traps are square or rectangular metal frames (of varying sizes, but normally 1 to two square metres) crossed by a series of vertical wires or string. A cloth or plastic capture bag is attached underneath. The trap can be placed on legs and used for ground trapping but can also be suspended by ropes in the canopy. *See figure 4.*

The wires are barely detectable by the bats, which fly into them and fall down into the capture bag. Once in the bag, the bats will rarely escape and can easily be collected.

See Wilson et. al (1996) for detailed description of set-up and dismantling.

Figure 4. Harp trap suspended by ropes.

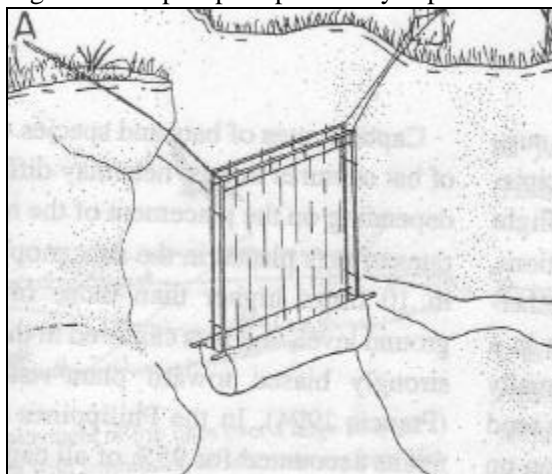


Figure from Wilson et al (1996).

Harp traps are good for catching larger species of bats, and bats that fly high in the canopy. They are also ideal for trapping around roosts where there are a large number of bats, as it is much easier and faster to collect individuals from the bag than untangling them from a mist net. They can be set in the openings of caves or hollow trees that function as roosts, and around buildings inhabited by bats. Placing the traps in clearings in the vegetation often gives the best results as the bats are channelled into the trap.

Harp traps are relatively heavy and awkward to carry around, so it is a good idea to identify a good trap location before bringing the traps. It takes a minimum of two people to set up the trap.

Using harp traps in conjunction with mist nets is a good combination, ensuring a high probability of catching most of the species that are present.

Bat dung collection

Dung from insectivorous bats have proved interesting to some scientists and the MGF projects helps collecting dung for analysis of food contents.

Dung can either be collected from individuals being released or from individuals kept as specimens.

The easiest is to keep the individual alive in a cloth bag over the night. If the individual is caught late in the evening it would have had time to forage before capture and will therefore produce more dung.

The dung is picked out of the bag with forceps and stored in butterfly envelopes. Make sure the dung is dry before packing, if not the sample will go mouldy. Store all dung from same one individual in the same bag. Make sure that the bag is clean before use, old dung from other individuals will make data unreliable.

Associated data is to be noted down on the envelope. If the animal is kept as a specimen note down on the specimen data sheet that dung has been collected and write the MGF collection number on the envelope.

Example of information noted down on envelope:

Frontier Madagascar Tulear Region Sept Lacs area S 23° 41' 32.9" E 044° 58' 12.7" 16.04.02 half moon 140 masl Gallery forest, 10m from lake Capture method: mist net 12 m. Time of capture: 8.30 pm. Time of processing: 9.00 am. <i>Myotis goudoti</i> . Male, adult Collection number: MGF 214
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1.5 Invertebrate collection

Invertebrate taxonomy is complex. The invertebrates of Madagascar are poorly understood and species lists for the majority of taxa are produced some time after the survey reports and are added as supplements.

This section outlines the simple, repeatable techniques required to compile inventories of the invertebrate groups encountered in each study site.

Butterfly collection

Introduction

Canopy traps and sweep-nets are used for the collection of butterflies. Some butterfly species have adapted to many different environments and are found throughout the Afrotropical region. This is especially true of many members of the family Pieridae, which are so well distributed that many of the species indicate very little about the areas in which they are

found. However, some butterflies are very sensitive to environmental disturbance. Many species from the sub-family Charaxinae and families Lycaenidae and Hesperidae are very specialised. Their presence or absence reflects the state of the environment, so they can be used as ecological indicators

The butterflies of Madagascar are very poorly understood. Collections at institutions worldwide are incomplete. New species are frequently being discovered. The species list and collection made by Frontier will contribute greatly to the overall knowledge of species from southwest Madagascar.

Canopy Trapping

Equipment

Canopy traps

Thin, strong nylon rope

Fermented banana bait

Butterfly envelopes

Killing pot

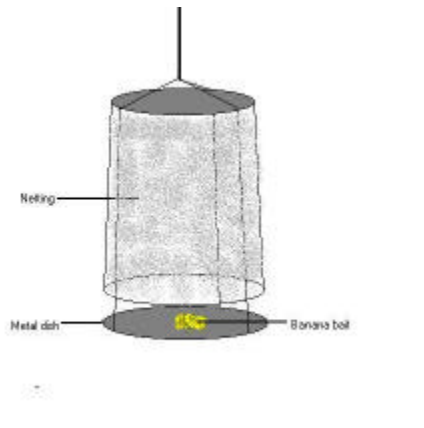
Butterfly forceps

Procedure

Canopy traps (also called blendon traps) are designed to catch many species of butterflies and are particularly useful for catching high-flying or fast species that would not otherwise be collected with a hand-held net. The traps consist of a circular metal dish hung below a net. Bait is placed on the metal dish to attract the butterflies, which then become trapped in the net (see figure 5).

Canopy traps are an integral part of biodiversity trapsites. The traps should be placed at different heights and in different habitats (e.g. near the ground in a clearing, or high up in thick vegetation). The trap is suspended over a suitable branch on a length of rope. The best way to do this is to tie a stone or piece of wood to the loose end of the rope and carefully throw it over the branch, then pull the net up to the desired height. Care should be taken to ensure that the trap can be raised and lowered without it snagging on branches, as this would lead to butterflies escaping as the trap is lowered, and may cause damage to the traps. Be sure to place the traps in a sheltered area. If the trap is blown around by the wind, the bait will slide out and the trap may become entangled in nearby vegetation. Also, butterflies cannot land on a swinging trap.

Figure 5. A canopy trap.



The bait used in canopy traps is usually a dollop of fermented banana. The banana bait should be mixed up in advance and left in a suitable container in the sun for at least 5 days to allow it to ferment. Care must be taken to ensure the container is large enough, as the mixture will swell. Also, be sure to open the container frequently to release the gases, or the resultant pressure will blow the container apart! Make sure that the mixture does not slide or dribble out of the trap, as butterflies must be induced to enter the trap to feed.

The traps work due to the fact that butterflies usually tend to fly upwards when they take off. So, the butterfly flies to the trap and lands on the edge of the metal dish. It then walks under the net and onto the middle of the dish to feed on the bait. It then flies upwards, into the net. It will sit there until it is released. Obviously, the size of the gap between the metal dish and the net is crucial. Too low and the butterflies cannot walk in. Too high and some will escape. The optimal gap size is about 5cm.

Traps are baited in the morning and checked at noon and at dusk. Butterflies should not be left in the traps overnight.

Sweep-netting

Equipment

Sweep-net
 Butterfly envelopes
 Killing pot
 Butterfly forceps

Procedure

Sweep-netting should focus on the peak activity time for most butterfly species, around midday. However, in order to maximise the chances of collecting all species present, opportunistic sweep-netting should be undertaken throughout the day. If the weather is rainy or overcast, wait until the sun comes out.

Hand-held nets are used to collect butterflies. It is a tricky business, as many species are fast and manoeuvrable. Take care not to snag and tear the nets on vegetation. Actively chasing specimens yields many captures, but standing and waiting in a sunny clearing, beside water or along a path can also prove successful, as specimens will come to you.

Keeping a Specimen

Firstly, be sure the specimen is a butterfly. Some butterflies, especially members of the family Hesperidae, look superficially similar to moths. In particular, species from the genus *Coeliades* appear moth-like in almost every way. The standard distinguishing feature for a butterfly is that they have clubbed antennae (i.e. the antennae have a small bulge at the end of a smooth stem), whereas moths have feathery antennae.

Try to identify the butterfly to genus (and preferably species), or at least try to identify whether it is one that has been collected already. It is important not to collect large numbers of the same species, but at the same time several genera have very similar species, so we do not want to release species not yet collected. For example, the genus *Acraea* contains many species that look superficially similar (red with clear patches on the wings and black spots). However, close examination often reveals noticeable differences in colouration and patterning.

If a butterfly is to be taken as a specimen, one way to quickly kill them is to squeeze the thorax (the place where the legs join the body) until a distinct popping is felt. Do not squeeze too softly or it will have no effect. Conversely, do not squeeze too hard or you will destroy the specimen. If the collector does not feel confident about killing the specimen by pinching the thorax, the butterfly can be killed by putting it in a 'killing pot'. This is a pot that contains cotton wool soaked in ethyl acetate.

When collecting butterflies, always take some of the greaseproof butterfly envelopes into the field. Placing a specimen in an envelope will prevent damage to the wings. (Butterfly wings are covered in tiny, coloured scales that fall off when the wing is touched.) Place only one specimen per envelope. Briefly but neatly label the envelope in the field, noting the date and location. The remaining information can be written on the envelope at base-camp (See figure 6). Take the butterfly out while you are writing on the envelope, or you will damage the specimen. Each envelope should be given a unique number, which will be recorded on a data sheet for that trap site or location. This produces a numbered species list for each area.

Figure 6. Example of a butterfly envelope.

Frontier Madagascar Tulear Region Sept Lacs area S 23° 41' 32.9" E 044° 58' 12.7" 16.04.02 (end of rainy season) 140m asl Gallery forest, valley floor, 10m from lake Capture method: canopy trap # 2 Trap height: 5m Bait: fermented banana <i>Charaxes zoolina</i> (male) Specimen # 145

Butterfly pinning

Butterflies are mostly identified by the size, colour, shape and patterns of the wings. Butterflies are easily pinned and dried so they can be displayed and studied.

Equipment

Insect pins
Polystyrene
Paper
Insect forceps
Insect proof, naphthalene impregnated storing boxes
Labels

Procedure

A little groove (about one centimetre wide and one centimetre deep, depending on the size of the butterfly) is cut in a piece of polystyrene. The wanted butterfly is caught and killed either by squeezing the thorax or by putting in a killing pot containing ethyl acetate. Pin the specimen soon after killing, if left for a while the body will go stiff and it will be difficult to spread the wings.

The butterfly is held gently with the forceps and a pin is pushed vertically down through the thorax from the dorsal to the ventral side. The butterfly is pinned on the polystyrene so the body rests in the groove and the wings are level with the surface of the polystyrene. The wings are gently spread out to the sides using forceps or pins, so that all four wings are visible. Cut out two pieces of paper and gently cover each side with one piece to hold the wings in position and to protect the wings. Pin the paper to the polystyrene on the edges of the wings. Do not pin through the wings, as this will damage the specimen. Adjust the legs, antennae and proboscis with pins so that the specimen looks 'natural'. Leave to dry in an insect-proof box for at least 10 days. After this time the paper and supporting pins can be removed, leaving only the one pin going through the thorax. The specimen can now gently be taken off the polystyrene and transferred into a storage box. Write one label with the species name of the specimen and another with description of the habitat it was found in. Slide the habitat label half way up the pin underneath the butterfly and leave the other label flush with the floor of the box.

Once the butterflies are dry they are very fragile and should be handled very carefully. Be especially careful when transporting the boxes, as too much movement may cause the wings and antennae to fall off, ruining the specimens.

The pinned specimens should preferably be stored in specially designed insect storing boxes impregnated with naphthalene. Bags with silica gel kept in the storage box will prevent the specimens from going mouldy in humid conditions.

Litter sifting methods for a 20 sample transect

This method samples the forest floor invertebrate community. It is particularly suitable for the collection of ants, spiders and scorpions, although many other taxa are collected.

Equipment

- 20 flags
- 20 leaf litter stuff sacks
- 20 mesh sacks for hanging extractor bags
- 20 hanging extractor bags
- Labels
- Gloves
- 2 hand-held rakes
- 2 sifter bags
- 2 machetes
- 5 m cord (to measure distance between samples)
- 20 bottom-less cups
- 2 normal cups
- 20 whirl back bags
- 1 large zip lock for whirl pack bags
- ½ to 1 litre of alcohol
- Masking tape
- Permanent ink (indian ink) pen
- Scissors
- Large bucket

Procedure

The aim of the leaf-litter transect is to standardise sampling between sites and to assure that enough samples are taken to cover representative microhabitats in the area.

Leaf-litter sampling cannot take place when the leaf litter layer is wet. Moist and damp leaf-litter is good, but when wet, the sifting process is not effective. Therefore, sifting cannot occur during the rain or soon after a rainfall.

Brief outline of steps:

- 1) Hang up all twenty of the extractor bags in a warm, dry place, out of the wind.
- 2) Choose a representative and diverse area of the forest and peg out the numbered flags in order. Place one flag every 5 metres.
- 3) Collect the leaf litter and put it into the numbered stuff sack bags.
- 4) Back at camp, fill the mesh sacks with leaf litter and place them in hanging extractor bags for 2 days.

Taking samples along the transect:

- 1) Tie off the bottom of the sifter collecting bag very securely (it can open up during vigorous sifting). Wear gloves during the sifting to avoid blisters.
- 2) Approximately 1 square metre of litter should be sifted for each sample. Include dead twigs, branches, rotten logs, and thick leaf litter at the base of trees. First chop the ground, twigs and logs with a machete. Rake the chopped material into a pile with the small hand-held rake. Place the edge of the sifter on the ground and rake the leaf litter and twigs into the sifter. Rake only down to the dirt layer. Do not overfill the sifter. Pull out the big sticks before shaking the sifter. Shake the sifter in a circular motion for at least 1 minute. It should take 3 to 6 fills of the sifter to cover 1 square metre.
- 3) The bag at the bottom of the sifter will fill up as you sift. You will learn to judge the amount needed to fill one mesh sack back in the lab. Once the leaf-litter has been sifted, pour the sifted litter into the corresponding numbered stuff sack for that sample. Tie the sack and place it next to the flag. If it begins to rain, quickly pick up all the filled sample bags and get them out of the rain.
- 4) When all 20 bags have been filled, carry them carefully back to camp without crushing the contents. The sifted litter is delicate and can easily be destroyed by rain or compression. Do not leave the bags in the sun. The samples should be processed the same day they are collected.

Processing the samples back at camp

- 1) Prepare all the whirl-pak bags, cups, and labels for the samples. Prepare the cups by attaching the whirl-pak to the bottomless cups with a piece of scotch tape. When you are ready for the cup, add about 2cm of alcohol to the whirl-pak and put the appropriate label number in the cup.
- 2) Organise the stuff sacks by number so you can quickly find the next sample.

3) Open the stuff sack and fill the mesh sack with the sifted litter. This is best done with two people, over a large bucket. Remove sticks by hand and after filling halfway, shake the mesh sack to settle the litter. Litter will fall into the bucket along with insects that are already escaping the mesh sack. The litter in the bucket usually contains a lot of insects so be sure to pour it into the mesh sack before hanging.

4) To hang the mesh sack, first place a normal cup at the bottom of the hanging extractor bag to catch insects and dirt that falls when hanging the mesh sack. Then hang the mesh sack in the hanging extractor bag. Soil will fall into the cup, along with some insects. Quickly take the cup from the hanging bag and pour the dirt and insects it contains back into the top of the mesh sack. Next, attach the whirl-pak cup to the bottom of the hanging bag. Tie the top of the hanging bag, being careful not to shake it and cause dirt to fill up the whirl-pak bag.

5) Be careful to avoid bumping the samples as dirt will fall into the samples and contaminate them. If you notice that dirt has fallen and has filled the whirl-pak bag to the level of the alcohol, remove the cup and add more alcohol to it.

6) After 2 days, remove the cups and whirl-paks. Securely tighten each whirl-pak, squeezing all air out of the sample. Place all samples from the transect into one ziplock bag and label the bag with the transect number. When transporting this bag, be careful that the samples are not crushed.

7) Remove the mesh sacks from the hanging bags and shake out the litter. Pack them away carefully.

7) Pack the hanging bags away only when they are dry. After a phase, be sure to dry the bags again in the sun.

Recording the Data

Individual sample labels should include a unique sample number. This will consist of three digits and three numbers e.g. MGF121. For leaf litter sample number 1, the label should read MGF121/01. Therefore, for every leaf litter transect, there will be twenty different numbered labels according to the samples. Using the example of MGF121, the twenty labels will be numbered MGF121/01 to MGF121/20.

The locality data should be recorded and stored in a database format. The following data must be included:

Specimen sample code
Country
Region
Area
Collection method
Date and season
Latitude and longitude
Altitude
Habitat

Malaise Trapping

Introduction

Malaise traps are large, stationary mesh nets that trap invertebrates and channel them up into a bottle containing ethanol. This method samples the flying invertebrate community. It is particularly suitable for collecting Hymenoptera (particularly wasps and flying ants) and

diptera (flies), but also proves a successful method for collecting Lepidoptera (butterflies and moths) and Coleoptera (beetles).

Equipment

Malaise trap

String

Tent-pegs (or similar)

Ethanol

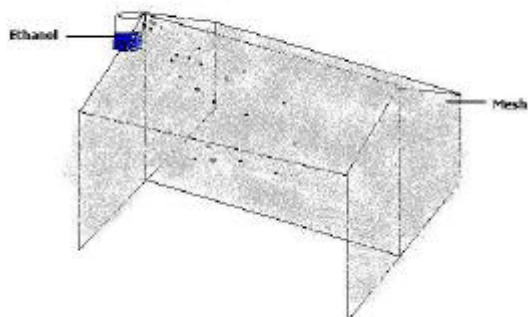
Procedure

Malaise traps are located in natural corridors for flying invertebrates such as the edge of a dense area of vegetation, gaps in vegetation, paths or the edge of streams. The trap is set up for 2-4 days in each location, and is placed in representative habitats in order to collect a wide variety of invertebrate species.

The trap is laid out at right angles to the natural corridor or flight path. The base is pegged out tightly along the ground then the malaise trap is lifted and tied to nearby vegetation, ensuring the sides of the mesh trap are tight. The bottle containing the ethanol must be placed at the highest point and in sunlight if possible (see figure 7).

About 5cm of ethanol should be placed in the trapping bottle. After 2-4 days, the bottle should be unscrewed and the contents poured into a whirl-pak bag. The bag should be sealed without trapping air inside.

Figure 7. A malaise trap.



Data recording

The whirl-pak bag must contain a label with a sample number that is unique for that malaise trap e.g. MGF132.

The locality data should be recorded and stored in a database format. The following data must be included:

Specimen sample code

Country

Region

Area

Collection method

Dates and season

Latitude and longitude

Altitude

Habitat

Spider and scorpion collection

Introduction

Spiders and scorpions are relatively un-studied in Madagascar. Few species are known. During the course of the biodiversity survey, many spiders and scorpions are encountered. The pitfall methodology outlined in Section 1.1 provides the ideal framework to produce a comprehensive collection with minimal effort. They are also often found during casual collections and night-walks (the eyes of a spider reflect blue in torchlight).

Procedure

Spiders and scorpions can be collected with relative ease. Carefully scoop the specimen into a pot - the venom toxicity of many species remains unknown, so handle them with care. Specimens should be stored in at least 75% ethanol. Placing them directly into the ethanol will kill them. All specimens from the same locality or trapsite should be put into one whirl-pak bag. The bag should be sealed without trapping air inside.

Data recording

Each whirl-pak bag must contain a label with a unique sample number e.g. MGF175. This will correspond with the trapsite or collection locality.

The locality data should be recorded and stored in a database format. The following data must be included:

Specimen sample code

Country

Region

Area

Collection method(s)

Dates and season

Latitude and longitude

Altitude

Habitat

1.6 Casual collections

We should not rely on traps to provide us with a representative sample of species from an area. Some species are too large or adept at escaping traps to be caught. For example, many geckos have toe-pads that enable them to climb out of buckets, and many of the snakes and mammals are large enough to climb out of the buckets. So, we undertake opportunistic day and night searches for specimens.

This involves 'sweeping' an area for large, visible species such as lemurs, snakes, chameleons or basking lizards. It also involves night-walks to look for frogs, nocturnal snakes, nocturnal mammals or chameleons (which become pale at night and can be spotted easily with a good head-torch). It is also necessary to look under fallen logs and in rotten tree-stumps, under bark, under rocks, in the leaf litter, in the soil or inside vegetation.

Any specimen found opportunistically should be collected and identified. Habitat notes (where it was found) should be recorded.

2.0 PRESERVING AND STORING SPECIMENS

Voucher specimens of most species are taken during every good biodiversity study. Without them, the data cannot be validated or referred to at a later date. The specimens collected by Frontier will verify the data collected in the field, and supplement incomplete specimen collections both in country and overseas.

As important as the specimen itself are the ecological notes and measurements taken at the time of capture. Without these notes, very little useful information can be gained from the specimen.

2.1 Specimen identification

When a specimen has been collected, an attempt should be made to identify it in the field. This is to ensure that a representative collection of many different species is made. Accurate field identification reduces the risk of repeatedly collecting specimens of the same species, and provides a preliminary assessment of the biodiversity of the area pending formal taxonomic identification.

Field identification is carried out using the following references:

Glaw, F. & Vences, M. (1994) A Field guide to the Amphibians and Reptiles of Madagascar

Hawkins, F. (1998) Birds of Madagascar, a Photographic guide

Sinclair, O. (1998) Birds of the Indian Ocean Islands

Butterflies of the Afrotropical Region (series)

Garbutt, N. (1998) Mammals of Madagascar

2.2 Equipment

For recording and taking a specimen

- Specimen pots for short-term specimen storage
- Plastic bags to make accurate colour notes easily while the animal is alive
- A colour chart to make colour notes that are reproducible
- Cotton wool for the chloroform
- Data sheets
- Weighing scales
- Calipers
- Tape measure
- Chloroform For killing reptiles or small mammals
- Chlorotone For killing frogs
- Formalin 10% solution
- Borax salt
- Dissection kit Including scissors, mounted needles, scalpel, syringes
- Dissection tray
- Parchment paper or labels

- White or black surgical thread
- Indian ink pen or soft pencil

2.3 Procedures

Recording specimen data

Specimen data must be recorded on the specimen sheets provided (see Appendices 3-8). Data are recorded both before and after the specimen is taken. The data required will vary depending on the species, but the following information should always be taken to ensure the correct specimen is associated with the correct data, and because the preserved specimen may change colour or size gradually over time:

<u>Field notes</u>	Date Time Weather Observers Location (GPS co-ordinates and general description) Method of capture (casual, pitfall trap, etc) Trap number (if applicable) Family, genus, species, subspecies (if known) New/recapture (if applicable) Marking code (if applicable)
<u>Topography</u>	Location, slope, aspect, altitude
<u>Ecology</u>	General habitat type, microhabitat, association to water Behaviour and other notes if relevant

NB When trapping bats it is important to draw a map of the area with important features (e.g. streams and paths) with the placement and configuration of the nets.

<u>Description</u>	Colour notes to be taken while the specimen is alive (head, back, belly, legs, tail, underside and topside, any distinguishing features) Adult, Juvenile, sub-adult Sex Dimensions (mm) (see specific data sheets) Weight (g) Other (e.g. scale counts for reptiles, etc) If a tissue sample was taken for DNA analysis
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General procedure for taking a specimen

1. Fill in the data sheets as fully as possible before taking the specimen. In particular, record all colour notes.
2. Kill the specimen (see notes for individual species).
3. Complete the remainder of the data sheet.
4. Tie a label with the unique specimen number written in pencil or indian ink to the back right leg of the animal or around the waist if the animal has no legs. Use white thread when labelling reptiles and amphibians, as black thread can stain and discolour these specimens.
5. Carefully inject the specimen with formalin, including the body cavity and all large areas of muscle.
6. Place the specimen in a natural posture in a box containing tissue paper saturated in 10% formalin.

7. Once fixed in that posture, (usually after 1 day), place the specimen in a solution of 10% formalin.

Reptiles

These can be killed with ether or chloroform by placing the animal in a sealable bag containing cotton wool soaked in the chemical. Seal the bag with the minimum amount of trapped air inside. Ether and chloroform can alter the colour of the specimen and cause muscle contortion, so be sure to take colour notes before taking the specimen. Some reptiles have a thick skin, such as the blind snake *Typhlops*. This reduces the rate of absorption of the chemical so the specimen may take a long time to die.

A different method for taking a reptile specimen to inject it with a saturated solution of chlorotone. Snakes are injected directly into the heart, and lizards through the armpit into the chest cavity. If the specimen is a small lizard, it can be placed directly into a container of saturated chlorotone solution.

When preserving a lizard, it is important for the specimen to retain the tail. This necessitates careful handling. The muscles that cause tail-loss can function for up to one hour after the animal is dead, so specimens should not be handled during this time. Also, when preserving specimens of the fish-scaled gecko (*Geckolepis*), do not handle them until they have been dead for at least one hour. The scales remain loose during this time, and will be lost through handling (they are an essential factor in specimen identification).

When preserving a snake, coil it carefully with the head on the outside. This makes the specimens easier to handle.

If the reptile specimen is male, the hemipeni should be everted before preservation. This is achieved by gently squeezing the base of the tail, pushing towards the vent, taking care not to lose the tail. When the hemipeni are everted they should be filled with formalin to prevent them from retracting. Hemipeni structure is often studied during species identification.

Amphibians

These should be killed by placing them into a strong solution of chlorotone (two heaped teaspoons-full in 150-200ml of water). Specimens from the genus *Scaphiophryne* may require a longer time in the solution, as they have tough skin that hinders absorption of the chemical. The specimen should then be placed in a life-like position, with the toes spread to show the webbing.

Small mammals

Small mammals may be collected as specimens. However, lemurs are primates and as such have protected status and cannot be taken as specimens without license.

When preparing a specimen, a small incision must be made in the lower abdomen to allow formalin to enter. The specimen should initially be stored upside down to allow gasses to escape.

See appendix 2 for example of measuring and sexing small mammals.

Specimen storage

This does not require much maintenance. Putting a pinch of borax salt in the formalin will help preserve the bones of the animal. Specimens need to be checked frequently at first to ensure adequate preservation, with additional injections of formalin if parts of the animal feel soft. The formalin should be changed when it becomes cloudy.

Additional information

- Ensure the animals collected from the field are dealt with as quickly as possible, with the minimum of handling. They should be kept out of direct sunlight.
- Always wear gloves when handling formalin

- To make a 10% solution of formalin, mix a standard 500ml bottle of locally purchased 'Formol' with 4 litres of water.
- Locate the heart of a snake by running your thumb down the vent until you can feel the pulse.
- Completely immerse the specimen in formalin for effective preservation.

2.4 DNA Samples

Formalin damages DNA. In order for phylogenetic studies to be carried out on specimens, a tissue sample must be removed before the specimen is preserved. One DNA sample should be taken from each species collected, so that a representative DNA library is built up. However, if a new or rare species is collected, several DNA samples should be taken.

Equipment

- 1ml eppendorf tubes
- A saturated solution of EDTA, or an 80% solution of ethanol.
- Specimen labels
- Indian ink pen or soft pencil

Procedure

The specimen should be killed using the methods described above. Immediately after it is dead, a tissue sample should be carefully removed without excessively damaging the specimen. If possible, a section of muscle from the hind leg or breast should be taken. Other tissue samples include the liver, the toe, the ear or the tip of the tail. The latter two have the advantage of being applicable for a specimen that cannot be taken as a specimen, such as a lemur or a boa. However, in all other situations a DNA sample must not be taken unless the specimen is to be kept.

The tissue sample must be removed with sterile dissection equipment, to prevent contamination. The sample should be put into an eppendorf tube containing a saturated solution of EDTA or 80% ethanol.

Data recording

Assign the DNA sample the unique code that corresponds to the tag attached to the specimen. Label the outside of the tube only (the ink contains organic material that may contaminate the sample). Record on the specimen data sheet that DNA has been taken.

Additional information

- Carefully wash implements and surfaces before taking a DNA sample to avoid contamination of DNA from other species and prevent the DNA being destroyed by formalin.
- DNA tissue samples should be taken soon after the death of the animal, because enzymes released at the time of death will damage DNA molecules.

3.0 METHODS BASED ON FIELD OBSERVATION

This is the least preferred method of data collection when carrying out a biodiversity study because it is extremely difficult to verify the data. However, it is very useful for other purposes, such as behaviour studies, or additional ecological notes about species with an already confirmed identification. Accurate field notes are the key to reliable field observations, and the information will vary depending on the aim of the study.

Sections 3.1, 3.2 and 3.3 describe examples of methods that require accurate field observation.

3.1 A species inventory for birds

Introduction

Birds form an integral part of the ecosystem. The variety and numbers of bird species present in an area will be indicative of the habitat and state of the environment. A species inventory will identify the presence of rare species, species important to a specific habitat, or unusual species in an area. This information will increase the current knowledge of the Madagascar avifauna and will highlight areas of the region that require further study or management. Birds are charismatic and popular with people interested in wildlife. Information about birds is often desired in studies related to tourism or conservation targeted to the general public. Birds are relatively easy to observe, and individual species can almost always be separated by sight and sound. Birds are well studied, and sufficient information about birds is available to make correct field identifications.

Aim

To obtain an inventory of the bird species in the survey area and record associated ecological data.

Equipment

Log book	to record all relevant data
Note book	to record information in the field
Pencil	Pen will run in the rain
Binoculars	8 x 40 are recommended for birding
Field Guide	To make identifications. Birds of Madagascar, a Photographic guide (1998, Frank Hawkins) Birds of the Indian Ocean Islands (1998, Sinclair)

Procedure

Specimens of birds are not taken, so it is vital that the bird data are reliable and accurate. One misidentification could jeopardise the validity of the inventory.

Notes for birds observed (and other associated data) are taken in the field and descriptions compared to the descriptions in the field guides. If the field notes match the notes in the guidebook, a positive identification can be made and the data is recorded in the Bird Log Book. Only positive identifications are recorded, or a full description if an identification cannot be made. One person is responsible for deciding which data are recorded. If the field notes are not adequate for the distinguishing features of the bird to match up to the description in the book, it is not classified as a positive identification. Relying on memory and identifying a specimen from the guidebook when returning from the field is not an acceptable way to make a positive identification.

Data recording

Daily Records:	Date Weather (Morning and afternoon)
Records per 'walk'	Observers (initials) Location GPS points and description, e.g. behind camp General habitat type e.g. coastal scrub Altitude
Records per observation	Time (to the nearest minute) Species name (if known) Full description Number of individuals
Record if appropriate	Sex, Juvenile, Breeding phase, colour phase Behaviour Microhabitat Vocalisation

Birding tips

- You are likely to see most birds early in the morning
- Wear clothing that is not white or reflective
- Some bird species will be seen by staying quietly in an area, while other species are more easily spotted while walking quickly through an area
- Get to know the general families found in the area, and you are more likely to identify individuals
- When making field notes, it is usually easier to make a drawing
- A very basic knowledge of bird anatomy will aid making a description
- Most bird books describe similar species and indicate features that distinguish one from another so that when you see a bird, you know what to look for in order to identify it
- Don't be discouraged about not being able to make identifications easily at first; after an initial learning period you will be able to identify a large variety of birds very easily

3.2 Tortoise behaviour

Introduction

Madagascar's radiated tortoise, *Geochelone radiata*, has long been thought to have robust populations throughout much of its range. However, new information that is emerging indicates that the species has suffered a major loss in important areas once believed to be strongholds for the species. Large-scale killing by organised groups has decimated the populations in these areas. Although *G. radiata* is listed under CITES index I, its protection is not enforced and a great many are collected and traded locally for food. They are now considered to be almost extinct north of the Onilahy river and the collectors are moving their efforts further south. Urgent action is needed to protect the tortoise. A recent study by the Wildlife Conservation Society (WCS) predicts that only a small number of viable populations will remain in the wild within 25 years, mostly in inaccessible or protected areas. Very little is known about the distribution, demographics or behaviour of radiated tortoises.

The status of the spider tortoise, *Pyxis arachnoides*, is also uncertain. With the population of *G. radiata* greatly reduced, there is now more pressure on the smaller *Pyxis arachnoides*. Its small size makes it less desirable as a food source but its main threat comes from habitat destruction due to charcoal production and the unsustainable collection for the pet trade. This

species has had very little research conducted on it and little is known about its ecology and behaviour. It is currently listed on CITES index II but it may well be reassigned to index I when more information about its status becomes available.

Informed management decisions regarding these tortoises will require knowledge of their basic ecology, as well as their distribution and density.

Aims

- To collect distribution, demographic and baseline measurement data
- To study tortoise behaviour

Equipment

For baseline measurements

- Permanent marker or nail varnish (for numbering tortoises)
- GPS
- Spring balances
- Tape measure
- Calipers
- Tortoise log book
- Pen
- Tag (to mark where the tortoises rest)

For behaviour work

- Tortoise measuring equipment (see above)
- Thermometer (to measure air temp)
- Compass (to take bearings)
- Tape measure (to measure distances)
- Pen
- Watch
- Binoculars

Procedure

Finding tortoises

This is achieved by using a number of people to systematically search the habitat in a 'sweep' formation. Tortoises are more active in the early mornings and late afternoons when it is not too hot. During the hot afternoons and at night, they seek the shade of a bush and settle into a shallow scrape in the sand. While keeping still, the tortoises are surprisingly difficult to see so the easiest times of the day to go looking for them is during their active periods of early mornings and late afternoons. However, the time of day at which an individual is active may depend, somewhat, on its size, sex or some other variable. To get a more reliable sample of the population, therefore, it would be better to go searching at all parts of the day. Tortoises can still be found even when they are inactive by carefully looking around the bases of bushes. It is possible to track a tortoise over long distances, by following its footprints in the sand. This method becomes difficult as the dry season ends and grass starts to grow.

Baseline measurements are taken of all tortoises found, and selected tortoises are followed until they rest for the night. The location is marked with a tag and a GPS point taken. This enables the specimen to be located the following morning.

Baseline measurements

These should be taken for every specimen. All individuals should be given a unique mark. Tortoise remains should be recorded when seen, including cause of death.

When a tortoise is found, note down:

1. which species it is,
2. the date,
3. the time,
4. whether it was active or inactive,
5. in shade or sunlight,
6. the weather conditions,
7. and the location.

This gives us useful information about the daily and seasonal activity patterns of the two species.

8. Check to see if it has already been caught and given a number in the past. If it has, then note this down. If it hasn't, then give it an individual number or code. Use nail varnish to write identification numbers on the tortoises.

There are a number of other observations and measurements that should be noted for every specimen. These will give us information about the age, sex ratio and size distribution of the population.

9. number of growth rings,
10. sex,
11. mass,
12. carapace height,
13. curved carapace length,
14. curved carapace width,
15. straight carapace length,
16. plastron length,
17. plastron width.
18. Lastly, study the specimen carefully and note anything unusual or distinguishing about it, e.g. an injury, cracked carapace, different colour or uncharacteristic behaviour. If it interacts with another tortoise, record which tortoise this was and the nature of the interaction, e.g. mating, or fighting with another male.

Tortoise behaviour

In addition to taking the above measurements and observations of any tortoises that are found, further information can be obtained by closer surveillance of a few individuals. By watching a tortoise throughout the day and following it at a discrete distance, its natural behaviour and activity patterns can be recorded.

It is best to locate an individual in the early evening while it is still active, and to take all the necessary measurements and give it its identification number. When it is released, it will probably be quite stressed due to the manhandling and its consequent behaviour is unlikely to be natural. As the sun gets low, however, the tortoise will seek shelter for the night, usually crawling under a bush where it will remain, completely inactive, until the morning. This makes it very simple for an observer to return to that spot before the tortoise awakes and be sitting, quietly waiting for it to start moving. Since all of the measurements were conducted during the previous evening, the tortoise will not be distressed and its behaviour can be considered natural.

There should now be an observer watching the tortoise and recording its behaviour throughout the day until it settles for the night again. The basic idea is to discover as much about these tortoises as possible. Nobody really knows what they eat, when they eat, at what temperature / season / time of day / humidity they are most active, when they mate, lay eggs, whether or not they are territorial, how long they live for, how large they get, their distribution or population size. They may show differences in behaviour depending on their

size, sex, age, hunger, the temperature, climate, season, time of day or a multitude of other variables. Any behaviour and the time at which this happens should be recorded. The more detail the better.

Examples of behaviour worth noting include:

- the time the sun first touches the tortoise,
- the time when the tortoise first moves,
- any consequent movements,
- when it eats,
- what it eats,
- contact with any other tortoises,
- which tortoises,
- what sort of interactions,
- the time it seeks shelter.

Every time the tortoise moves, it is useful to record the distance it travels before it stops and at what time it starts and stops. This not only tells us when the tortoise is most active but also whether it is covering the distance in one continuous stretch or stopping periodically to rest or forage. It is also informative to take a compass bearing of the direction in which the tortoise is travelling. When combined with the distances, a map can be drawn of the route taken. When this is repeated, over several days, it can be seen if the tortoise is remaining within a home range or wandering around randomly. We have noticed some individuals returning to the exact spot night after night while others kept walking in a straight line for days until we lost them.

Being cold blooded, the tortoises need to warm up in the sun before they become very active. As the ambient temperature rises in the afternoons, however, they risk overheating and will seek the shade. The amount of time spent in the sun or shade may also depend somewhat on the size of the individual. It is informative, therefore, to divide the day into 15-minute periods and record the total time that the tortoise has spent in direct sunlight, dappled light and in the shade during each of those periods. The total distance travelled during the 15 minutes should also be noted. These 15-minute summaries make it possible to deduce during which parts of the day a tortoise spends more time basking in the sun, when it seeks shade and when it is most active. By also recording the air temperature every 15 minutes throughout the day, it can be seen if any of the behaviour is dictated by the temperature.

If the tortoise eats, record when this happens and try to identify the item. If the species of plant is not known, it may be possible to take a sample of some leaves that can be pressed, labelled and identified at a later date.

When the tortoise seeks the shelter of a bush and becomes inactive either during the day or in the evening, make a brief description of the vegetation it has chosen. Note the height and species of the bush, if it is dense foliage, or if the tortoise is quite exposed. Also look back to the position where the tortoise started from, that morning, and estimate the distance as the

crow flies and take a compass bearing. Using a GPS can be much more accurate. Remember to clearly mark the site so that it can be found again the following morning.

One of the concerns is that the tortoises may be aware of the observer and not react in a natural way. It has been noticed that if somebody walks near to a tortoise or makes a sudden movement, it often stops walking and looks at that person. Usually after a pause of only a few seconds the tortoise realises that it is not a threat and continues walking in the same direction as it was originally heading, even if this is towards the moving person. On one occasion a tortoise even passed between the observer's legs. So it appears that the tortoises do not notice or at least are not concerned by the observers. Even so, it is better to remain at a discrete distance away and behind the tortoise (outside of its field of vision), so long as you still have a clear view. Binoculars can be used to get a closer look if necessary. It is also wise to position yourself downwind, keep as still as possible and walk gently to cause the minimum of vibrations. Talking should be avoided.

3.3 Lemur observation

Equipment

- GPS
- Compass
- Watch
- Notebook and pen

Procedure

Studies of Verreaux's sifaka (*Propithecus verreauxi verreauxi*) and ring-tailed lemur (*Lemur catta*) individual and group behaviour patterns, spatial distribution and population estimates are carried out.

Behaviours are simplified into categories – 'active', 'non active', 'resting', 'feeding' and 'other'. All observers spend time with the same lemur group to define each activity category and improve observer reliability. Scan samples - the activity of all visible individuals in the group at a precise moment - are taken every ten minutes.

A second methodology is employed to work on the spatial distribution of groups of lemurs and to delineate rough home ranges using GPS units. Groups of observers follow groups of lemurs for as long as possible. All groups are located the previous evening and followed to their respective 'sleep trees'. From their first movements at dawn they are followed and a GPS reading taken every ten minutes. This provides not only the location of the group but also the temporal distribution, determining the length of time each group spends in a certain area.

Populations of lemur groups are estimated through casual observations. This is based upon individual sightings and counts. In addition, triangulation of non-visible groups is possible through their vocalisations.

While following the groups of lemurs, a GPS point and compass bearing must be taken for each vocalisation. Three separate groups of observers are needed to produce three separate bearings enabling the vocalising group to be pinpointed.

4.0 RESOURCE-USE SURVEYS

Environmental disturbance caused by man has an enormous effect on the biodiversity of an area. Charcoal burning, logging, slash-and-burn agriculture, trapping and cattle grazing all contribute to habitat destruction, which in turn reduces biodiversity.

Standard and repeatable methods must be used to quantify levels of human disturbance in order to make sense of future surveys and to allow comparable monitoring of previously surveyed habitats. The following methods provide baseline land-use data and can be used in conjunction with biodiversity and eco-tourism survey data to identify areas of focus for management plans.

4.1 Disturbance transects

Aims

This survey aims to quantify and record the number of trees and saplings along transects, along with the level of tree cutting (for timber) and sapling cutting (for poles). It also aims to record different types of disturbance occurring in the survey area.

The survey area consists of the area 5m each side of a transect line, so that a 10m wide swathe of vegetation is surveyed along the transect. The survey data will show the number of live trees and saplings, the number of naturally dead trees and saplings, and the number of old cut and new cut trees and saplings. It will also record the incidence of other forms of disturbance, such as trapping, cattle grazing, charcoal production or cultivation.

Equipment

Below is a list of the necessary equipment required to conduct a disturbance transect survey:

- A thin, strong nylon rope, 50m in length.

Thick ropes tend to twist and snag on vegetation. Make sure the rope does not stretch. A brightly coloured rope is preferable so that it can be clearly seen amongst thick vegetation.

- A notepad and several pencils (with eraser) and pens.

The notepad should be waterproof if possible. The page layout should be drawn up as described in the *Data Recording* section (see below).

- A GPS and spare AA batteries.

When returning to an area to continue an on-going transect, be sure to take the same GPS that was used there previously. This ensures that the GPS will have the relevant waypoints recorded, so the location of the previous day's work can be found.

- A DBH (Diameter at Breast Height) tape

This tape is used to measure the diameter of trees and saplings.

- A Tape-measure (at least 5m in length)

This is to measure the distance from the transect rope to the tree or sapling.

- An altimeter.

This is used to record the altitude at each 50m point along the disturbance transect.

- A compass

Some disturbance transects are aligned east-west or north-south. A compass is necessary to maintain a straight transect line.

- Plastic tags (and permanent marker pens)

These are used to tag and label the 50m points and are also used to mark the end-point of incomplete transects. The tags should be brightly coloured, to allow returning teams to easily locate the transect route.

- A machete

This is used where the vegetation is very dense, and should be used only when absolutely necessary.

It is also advisable to take a butterfly net, butterfly envelopes and specimen pots. Many casual zoological specimens are collected during disturbance surveys.

Procedure

Transect Location

When undertaking a disturbance survey in an area, the first step is to locate the direction and position of the transect. If the area to be surveyed is large, it is often a good idea to lay down a grid system of disturbance transects across the survey region.

In small areas such as narrow valleys or lakeshores, it is possible to wind the transect through the survey area in order to build up a picture of the disturbance in that area. For example, three disturbance survey transects could be positioned in a valley, one to run up the left, one up the middle and one up the right of the valley. This would build up a good data set for the incidence of disturbance along and across the entire valley.

Methods

The first step is to lay out the transect rope. This is done by one person, who pulls the rope from the starting point of the survey until all 50 metres are laid out in the desired direction. If the transect follows compass directions, the rope must be laid accurately along the chosen angle. If the transect follows the course of a valley, river or lakeshore, the rope should be laid in the direction of the survey area.

The second step is to begin the survey along the transect line. The aim is to record the number of live trees and saplings, dead trees and saplings and cut trees and saplings for each 50 metre section of the transect. This requires one person to count the all trees located within five metres either side of the transect rope, and another person to do the same for saplings. For the purposes of the survey, a sapling is any plant with a diameter at breast height (DBH) greater than 5cm, but less than 20cm. A tree has a DBH greater than 20cm.

Each tree or sapling is classified as live, dead, old cut or new cut. The differentiation between an old and a newly cut tree or sapling is identified in the colour of the cut stump. If the cut wood is the natural colour of the wood, it is a fresh cut. If the stump has blackened, it is classified as an old cut. The transect is then extended by a further 50 metres, and the process begins again.

At the end of the entire transect, the person recording the data will have figures indicating the composition and relative disturbance for that area, divided into 50 metre sections. This enables variation in levels of disturbance and forest structure to be seen.

Data Recording

Data are recorded in the field (as a running tally) using the following format:

<u>Distance</u> <u>(m)</u>	<u>Altitude</u>	<u>Live</u> <u>saplings</u> <u>(poles)</u>	<u>Naturally</u> <u>dead</u> <u>saplings</u>	<u>Old cut</u> <u>saplings</u>	<u>New cut</u> <u>saplings</u>	<u>Other</u> <u>disturbance</u>
0-50						
50-100						
100-150						
150-200						
200-250						
250-300						
300-350						
350-400						
400-450						
450-500						

<u>Distance</u> <u>(m)</u>	<u>Altitude</u>	<u>Live trees</u> <u>(timbers)</u>	<u>Naturally</u> <u>dead trees</u>	<u>Old cut</u> <u>trees</u>	<u>New cut</u> <u>trees</u>	<u>Other</u> <u>disturbance</u>
0-50						
50-100						
100-150						
150-200						
200-250						
250-300						
300-350						
350-400						
400-450						
450-500						

When returning from the field, the data should be immediately copied onto a Disturbance Data Sheet and filed away accordingly. The data must also be entered onto an excel spreadsheet with GIS capabilities; latitude and longitude must be recorded.

4.2 Mapping

Aims

This survey aims to map the periphery of areas of human land-use, such as maize fields, charcoal mounds or villages. The data will then be transferred onto a GIS map. The GIS map will be based on a satellite image of the area. The map will also incorporate data from the biodiversity surveys, giving the distribution of species throughout the area and showing the biological importance of the surveyed habitats.

Equipment

- GPS and spare AA batteries
- Notebook and pen
- Camera
- Compass

Procedure

The survey area must be divided into manageable sections. A team will then systematically sweep the designated section of the survey area.

When an area of human disturbance has been identified, it should be described and photographed. For example, if a maize field is found, notes should be taken describing the area, how recently the area has been cleared for cultivation, and whether the area was cut or burnt. The periphery of the disturbed area should then be mapped using the GPS. To produce an accurate map, waypoints should be taken frequently and regularly.

Data Recording

Mapping must be undertaken in a careful, organised manner. All waypoints and descriptions for each specific area of disturbance must be recorded together in a block of data and labelled accordingly. The accompanying photo number should also be recorded, so that the photo can be linked to the area after development.

All waypoints must be taken using the correct GPS setting: latitude and longitude in degrees, minutes and seconds. No other setting is acceptable.

When an area has been mapped, the data must be recorded in a specific format on an Excel Database. Appendix 9 shows the required layout and shows examples.

5.0 Taxonomic verification

Botany:

Pierre Jules Rataromalaza	WWF Tulear WWF Toliara, B.P. 220 Tulear (1), Madagascar
Mme Felicité Raja Fienina	University of Tulear University of Tulear, B.P. 412 Tulear, Madagascar

Zoology:

Bats and other Small mammals:

Steven Goodman	Field Museum of Natural 1400Roosevelt Road Chicago, Illinois 60605, USA
Dr Daniel Rakotondravony	Dept of Animal Biology University of Antananarivo, B.P. 906, Antananarivo, Madagascar

Reptiles and Amphibians:

Chris J. Raxworthy	US Museum of Natural History American Museum of Natural History New York, USA
Achille Raselimanana	WWF Antananarivo Biodiversity Officer, WWF Antananarivo

Invertebrates:

Butterflies:

Alison Cameron David Lees	Natural History Museum Dept of Emtomology, The Natural History Museum, Cromwell Road, London, SW7 5BD
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Ants and other invertebrates:

Brian Fisher	Dept of Entomology	California Academy of Sciences, Golden gate Park, San Francisco, California 94118, USA
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6.0 SUGGESTED READINGS

Garbutt, N. 1999. Mammals of Madagascar. Pica Press.

Rabinowitz, A. 1997. Wildlife Field Research and Conservation Training Manual. Wildlife Conservation Society

Wilson, D. E. (Ed.) 1996. Measuring and Monitoring Biological Diversity. Standard Methods for Mammals. Smithsonian Institution.

7.0 BIBLIOGRAPHY

Fisher, B.L., 1999 Improving inventory efficiency: a case study of leaf litter ant diversity in Madagascar. *Ecological Applications*, **9**: 714-731.

Glaw, F. & Vences, M. (1994) A Fieldguide to the Amphibians and Reptiles of Madagascar

Goodman, S.M., M.D. Carleton & M. Pidgeon, 1999. Rodents of the Reserve Naturelle Integrale d'Andohahela, Madagascar. Pp. 217-249, *In* Goodman, S.M. (Ed.), A floral and faunal inventory of the Reserve Naturelle Integrale d'Andohahela, Madagascar: with reference to elevational variation. Fieldiana: Zoology, new series, 94.

Hawkins, F. 1998. Birds of Madagascar, A photographic guide

Kremen, C. Biological inventory using target taxa: a case study of the butterflies of Madagascar. *Ecological Applications*, **4**(3), 1994, pp. 407-422.

Landre, P.B., Verner, J. & Thomas, J.W. 1988. Ecological use of vertebrate indicator species: a critique. *Conservation Biology* **2**: 316-328.

Raxworthy, C.J., Nussbaum, R. A. A rainforest survey of Amphibians, Reptiles and Small mammals at Montagne D'Ambre, Madagascar. *Biological Conservation* **69**, (1994), pp 65-73.

Sinclair, O. 1998. Birds of the Indian Ocean Islands.

Garbutt, N. (1998) Mammals of Madagascar

Appendix 1

Assessment lines and density estimates

(Retrieved from Rabinowitz, A. R. 1997)

Estimates of density can only be made if there is some way to determine movements of the target species away from the capture area. The purpose of assessment lines is to determine how far away the animals are coming in from, and thus get a better idea of the true trapping area - called the Area of Effect. The proportion of new to previous marked animals of any one species along these lines determines the area of effect, which can then be used for density estimates.

Assessment lines are generally placed at acute angles to the census line and trapping is conducted for a short period of time. The angle depends upon the expected area of effect, and the spacing of the trap stations on the assessment lines. Assessment lines must be arranged so that they do not modify the capture rates on other lines nearby. Trap spacing depends upon the estimates or known movements of the dominant species (a spacing of one 1/6 the average diameter of the home range has been suggested). For most small mammal species a trap spacing of 15 to 20 metres is used, with assessment lines at approximately 45 ° angle to the census lines.

Data analysis

1. Determine the width of the extension outside of the actual trapping configuration (W_A) by examining the assessment line capture data visually or graphically. *See figure 1.* Remember that area of effect varies not only between species but may have to be calculated separately for different sexes of species.
2. Determine area of effect (A) or the real extent of the trapping area that goes beyond the area of your grid.
3. Determine the number of animals caught within the area of effect (N_A), which is the number of animals caught within your configuration adjusted to include animals within all of A.
4. Determine the true species density based on the figures from steps two and three.

Determining W_A

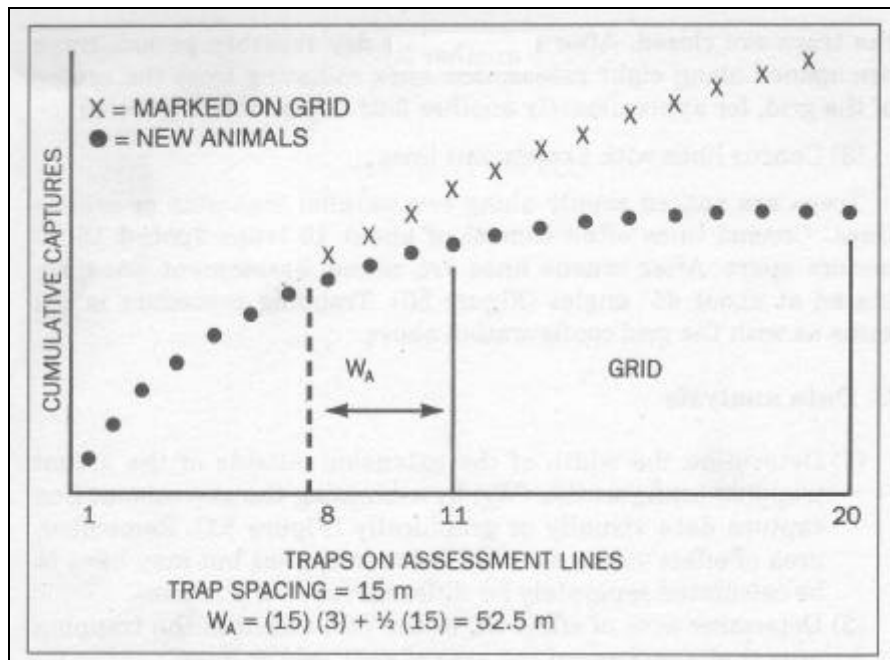
W_A = width of the extension outside of the actual trapping configuration which is determined by the movement of animals away from the trapping configuration. To determine this width, use the distance from the grid to the midpoint between the two trap stations where the break in slope occurs between you and marked animals, as seen graphically. *See figure 1.*

Determining A

$$A = W_G^2 + 4W_G W_A + \pi W_A^2$$

W_G = Width of grid

Figure 1. Determination of W_A from animals marked on grid to new animals. The distance from the grid to the midpoint between the two trap stations where the break in slope occurs between new and marked animals is the width (W_A).



Determining N_A

Proportion of animals removed (sampled) from Area of effect:

$$R_p = M/T$$

M = Number of animals caught in the area of effect that had already been marked on the grid

T = Total animals (marked and unmarked) caught on the assessment lines, within the area of effect

$$N_A = N_G / R_p$$

N_G = Total number of animals captured and marked during grid trapping

R_G = Ratio of marked to total animals in area of effect

Determining species density

$$D = N_A / A$$

N_A = Adjusted number of animals captured

A = Area of effect

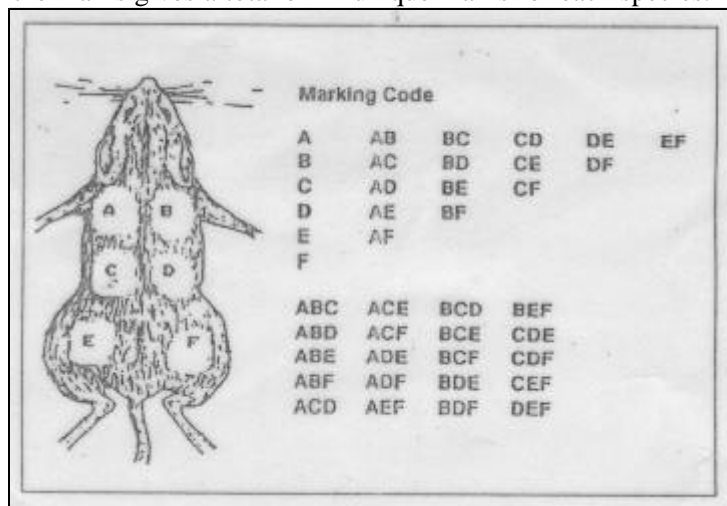
Appendix 2

Marking, measuring and sexing small mammals

Fur clip marking method

A cheap and easy way of temporarily marking small mammals is cutting the fur, giving each individual a unique code. Cutting the fur will give a mark that will remain visible from three to eight weeks, depending on the species and age. Young individuals re-grow fur faster than adults. The mark can be applied to the animal while gently holding it by the scruff of the neck. With the use of mesh traps the animals can be marked without handling, while they are still sitting in the trap. This method causes minimal harm and stress to the animals. *See figure 1.*

Figure 1. Fur clip method for small mammals. A combination of the marks gives a total of 41 unique marks for each species.



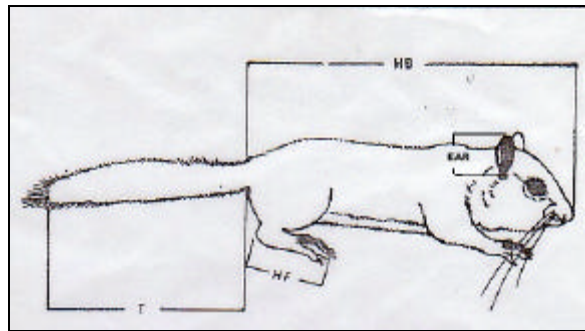
Using the letter system showed in figure 1, each individual of each species can be given an unique mark, with a total of 41 combinations. If there are many individuals of one species, the system can be applied for each sex. For example there can be both a female AB and a male AB of the species *Eliurus myoxinus* in a trapping area, giving the possibility of 82 combinations for one species.

When cutting the fur, cut as small an area as possible so that it does not affect the thermoregulation. If the survey is continuing for more than three weeks, be sure to cut the underfur, otherwise the fur may grow out and the mark disappear too quickly.

Measuring small mammals

When surveying small mammals there are standardised methods for measuring specimens. This is to make identification easy and to ensure comparable data. *See figure 2.*

Figure 2. Measuring small mammal dimensions.



HB= Head-body, T= tail, HF= hindfoot.

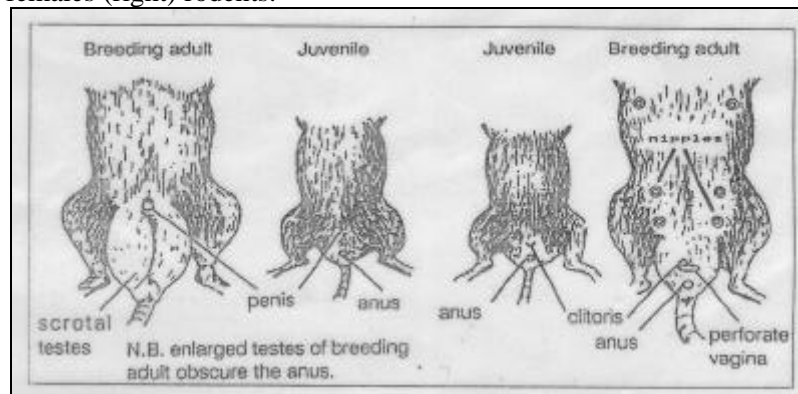
- The most important measurements for field identifications are the head-body, tail, hindfoot and ear.
- The head-body is the length of the animal from the tip of the nose (without whiskers) to the anus. Measure the animal when it is in a natural position.
- The tail is measured from the anus to the tip of the tail (not including hairs).
- The hindfoot is measured from the back of the heel to the tip of the longest toe (without the claw).
- The ear is measured from the tip diagonally across to the bottom, the longest stretch.
- Measuring is preferably done when the animal is either held in the hand, under anaesthetic, or dead. If in a mesh trap the animal will move around and the measurements will not be precise.
- The smallest measurements (hindfoot and ear) should be precise, so callipers must be used.

Sexing small mammals

Sexually dimorphic characters such as genitalia, body size, pelage, scent glands and behaviour can normally be used to distinguish between male and female mammals *See figure 3.*

Sexing small mammals is often difficult, as sexual characteristics can be hard to identify. In juveniles and non-breeding adult rodents, the distance between anus and the penis or vagina can distinguish between males and females. The distance is longer in males than in females.

Figure 3. Sexual characteristics in adult and juvenile males (left) and females (right) rodents.



In females, the nipples are not very distinct but can often still be seen. Depending on the species, the females usually have between three and five pairs of nipples. One or two pairs can sometimes be located between the front legs, and a pair far behind, between the hind legs (be aware that males of some species have visible nipples-always look for other characteristics as well). When the males are in breeding season, the testes are often enlarged in the scrotal sac and are easily identified. Pregnant females have a swollen abdomen (are heavier) and have visible nipples. When the female is lactating, the nipples are large and have a ring of bare skin around them, and the vagina is perforated.

Sexing insectivore species such as shrews and shrew tenrecs is often problematic. The penis is not visible externally and only protrudes from the cloaca during erection. Pressing the sides of the cloaca can physically evert the penis.

Appendix 3: Mammal Data Sheet

Frontier Madagascar Wilderness Project

MAMMALS (not bats)

Biodiversity Survey

Region:	<input type="text"/>	Latitude:	<input type="text"/>	Taxon ID:	<input type="text"/>
Area:	<input type="text"/>	Longitude:	<input type="text"/>	Genus:	<input type="text"/>
MGF No:	<input type="text"/>	Weather:	<input type="text"/>	Species:	<input type="text"/>
Collectors:	<input type="text"/>	Sub-species:	<input type="text"/>		
Day:	<input type="text"/>	Month:	<input type="text"/>	Year:	<input type="text"/>
Capture Method:	<input type="text"/>	Bait used:	<input type="text"/>	Time:	<input type="text"/>

Topography

Valley:	<input type="text"/>	Hill-top:	<input type="text"/>	Gentle hillside:	<input type="text"/>	Altitude:	<input type="text"/>
				(Slope less than 25 degrees)			
Ridge:	<input type="text"/>	Plateau:	<input type="text"/>	Steep hillside:	<input type="text"/>	Slope (deg):	<input type="text"/>
				(Slope more than 25 degrees)			
Gully:	<input type="text"/>	Plains:	<input type="text"/>	Other:	<input type="text"/>	Aspect:	<input type="text"/>
						(Direction the slope is facing)	

Vegetation Type

Vegetation Cover

Spiny scrub:	<input type="text"/>	Lowland forest:	<input type="text"/>	Ground layer (% cover):	<input type="text"/>
Transitional (scrub):	<input type="text"/>	Submontane forest:	<input type="text"/>	Shrub layer (% cover):	<input type="text"/>
Gallery forest:	<input type="text"/>	Montane forest:	<input type="text"/>	Tree canopy (% cover):	<input type="text"/>
Dry spiny forest:	<input type="text"/>	Marsh / Swamp:	<input type="text"/>	Canopy Height (m):	<input type="text"/>
Rocky / Barren:	<input type="text"/>	Cultivation:	<input type="text"/>		
Grassland:	<input type="text"/>	Disturbed:	<input type="text"/>		

Microhabitat

Water Association

Tree bark:	<input type="text"/>	Leaf litter:	<input type="text"/>	Water edge:	<input type="text"/>	River:	<input type="text"/>
Tree base:	<input type="text"/>	Grass:	<input type="text"/>	Bare ground:	<input type="text"/>	Stream:	<input type="text"/>
Branch:	<input type="text"/>	Rock:	<input type="text"/>	Burnt land:	<input type="text"/>	Pond/lake:	<input type="text"/>
Leaf:	<input type="text"/>	Path:	<input type="text"/>	Other:	<input type="text"/>	Marsh:	<input type="text"/>
Log:	<input type="text"/>	Water:	<input type="text"/>			None:	<input type="text"/>
Metres above ground:	<input type="text"/>	Distance to water (if under 50m):	<input type="text"/>	m			
Associated plant sp.:	<input type="text"/>						

Sex:

Colour Notes

Pregnant:
Lactating:

Belly:

Age

Juvenile
Subadult
Adult
Unknown

Back:

Biometrics

Head + Body: mm
Tail: mm
Hind foot: mm
Ear: mm
Weight: g
Other:

Head: Above:
Below:

Leg: Above:
Below:

Tail: Above:
Below:

DNA sample taken:

Appendix 4: Bats Data Sheet

Frontier Madagascar Wilderness Project

BATS

Biodiversity Survey

Region: <input style="width: 90%;" type="text"/>	Latitude: <input style="width: 90%;" type="text"/>	Taxon ID: <input style="width: 95%;" type="text"/>
Area: <input style="width: 90%;" type="text"/>	Longitude: <input style="width: 90%;" type="text"/>	Genus: <input style="width: 95%;" type="text"/>
MGF No: <input style="width: 90%;" type="text"/>		Species: <input style="width: 95%;" type="text"/>
Collectors: <input style="width: 90%;" type="text"/>		Sub-species: <input style="width: 95%;" type="text"/>
Day: <input style="width: 20%;" type="text"/>	Month: <input style="width: 20%;" type="text"/>	Year: <input style="width: 20%;" type="text"/>
Capture Method: <input style="width: 90%;" type="text"/>		Time: <input style="width: 95%;" type="text"/>
		Weather: <input style="width: 95%;" type="text"/>

Topography

Valley: <input style="width: 90%;" type="text"/>	Hill-top: <input style="width: 90%;" type="text"/>	Gentle hillside: <input style="width: 90%;" type="text"/>	Altitude: <input style="width: 95%;" type="text"/>
		<small>(Slope less than 25 degrees)</small>	
Ridge: <input style="width: 90%;" type="text"/>	Plateau: <input style="width: 90%;" type="text"/>	Steep hillside: <input style="width: 90%;" type="text"/>	Slope (deg): <input style="width: 95%;" type="text"/>
		<small>(Slope more than 25 degrees)</small>	
Gully: <input style="width: 90%;" type="text"/>	Plains: <input style="width: 90%;" type="text"/>	Other: <input style="width: 90%;" type="text"/>	Aspect: <input style="width: 95%;" type="text"/>
<small>(Direction the slope is facing)</small>			

Vegetation Type

Spiny scrub: <input style="width: 90%;" type="text"/>	Lowland forest: <input style="width: 90%;" type="text"/>
Transitional (scrub): <input style="width: 90%;" type="text"/>	Submontane forest: <input style="width: 90%;" type="text"/>
Gallery forest: <input style="width: 90%;" type="text"/>	Montane forest: <input style="width: 90%;" type="text"/>
Dry spiny forest: <input style="width: 90%;" type="text"/>	Marsh / Swamp: <input style="width: 90%;" type="text"/>
Rocky / Barren: <input style="width: 90%;" type="text"/>	Cultivation: <input style="width: 90%;" type="text"/>
Grassland: <input style="width: 90%;" type="text"/>	Disturbed: <input style="width: 90%;" type="text"/>

Vegetation Cover

Ground layer (% cover):	<input style="width: 95%;" type="text"/>
Shrub layer (% cover):	<input style="width: 95%;" type="text"/>
Tree canopy (% cover):	<input style="width: 95%;" type="text"/>
Canopy Height (m):	<input style="width: 95%;" type="text"/>

Microhabitat

Tree bark: <input style="width: 90%;" type="text"/>	Leaf litter: <input style="width: 90%;" type="text"/>	Water edge: <input style="width: 95%;" type="text"/>
Tree base: <input style="width: 90%;" type="text"/>	Grass: <input style="width: 90%;" type="text"/>	Bare ground: <input style="width: 95%;" type="text"/>
Branch: <input style="width: 90%;" type="text"/>	Rock: <input style="width: 90%;" type="text"/>	Burnt land: <input style="width: 95%;" type="text"/>
Leaf: <input style="width: 90%;" type="text"/>	Path: <input style="width: 90%;" type="text"/>	Other: <input style="width: 95%;" type="text"/>
Log: <input style="width: 90%;" type="text"/>	Water: <input style="width: 90%;" type="text"/>	

Water Association

River: <input style="width: 95%;" type="text"/>
Stream: <input style="width: 95%;" type="text"/>
Pond/lake: <input style="width: 95%;" type="text"/>
Marsh: <input style="width: 95%;" type="text"/>
None: <input style="width: 95%;" type="text"/>

Metres above ground:

Associated plant sp.:

Distance to water (if under 50m):

Sex:

Pregnant:

Lactating:

Age

Juvenile

Subadult

Adult

Unknown

Biometrics

Head/body: <input style="width: 95%;" type="text"/>	mm
Tail: <input style="width: 95%;" type="text"/>	mm
Hind-foot: <input style="width: 95%;" type="text"/>	mm
Ear: <input style="width: 95%;" type="text"/>	mm
Forearm: <input style="width: 95%;" type="text"/>	mm
Thumb: <input style="width: 95%;" type="text"/>	mm
3rd Finger: <input style="width: 95%;" type="text"/>	mm
4th Finger: <input style="width: 95%;" type="text"/>	mm
5th Finger: <input style="width: 95%;" type="text"/>	mm
Weight: <input style="width: 95%;" type="text"/>	g
Other: <input style="width: 95%;" type="text"/>	

Colour Notes

Belly:	<input style="width: 95%; height: 60px;" type="text"/>
Back:	<input style="width: 95%; height: 60px;" type="text"/>
Head:	Above: <input style="width: 95%; height: 20px;" type="text"/>
	Below: <input style="width: 95%; height: 20px;" type="text"/>
Wing:	<input style="width: 95%; height: 30px;" type="text"/>

DNA sample taken:

Appendix 5: Reptile Data Sheet

Frontier Madagascar Wilderness Project

REPTILES

Biodiversity Survey

Region: <input style="width: 80%;" type="text"/>	Latitude: <input style="width: 80%;" type="text"/>	Taxon ID: <input style="width: 95%;" type="text"/>
Area: <input style="width: 80%;" type="text"/>	Longitude: <input style="width: 80%;" type="text"/>	Genus: <input style="width: 95%;" type="text"/>
MGF No: <input style="width: 80%;" type="text"/>		Species: <input style="width: 95%;" type="text"/>
Collectors: <input style="width: 80%;" type="text"/>		Sub-species: <input style="width: 95%;" type="text"/>
Day: <input style="width: 20%;" type="text"/>	Month: <input style="width: 20%;" type="text"/>	Year: <input style="width: 20%;" type="text"/>
Capture Method: <input style="width: 80%;" type="text"/>		Time: <input style="width: 20%;" type="text"/>
		Specimen active/inactive: <input style="width: 20%;" type="text"/>

Topography

Valley: <input style="width: 80%;" type="text"/>	Hill-top: <input style="width: 80%;" type="text"/>	Gentle hillside: <input style="width: 80%;" type="text"/>	Altitude: <input style="width: 95%;" type="text"/>
		(Slope less than 25 degrees)	
Ridge: <input style="width: 80%;" type="text"/>	Plateau: <input style="width: 80%;" type="text"/>	Steep hillside: <input style="width: 80%;" type="text"/>	Slope (deg): <input style="width: 95%;" type="text"/>
		(Slope more than 25 degrees)	
Gully: <input style="width: 80%;" type="text"/>	Plains: <input style="width: 80%;" type="text"/>	Other: <input style="width: 80%;" type="text"/>	Aspect: <input style="width: 95%;" type="text"/>

Vegetation Type

Spiny scrub: <input style="width: 80%;" type="text"/>	Lowland forest: <input style="width: 80%;" type="text"/>
Disturbed spiny scrub: <input style="width: 80%;" type="text"/>	Submontane forest: <input style="width: 80%;" type="text"/>
Gallery forest: <input style="width: 80%;" type="text"/>	Montane forest: <input style="width: 80%;" type="text"/>
Disturbed gallery forest: <input style="width: 80%;" type="text"/>	Marsh / Swamp: <input style="width: 80%;" type="text"/>
Dry spiny forest: <input style="width: 80%;" type="text"/>	Grassland: <input style="width: 80%;" type="text"/>
Rocky / Barren: <input style="width: 80%;" type="text"/>	Cultivation: <input style="width: 80%;" type="text"/>

Vegetation Cover

Ground layer (% cover): <input style="width: 95%;" type="text"/>
Shrub layer (% cover): <input style="width: 95%;" type="text"/>
Tree canopy (% cover): <input style="width: 95%;" type="text"/>
Canopy Height (m): <input style="width: 95%;" type="text"/>

Microhabitat

Tree bark: <input style="width: 80%;" type="text"/>	Leaf litter: <input style="width: 80%;" type="text"/>	Water edge: <input style="width: 80%;" type="text"/>
Tree base: <input style="width: 80%;" type="text"/>	Grass: <input style="width: 80%;" type="text"/>	Bare ground: <input style="width: 80%;" type="text"/>
Branch: <input style="width: 80%;" type="text"/>	Rock: <input style="width: 80%;" type="text"/>	Burnt land: <input style="width: 80%;" type="text"/>
Leaf: <input style="width: 80%;" type="text"/>	Path: <input style="width: 80%;" type="text"/>	Other: <input style="width: 80%;" type="text"/>
Log: <input style="width: 80%;" type="text"/>	Water: <input style="width: 80%;" type="text"/>	
Metres above ground: <input style="width: 80%;" type="text"/>		
Associated plant sp.: <input style="width: 95%;" type="text"/>		

Water Association

River: <input style="width: 95%;" type="text"/>
Stream: <input style="width: 95%;" type="text"/>
Pond/lake: <input style="width: 95%;" type="text"/>
Marsh: <input style="width: 95%;" type="text"/>
None: <input style="width: 95%;" type="text"/>
Distance to water: <input style="width: 95%;" type="text"/>

Sex:

Colour Notes

Age

Juvenile

Subadult

Adult

Unknown

Belly:	<input style="width: 95%;" type="text"/>
Back:	<input style="width: 95%;" type="text"/>

Biometrics

Head + Body (mm):	<input style="width: 95%;" type="text"/>	mm
Tail (mm):	<input style="width: 95%;" type="text"/>	mm
Weight (g):	<input style="width: 95%;" type="text"/>	g
Pupil shape:	<input style="width: 95%;" type="text"/>	

Head:	Above: <input style="width: 95%;" type="text"/>
	Below: <input style="width: 95%;" type="text"/>
Leg:	Above: <input style="width: 95%;" type="text"/>
	Below: <input style="width: 95%;" type="text"/>
Tail:	Above: <input style="width: 95%;" type="text"/>
	Below: <input style="width: 95%;" type="text"/>

Additional measurements

Ventral scale count:	<input style="width: 95%;" type="text"/>
Mid-body scale count:	<input style="width: 95%;" type="text"/>
Other:	<input style="width: 95%;" type="text"/>

DNA sample taken:

Appendix 6: Amphibian Data Sheet

Frontier Madagascar Wilderness Project

AMPHIBIANS

Biodiversity Survey

Region: <input style="width: 90%;" type="text"/>	Latitude: <input style="width: 90%;" type="text"/>	Taxon ID: <input style="width: 90%;" type="text"/>
Area: <input style="width: 90%;" type="text"/>	Longitude: <input style="width: 90%;" type="text"/>	Genus: <input style="width: 90%;" type="text"/>
MGF No: <input style="width: 90%;" type="text"/>	Weather: <input style="width: 90%;" type="text"/>	Species: <input style="width: 90%;" type="text"/>
Collectors: <input style="width: 90%;" type="text"/>	Sub-species: <input style="width: 90%;" type="text"/>	
Day: <input style="width: 20%;" type="text"/>	Month: <input style="width: 20%;" type="text"/>	Year: <input style="width: 20%;" type="text"/>
Capture Method: <input style="width: 90%;" type="text"/>		Time: <input style="width: 90%;" type="text"/>
		Active/Inactive: <input style="width: 90%;" type="text"/>

Topography

Valley: <input style="width: 90%;" type="text"/>	Hill-top: <input style="width: 90%;" type="text"/>	Gentle hillside: <input style="width: 90%;" type="text"/> <small>(Slope less than 25 degrees)</small>	Altitude: <input style="width: 90%;" type="text"/>
Ridge: <input style="width: 90%;" type="text"/>	Plateau: <input style="width: 90%;" type="text"/>	Steep hillside: <input style="width: 90%;" type="text"/> <small>(Slope more than 25 degrees)</small>	Slope (deg): <input style="width: 90%;" type="text"/>
Gully: <input style="width: 90%;" type="text"/>	Plains: <input style="width: 90%;" type="text"/>	Other: <input style="width: 90%;" type="text"/>	Aspect: <input style="width: 90%;" type="text"/> <small>(Direction the slope is facing)</small>

Vegetation Type

Spiny scrub: <input style="width: 90%;" type="text"/>	Lowland forest: <input style="width: 90%;" type="text"/>
Transitional (scrub): <input style="width: 90%;" type="text"/>	Submontane forest: <input style="width: 90%;" type="text"/>
Gallery forest: <input style="width: 90%;" type="text"/>	Montane forest: <input style="width: 90%;" type="text"/>
Dry spiny forest: <input style="width: 90%;" type="text"/>	Marsh / Swamp: <input style="width: 90%;" type="text"/>
Rocky / Barren: <input style="width: 90%;" type="text"/>	Cultivation: <input style="width: 90%;" type="text"/>
Grassland: <input style="width: 90%;" type="text"/>	Significantly disturbed: <input style="width: 90%;" type="text"/>

Vegetation Cover

Ground layer (% cover): <input style="width: 90%;" type="text"/>
Shrub layer (% cover): <input style="width: 90%;" type="text"/>
Tree canopy (% cover): <input style="width: 90%;" type="text"/>
Canopy Height (m): <input style="width: 90%;" type="text"/>

Microhabitat

Tree bark: <input style="width: 90%;" type="text"/>	Leaf litter: <input style="width: 90%;" type="text"/>	Water edge: <input style="width: 90%;" type="text"/>
Tree base: <input style="width: 90%;" type="text"/>	Grass: <input style="width: 90%;" type="text"/>	Bare ground: <input style="width: 90%;" type="text"/>
Branch: <input style="width: 90%;" type="text"/>	Rock: <input style="width: 90%;" type="text"/>	Burnt land: <input style="width: 90%;" type="text"/>
Leaf: <input style="width: 90%;" type="text"/>	Path: <input style="width: 90%;" type="text"/>	Other: <input style="width: 90%;" type="text"/>
Log: <input style="width: 90%;" type="text"/>	Water: <input style="width: 90%;" type="text"/>	

Water Association

River: <input style="width: 90%;" type="text"/>
Stream: <input style="width: 90%;" type="text"/>
Pond/lake: <input style="width: 90%;" type="text"/>
Marsh: <input style="width: 90%;" type="text"/>
None: <input style="width: 90%;" type="text"/>

Metres above ground: <input style="width: 90%;" type="text"/>	Distance to water (if under 50m): <input style="width: 90%;" type="text"/> m
Associated plant sp.: <input style="width: 90%;" type="text"/>	

Sex:

Colour Notes

Age

Juvenile <input style="width: 90%;" type="text"/>
Sub-adult <input style="width: 90%;" type="text"/>
Adult <input style="width: 90%;" type="text"/>
Unknown <input style="width: 90%;" type="text"/>

Belly:

Back:

Biometrics

Snout-vent (mm): <input style="width: 90%;" type="text"/> mm
Tail (mm): <input style="width: 90%;" type="text"/> mm
Weight (g): <input style="width: 90%;" type="text"/> g

Head: Above:
Below:

Leg: Above:
Below:

Eyes

Pupil shape: <input style="width: 90%;" type="text"/>
Iris colour: <input style="width: 90%;" type="text"/>

Tail:

Other: <input style="width: 90%;" type="text"/>	DNA sample taken: <input style="width: 90%;" type="text"/>
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Appendix 8: Butterfly Specimen Summary Sheet

FRONTIER MADAGASCAR

Butterfly Trapsite Summary Sheet

Phase No: Trapsite No:

Region:

Latitude:

Area:

Longitude:

From: Day: Month: Year:

To: Day: Month: Year:

Frontier Madagascar Wilderness Project

Biodiversity Survey

NUMBER OF BUTTERFLIES KEPT AS SPECIMENS

Date	Canopy Trap No.				Sweepnetting - bucket line No.			Casual	Total
	1	2	3	4	1	2	3		
Overall Total									

Appendix 9: Example of GPS Data Recording for GIS use

Latitude	Longitude	Date	Season	Point description	Photo no.	Latitude			Longitude		
						Degrees	Minutes	Seconds	Degrees	Minutes	Seconds
23° 28' 49.6"	44° 03' 59.6"	17.08.01	Dry season	Periphery of Antafoky lake	1	23	28	49.6	44	3	59.6
23° 28' 50.9"	44° 04' 01.7"	17.08.01	Dry season	Periphery of Antafoky lake	1	23	28	50.9	44	4	1.7
23° 28' 55.0"	44° 04' 04.0"	17.08.01	Dry season	Periphery of Antafoky lake	1	23	28	55	44	4	4
23° 29' 00.4"	44° 04' 01.1"	17.08.01	Dry season	Periphery of Antafoky lake	1	23	29	0.4	44	4	1.1
23° 29' 29.5"	44° 04' 02.6"	17.08.01	Dry season	Periphery of Antafoky lake	1	23	29	29.5	44	4	2.6
23° 29' 11.4"	44° 04' 06.4"	17.08.01	Dry season	Periphery of Antafoky lake	1	23	29	11.4	44	4	6.4
23° 29' 15.3"	44° 04' 01.6"	17.08.01	Dry season	Periphery of Antafoky lake	1	23	29	15.3	44	4	1.6
23° 29' 16.0"	44° 03' 56.8"	17.08.01	Dry season	Periphery of Antafoky lake	1	23	29	16	44	3	56.8
23° 29' 22.5"	44° 04' 01.4"	17.08.01	Dry season	Periphery of Antafoky lake	1	23	29	22.5	44	4	1.4
23° 29' 29.6"	44° 04' 05.9"	17.08.01	Dry season	Periphery of Antafoky lake	1	23	29	29.6	44	4	5.9
23° 29' 26.2"	44° 04' 12.2"	17.08.01	Dry season	Periphery of Antafoky lake	1	23	29	26.2	44	4	12.2
23° 29' 24.1"	44° 04' 18.3"	17.08.01	Dry season	Periphery of Antafoky lake	1	23	29	24.1	44	4	18.3
23° 29' 21.3"	44° 04' 24.5"	17.08.01	Dry season	Periphery of Antafoky lake	1	23	29	21.3	44	4	24.5
23° 29' 22.4"	44° 04' 30.4"	17.08.01	Dry season	Periphery of Antafoky lake	1	23	29	22.4	44	4	30.4
23°29' 29.8"	44°04' 06.5"	14.02.02	Rainy Season	Road, start of village of Antafoky	59	23	29	29.8	44	4	6.5
23°29' 31.5"	44°04' 06.6"	14.02.02	Rainy Season	Road through Antafoky	59	23	29	31.5	44	4	6.6
23°29' 33.7"	44°04' 07.8"	14.02.02	Rainy Season	Road through Antafoky	81	23	29	33.7	44	4	7.8
23°29' 35.7"	44°04' 09.2"	14.02.02	Rainy Season	Road through Antafoky	81	23	29	35.7	44	4	9.2
23°29' 36.8"	44°04' 09.9"	14.02.02	Rainy Season	Road, end of village of Antafoky	81	23	29	36.8	44	4	9.9
23° 29' 08.5"	044° 04' 38.0"	23.01.02	Rainy season	Sink hole	94	23	29	8.5	44	4	38
23°28'27.3"	44°04'27.6"	2/3/02	Rainy Season	Exposed subterraneous cave	33	23	28	27.3	44	4	27.6
23°28'23.2"	44°04'23.8"	1/31/02	Rainy Season	Fossil Coral Reef	2, 35, 57	23	28	23.2	44	4	23.8