

## Chapter 19

# Field Methods and Techniques for Monitoring Mammals

by

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## **Abstract**

This chapter is a brief introduction to inventory methods for mammals in terrestrial habitats, with a focus on trapping methods for terrestrial small mammals, bats and medium-sized (meso-) mammals. For large mammals we refer the reader to more detailed sources. We suggest guidelines for designing a study, introduce selected trapping and handling procedures, and make recommendations for field equipment and data recording. Practical notes and hints based on authors' field experience are integrated in all sections of the chapter. Additionally, the authors review safety precautions and cover practical aspects for what to do "before launching" an expedition.

**Key words:** animal handling, bats, preservation, small mammals, trapping

## 1. Introduction

Aim of this chapter is to describe inventory methods for mammals in terrestrial habitats, with an emphasis on small- and medium-sized (meso-) mammal and bat surveys through a variety of trapping methods. We provide guidelines for designing a study, specify trapping and handling procedures, and make recommendations for field equipment and primary data records. Practical notes and hints based on our own field experience are integrated in all sections of the chapter.

For practical aspects the outline of the chapter is based on three non-taxonomic groupings. Due to the difference in mammal body size and their mode of life, such as volant and non-volant, specific methods, techniques and approaches are required and are therefore treated separately. We give a brief introduction on mammalian diversity and define operational terms for “Small-, Medium-sized and Large Mammals”.

### 1.1. Mammalian diversity

The Class Mammalia can be coarsely divided as follows (Simpson, 1931):

- Subclass Prototheria (Monotremes, one Order)
- Subclass Theria
  - Infraclass Metatheria (Marsupial Mammals, seven Orders)
  - Infraclass Eutheria (Higher Mammals - Placentalia; 21 Orders)

According to the most recent (3<sup>rd</sup>) edition of the standard taxonomic reference work, *Mammal Species of the World* (Wilson & Reeder, 2005; hereafter: MSW3), the class Mammalia comprises 5416 species (Tab. 1). Of these 2277 (42 %) are rodents (Rodentia), 1116 (20.6 %) are bats (Chiroptera) and 428 (7.9 %) are shrews and allies (Soricomorpha). However, these numbers are a taxonomic “snapshot” in time. Taxonomy is extremely dynamic, especially since the advent of molecular genetics has accelerated the revision of taxonomic groups and species delineations, but also with increased efforts to survey the last undisturbed places in a race against accelerating extinctions (Wilson, 1992).

MSW3 includes 787 more species than the 2nd (1993) edition, 260 species of which are newly described species. Ten of these were large mammals (8 artiodactyls, 1 carnivoran, and 1 whale). The vast majority were small mammals: 49 bats, 18 soricomorphs (17 shrews, 1 solenodont), and 128 rodents. This rapid increase in known mammal species highlights the importance of continued, standardized survey work throughout the world, particularly in habitats that are little known and/or in danger of being destroyed due to logging, mining, or other forms of “development”. Since much of All Taxa Biodiversity Inventory and Monitoring (ATBI+M) focuses on biodiversity surveys, conservation assessments and baseline data collections, we recommend using MSW3 (or its subsequent editions) as a taxonomic standard and referring to more recent taxonomic changes in the primary literature only in specific cases. For more information on mammalian diversity and natural history consult Cole & Wilson (1996).

Order	No. of Species	% of Total	Order	No. of Species	% of Total
Rodentia	2277	42.04	Scandentia	20	0.37
Chiroptera	1116	20.61	Perissodactyla	17	0.31
Soricomorpha	428	7.90	Macroscelidea	15	0.28
Primates	376	6.94	Pilosa	10	0.18
Carnivora	286	5.28	Pholidota	8	0.15
Artiodactyla	240	4.43	Paucituberculata	6	0.11
Diprotodontia	143	2.64	Monotremata	5	0.09
Lagomorpha	92	1.70	Sirenia*	5	0.09
Didelphimorphia	87	1.61	Hyracoidea	4	0.07
Cetacea*	84	1.55	Proboscidea	3	0.06
Dasyuromorphia	71	1.31	Notoryctemorphia	2	0.04
Afrosoricida	51	0.94	Dermoptera	2	0.04
Erinaceomorpha	24	0.44	Microbiotheria	1	0.02
Paramelemorphia	21	0.39	Tubulidentata	1	0.02
Cingulata	21	0.39			
Total Number of Mammal Species				<b>5416</b>	

**Table 1.** Mammalian Orders based on Mammal Species of the World 3rd Edition listed in descending order of species diversity with percentage of total mammal species. \*Two orders are comprised entirely of species that are highly adapted for life in aquatic (primarily marine) environments.

## 1.2. Definition of “Small, medium-sized and large mammals”

We will frequently refer to small and medium-sized mammals not as taxonomic groupings, but as practical subdivisions that require different methods and approaches.

“Small mammals” are usually divided into small terrestrial and volant mammals (bats). Terrestrial small mammals commonly refer to everything smaller than the largest rodents (capybara, nutria, grasscutter) or lagomorphs (hares, rabbits and pikas). Although some authors (e.g. Bourlière, 1975; Stoddart, 1979; Gaines & McClenaghan Jr., 1980) include in “small mammals” all mammal species, whose weight or size is less than a hare (3-5 kg), we include in “small mammals” only species weighing less than 500 g, the upper size limit that can easily be caught in commercially produced live traps (see Section 2.2) used in a standard small mammal survey. There is a considerable range of species within

this limit, including shrews, moles, most rats, mice, lemmings, gerbils, jerboas, dormice and many squirrels (Delany, 1974).

“Medium-sized mammals” is often used for small carnivores, small primates, large rodents, hyraxes, and pangolins that are not adequately covered by small mammal trapping arrays and require larger (wire mesh) traps. Some of these mammals can also be detected through non-trapping “observational” methods, such as track censuses or automatic camera traps. This group includes some of the most secretive, hard to survey, and hence still poorly known species.

“Large mammals” include most diurnal primates, most carnivores larger than a fox or house cat, all perissodactyls (horses, rhinos, tapirs) and artiodactyls (including the relatively small duikers). There will be some overlap between these broad categories. For example, in North America the smaller weasels (*Mustela* sp.) are caught in traplines set for rodents and shrews (about 1 weasel per 200 rodent captures, J. Decher, unpubl. data), but these traps exclude the larger mustelids like mink *Neovison* (*Mustela*) *vison* or Marten and Fisher (*Martes* sp.). In Africa the largest rodents (*Atherurus*, *Cricetomys*, *Thryonomys*, etc.) are best caught in large wire traps (Tomahawk, Havahart) and often show up on automatic camera trap pictures.

In section 2.1 we provide a brief summary of field techniques for medium-sized mammals in species inventories. Medium-sized mammals are generally much less known than larger mammals. We do not address the vast methodology on large mammals, because it is adequately presented elsewhere (e.g. Caughley, 1977; Davis, 1982; Wilson *et al.*, 1996; Martin *et al.*, 2000). A specific chapter in this volume is dedicated to camera-trapping (see Chapter 6), which in recent years has been developed into an efficient method for surveying both medium-sized and large mammals.

## **2. Field techniques**

### **2.1. Medium-sized mammals**

#### **2.1.1. Trapping methods**

Trapping of medium-sized mammals is generally more challenging and costly than trapping of small mammals, and therefore it is more often used for focal studies (e.g. radio-tracking or collection of DNA samples) than for species inventories. Moreover, some species or groups of species will require *ad-hoc* trapping methods: for example, small nocturnal primates can be trapped using Chardonneret traps (15 x 15 x 23 cm) baited with bananas and placed in trees (Doggart *et al.*, 2006); the larger elephant-shrews of the genus *Rhynchocyon* have been successfully trapped using fishing nets strung along the forest floor (Rathbun, 1979). For general trapping methodology and procedures we refer to the detailed account on small mammals presented in Section 2.2. Suitable traps for medium-sized mammals are commercially available, e.g. Tomahawks and Havaharts (details in Section 2.2). They consist of foldable cages made of galvanized wire mesh and can be single or double-door; the size varies depending on target species, with 18 x 18 x 61 or 23 x 23 x 66 cm being a

standard size for trapping small carnivores and large rodents. Because of limited survey budgets, it is rarely possible to purchase large numbers of these traps and therefore trapping will be more successful when animal's trails, nests or burrows can be found, which in tropical countries is usually facilitated by local hunters. Medium-sized mammal traps need to be checked very frequently as captured animals may become stressed quickly and hurt themselves trying to bite or dig through the mesh.

### **2.1.2. Observational methods**

“Observational methods”, including camera-trapping, can be used to survey some groups of medium-sized mammals. There are three types of observational methods: (1) direct observations, (2) identification of dung, tracks and other signs, and (3) camera-trapping, *i.e.* the use of remotely set, automatic cameras. Because of the more challenging technological implications and recent advances and applications in camera-trapping, this method is described in full detail in Chapter 6. There are firm indications that camera-trapping is a very cost-efficient method of surveying both medium-sized and large mammals, especially in forest habitats, where visibility and track/dung detection may be difficult (see Chapter 6 and Bowkett *et al.*, 2006). Nevertheless, both direct sightings and signs should be considered either as an alternative method or to complement camera-trapping surveys.

In general, direct observation is not an efficient method to detect medium-sized mammals. There are a few exceptions, for example the nocturnal primates, whose eye shine is easily sighted at night using head torches (Doggart *et al.*, 2006). Some of the locally common small African carnivores, such as palm civets and genets, can also be sighted with torches during night walks. Tracks, scats and other signs can, instead, be more easily recorded in the course of any survey. Photographs, possibly including a scale reference, can assist with later identification confirmations, and localities should be recorded with a handheld GPS unit. Sometimes indigenous knowledge (especially from hunters) can be useful for a preliminary list of species and/or help with identification of signs. Chances to detect tracks can be increased with tracking stations, by clearing portions of an animal trail and covering the ground with fine sand or with special track plates with surface blackened using smoke or printer toner (Zielinski, 1995; Wemmer *et al.*, 1996; Foresman & Pearson, 1998). Synthetic attractant or natural lures can be placed at tracking stations, especially to attract carnivores.

An introduction to mammalian signs including tracks, nest and burrows, scats and food caches is provided by Wemmer *et al.* (1996). A more detailed treatment on tracking in the North American temperate environment is found in Rezendes (1999). Field guides to the identification of signs and tracks for different parts of the world can be found in corresponding specialised field guides for mammals (Stuart & Stuart, 1994; Rezendes, 1999; Bang & Dahlstrom, 2001; MacDonald & Barrett, 2002; Ohnesorge & Scheiba, 2007).

### 2.1.3. Indirect methods

Indirect surveys of small carnivores may involve hunting or fur harvest records such as the classic study of Canada lynx (*Lynx canadensis*) cycles and more recent work on mink (*Neovison vison*) in Canada (Elton & Nicholson, 1942; Shier & Boyce, 2009), surveys of meat markets in Africa (Anadu *et al.*, 1988; Angelici *et al.*, 1999; Crookes *et al.*, 2005), or setting up scent marking stations with hair traps to monitor small carnivores (Schmidt & Kowalczyk, 2006).

## 2.2. Small mammals

Even though terrestrial small mammals are often quite abundant, they are rarely observed and (except in snow or sand) their tracks are rarely seen and hard to identify to species. However, they can be easily sampled with sufficient numbers of traps or pitfalls, and the most abundant species in a small mammal assemblage allow for population estimates using capture-mark-recapture protocols (Smith *et al.*, 1975; Caughley, 1977; Krebs, 1989). Most small mammals are easily handled requiring relatively little specialized equipment.

### 2.2.1. Traps and bait

For most terrestrial small mammals the Sherman live trap (<http://www.shermantraps.com>) has become the standard foldable, very portable and efficient trap of choice (Fig. 1). H. B. Sherman makes several sizes and has recently started to offer most models with perforated walls, which should help prevent overheating in hot grassland or semi-desert environments.

The standard model (LFA-TDG, 7.5 x 9 x 23 cm) is the most widely used trap, especially in the United States. In tropical environments we have found the extra long model (XLK, 7.7 x 9.5 x 30.5 cm) to be preferable given the larger average size of tropical mammals and the long tails of many genera (*e.g.* *Malacomys*, *Dephomys*). If only the standard model is available, but capture of long-tailed species is expected, traps could be modified to avoiding injury of the animals by attaching a small spacer piece at the top rim of the trap which creates a narrow gap (*ca.* 3 mm) for the animals to safely pull their tails into the trap. The largest model (XLF15, 10 x 11.5 x 38 cm) has been tested successfully by one of us (J. Decher) with small mammals. It should theoretically be useful in a study focusing on small mustelids or herpestids, but many medium-sized mammals can be extremely shy to enter a trap with solid walls and a wire or cage trap like the Tomahawk models (<http://www.livetraps.com>) is preferable.

In colder climates the small mammal trap of choice may be one that has a nest box attached, such as the British Longworth trap (Penlon Ltd., Oxford, U.K., <http://www.alanaecology.com>). However, the Longworth design is not collapsible, and the traps are considerably more expensive than the standard Sherman trap. The usability of Sherman traps in colder climates can be extended by placing the traps into “waxed cardboard” containers (Fig. 2) saved

**Fig. 1.** Popular trap types. Back: Collapsible Tomahawk trap for squirrels, small carnivores, and large rats. Centre: Standard-sized collapsible Sherman trap. Front left: Victor Rat trap. Front right: Museum Special snap trap. (Photo by Jan Decher).



**Fig. 2.** Standard Sherman Live Trap (LFA-TDG, 7.5 x 9 x 23 cm) protected in a 2 litre milk carton. (Photo by Anke Hoffmann).



from milk or juice products. Some bedding like cotton or shredded paper can be stuffed into the very back of Sherman traps as well as additional food, as long as it does not block the treadle mechanism. In general, live trap survival can be improved if covers are used to protect traps from the elements (sun, snow, rain).

In one comparison of small non-folding (5.4 x 6.5 x 17 cm), and large (7.7 x 9.1 x 23 cm) folding Shermans with two-piece Longworth traps (13.8 x 6.4 x 8.4 cm), small Shermans captured the most animals and appeared to be the most effective traps for smaller-sized mammals. Longworth and Sherman traps exhibited species-specific differences in capture rates suggesting that they should be used in combination to reduce overall bias (Hoffmann, 1995; Anthony *et al.*, 2005). Similarly Nicolas & Colyn (2006) compared the efficiency of Sherman traps, metal snap and pitfall traps and concluded that an assortment of traps should always be employed in studies of small mammal communities in African rainforest in order to obtain a wider range of taxa, and thus a better representation of the community.

Larger wire traps are offered in numerous sizes, and in single or double door and rigid or collapsible versions by the Tomahawk (<http://www.livetraps.com>, see Fig. 1) and Havahart (<http://www.havahart.com>) trap companies. Some rapid biodiversity assessments when maximum trap success is important may justify the use of snap traps of various types. Because a standard mouse trap from the hardware store is often too weak for wild rodents and shrews and the larger rat trap is too large for smaller species, a medium sized trap was developed known as the "Museum Special" trap (Fig. 1). It also has a better probability for leaving small mammal skulls (the most important museum-diagnostic structure in mammals) intact (Smith *et al.*, 1971, but also see Perry *et al.*, 1996). When trapping in protected areas check with authorities if the use of removal traps is permitted.

Recent studies have emphasized the need to avoid bias towards certain species by trapping only on the forest floor in tropical environments. For this reason a number of workers have taken to placing traps on platforms that can be lowered with a pulley system high in the canopy to sample for scansorial or arboreal species. However, initial placement of the trap platforms (or pulley attachment) requires special climbing gear and considerable athletic skills (Malcolm, 1991, 1995; also see Jay Malcolm checking his arboreal traps in the video *Rain Forest*, National Geographic Society, 1998).

The use of bait versus no bait and the advantages of pre-baiting (baiting for several days prior to placing or setting the traps), when survey time allows for it, have been discussed elsewhere (Smith *et al.*, 1975; Jones *et al.*, 1996). Numerous favourite recipes exist on the subject of bait preparation. Standard bait among many mammalogists is oatmeal flavoured with peanut butter. We have also known a mammalogist who routinely chewed (!) the oatmeal to prepare it for use on snap traps. One of us (A. Hoffmann) prepares a "sticky cake" from oatmeal, peanut butter (or locally sold "groundnut paste" in Africa) and bananas, if available, which can be formed into adhesive balls that can easily be attached to the back of Sherman traps or on the treadle of a snap trap. Another effective recipe, if no peanut butter is available, is a sticky dough made from maize flour, ripe bananas and (roasted) peanuts (Hoffmann, 1999). We

have also successfully used shavings of the outer fibrous and oily (pericarp) layer of oil palm nuts (*Elaeis guineensis*) in Africa. Their scent seems to equally attract insectivorous shrews and rodents.

Pitfall traps may be the most effective trap for mammals under 10 g. Pitfalls can be made from 5-10 litre buckets, large yogurt containers or specially made cones (Pankakoski, 1979). Cones are very useful in marshy habitat where they can just be pushed into the ground. In rocky or laterite soil, pitfalls can mean a large investment of labour, but their placement is often rewarded by the capture of small shrews not sampled with any other method (Handley & Kalko, 1993; Kalko & Handley, 1993; Nicolas & Colyn, 2006). Pitfalls work most efficiently if they are connected by a plastic or mesh drift fence running across each pitfall. For an example of a very thorough application of pitfalls and drift fences to shrew diversity and abundance in different habitats in Guinea, see the recent work by Nicolas *et al.* (2009).

Pitfalls work well unbaited but we have also baited them in certain situations. They should be checked often to avoid multiple animals captured from attacking each other or being taken by predators. Buckets should be punctured to reduce the chance of drowning during heavy rains. Buckets can also be covered with small boards spaced with a gap above the buckets using three or four rocks to reduce flooding and predator impact. Some plant material and little stones in the bucket can also provide hiding places and protection against sun and rain for the animals. For pitfall traplines shorter spacing distances ( $\leq 5$  m) have been recommended, because of the smaller size of the target species (Handley & Kalko, 1993). The array of a pitfall trapline depends much on habitat, substrate and man power. The length per line can vary between 10-50 m, whereas the set-up of the drift fence must still be practicable. Some workers have recommended more elaborate drift fence and pitfall arrays such as a Y-shaped design with a pitfall at each end of the fence arms and one in the centre (Kirkland & Sheppard, 1994). Pitfall set-up and results can sometimes be shared with entomologists and herpetologists who might be working on the same inventory (see Chapters 9, 14 & 20).

### **2.2.2. Trapping procedure**

The way traps are arrayed in the habitat depends on the question being asked and the estimation methods used. For inventories, accurate estimates of abundance (total number of animals) or density (numbers per unit area) are not necessary: the primary concerns are assessing the true mammal diversity of an area by sampling a sufficiently large area with a diverse array of methods. In any case, a standardised design should be used and carefully documented to allow for future repetition and facilitate a meaningful long-term monitoring effort.

#### ***Trapline designs***

For inventories of small terrestrial mammals the easiest approach is to place traps at equal intervals along a line, which ideally should cover all habitat types, ideally with one or two replicate lines. Spacing distances are a function of habitat complexity. Traps in more complex habitats should be more closely

placed. Size of the target species is also a consideration, because smaller mammals tend to travel shorter distances than larger mammals (Jones *et al.*, 1996). We recommend that a trapline ideally be about 150 m long, with traps placed every 10 to 15 m (Mühlenberg, 1993; Jones *et al.*, 1996), but this design has to be adapted to the respective habitat conditions and target species. Whatever the spacing, to increase the trap success, traps should be placed at habitat features (*e.g.* log, rocks, tree, runways, burrows, bush clusters) as long as they lie within 2 m of the point. Where possible, a subset of traps should also be placed on branches of trees in order to catch scansorial species. If freshwater habitat (stream, pond, lake) is present, we recommend placing several traps near these bodies of water. Traplines near water and in trees should be tethered to reduce loss due to sudden water level changes or traps falling out of trees. For replicate traplines, we recommend a minimum distance of at least 100 m between the traplines to avoid an impact on the trap success.

Trapping effort is commonly expressed in “trap-nights”, that is the number of traps multiplied by the number of daily trap periods (*e.g.* sunset to sunrise). A minimum of 400-500 “trap-nights” has been recommended for a preliminary inventory of a habitat (Jones *et al.*, 1996; Fraser *et al.*, 2003). Thus, at least 100-150 traps are needed for an efficient inventory survey so that the trapping period can be limited to three or four consecutive nights in each habitat and season. More traps reduce the number of daily trap periods, but are difficult to check efficiently in one trap inspection especially if many measurements and habitat data are recorded at each trap station. The required trapping effort can be determined with a species accumulation curve (Colwell *et al.*, 2004; Decher *et al.*, in press).

We recommend placing two traps at every station to reduce the saturation of traps by “trap-happy” individuals or very abundant species. This practice increases the chance trapping animals that are less active, less attracted to traps (Drickamer, 1987), or “trap-prone” (Andrzejewski *et al.*, 1971). Each trap station should have at least one Sherman trap, which can be combined with any other trap type available. If 80% of the traps are occupied it is recommended to increase their number (Corbet & Harris, 1990).

Whether traps follow a rigid grid arrangement or a linear trapline, individual traps can often be set opening towards, or in line with a rodent runway, along a log that can act like a drift fence, or near a hole/hiding place. Trap stations should be marked with a flagged pole (in grassland habitat) or flagged tree (in forest) which should be visible from one trap station to another to facilitate orientation. This prevents loss of traps and makes the trapline easy to follow and re-bait. We recommend marking each trap with a unique identifier for each trapline and station (*e.g.* A1, A2F... A15; B1, B2F... B15; etc). If two traps are placed at one station they can be distinguished by a small letter (*e.g.* A1a, A1b, A2a, A2b, etc). This is especially important if animals are brought to a central processing place to be released later at the same trap site. Marking tape and marking pen should be water resistant. Reflective station markers (*e.g.* 3M-Scotchlite™; <http://solutions.3m.com> or <http://www.amazon.com>) can be useful, if traps need to be checked at night. Marking devices should be removed after the study, unless biodegradable, non-polluting tape is used. If large herbivores

(esp. cattle) are present in the survey area, aluminium tags could be used to prevent ingestion of marking tape. In open habitats (grassland, desert) it might be necessary to tie traps to poles in order to avoid displacement by wind.

### ***Trap inspection***

Depending on trap success and habitat conditions 100-200 traps can be checked within one trap inspection. Ideally, traplines are run for a period of 3-5 days (Mühlenberg, 1993; Jones *et al.*, 1996) to reduce stress on the animals. Traps are set before sunset and checked as early as possible the following morning. All traps are then closed for the day, unless day trapping is planned. At sites where many shrews (esp. Soricinae) are expected, trap inspection at shorter intervals can prevent the animals from dying in live traps or being eaten by ants or predators in snap traps. If personnel allow it, we recommend daytime trapping at least for two days to check for diurnal species. Depending on weather conditions (*e.g.* heat) during the day, trap inspection at short intervals should be considered. Traps are baited the first day and as necessary re-baited the following days.

### **2.2.3. Animal handling**

Most animals trapped in box (live-) traps will be alive, and a decision has to be made if a particular animal will be released after treatment or if it will be kept as a voucher specimen. Before starting a capture programme the risks of disease transmission from wildlife species (see Section 4) should be assessed. As a general precaution we recommend that the investigator wear sturdy protective gloves for handling live animals and disposable laboratory gloves during processing of dead animals. In regions with specific risks (*e.g.* hantavirus, Lassa fever) a mask or full protective gear is recommended (Mills *et al.*, 1995).

### ***Voucher specimens***

If and how many voucher specimens are to be taken from each inventory site depends on the study objectives, and also on the particular regulations and permit specifications of each site and country. Many small mammal species can not confidently be identified in the field. This can be particularly problematic for shrews. Sometimes researchers should even consider taking a larger series of hard to identify sympatric taxa. There may be a diversity of colour morphs or other phenotypically unique forms present in an area. The most interesting aspects of small mammal biology and diversity are often easily overlooked in the field. In general, we recommend keeping at least one adult male and one adult female per species from each inventory site. After the euthanasia processes (see Section 3), the animal should be accurately measured (see Section 2.2.4), prepared for preservation (see Section 3), and have tissue samples taken (Chapter 7). Finally, even when live traps are used, there is almost always some mortality. Ethically speaking, animals which die in the course of a study belong in a collection.

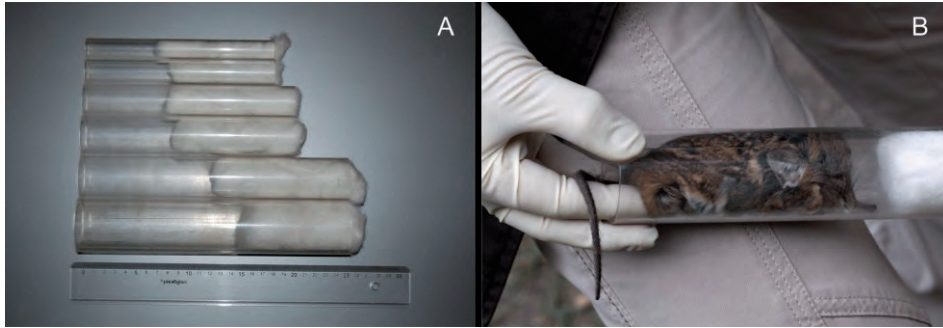
## **Animal release**

The aim, when handling the animals, is to obtain the necessary information rapidly, without undue stress or injury to either the animal or the researcher. First the trapped animal is transferred from the trap into a clear, strong plastic (size 3 litres) or cloth bag. For this the door end of the trap is inserted into the opening of the bag, the door pushed open through the bag fabric, and the animal is shaken into the bag. Take care to prevent a gap between the bag and the trap through which the animal can escape. Animal and bag are weighed together with an accuracy of 0.5-1.0 g and the bag weight is subtracted. Bags used need to be re-weighed frequently because of moisture and debris (bait, faeces) from the traps altering their weights. Spring balances (Pesola, etc.) of different weight classes (30 g, 100 g, 300 g) should be available depending on the size range of species captured.

Preliminary species identification can be done while the animal is held in the plastic bag until processed. In order to establish the best field identification, some body measurements besides the weight (tail, hind foot, and ear length) are taken. If more time for identification is needed the animal can temporarily be kept in a cage for observation. Photos as references can be useful. If identification remains uncertain then a representative individual should be taken as a voucher specimen.

There are two ways to handle live animals, and in our view the second option is less stressful for the animal and the researcher, and especially recommended for use by inexperienced persons.

- Grasping the animals by the nape of the neck is described by Jones *et al.* (1996). Therefore the animal is initially grasped through the bag and then bag is peeled back. After placing the animal on a flat surface, the investigator positions his/her thumb and forefinger on each side of the neck, against the back of the skull, squeezes, and pulls back, so that the fingers close only on the skin. Firmly grasping all the loose skin across the upper back, especially the skin behind the neck restricts the movement of the animal's head and allows the researcher to lift the animal and turn it to view the ventral surface for determination of sex and reproductive conditions. Several species have loose skin and cannot be grasped in this way. Likewise, holding the animal by the tail should be avoided.
- A tube of acrylic glass of an adequate calibre, both sides open, one side closed with cotton batting, is placed into the bag. The animal is then gently guided into the tube held upright and in this way calmed for further treatment. Hind feet and tail should be positioned outside the tube, and the cotton wool prevents animal's movement too far into the tube, but allows it to breathe. One finger of the investigator is always placed to prevent the animal from escaping backwards. Tubes (5-6 pieces) in different sizes (15-50 mm diameter, length 15-25 cm, Fig. 3) should be available. Avoid holding tubes with animals for extended periods to prevent overheating inside.



**Fig. 3.** A. Tubes of acrylic glass in different size; B. example of usage. (Photos by Anke Hoffmann).

After restraining the animal in this way, sex and reproductive status can be recorded and selected body measurements can be taken (see Section 2.2.4) with a calliper or ruler. If needed a tissue sample for DNA analysis can be taken from live animals (see Section 3; Chapter 7). After all data has been collected the animal should be released at the site of capture. The handling procedure usually lasts 5-10 min per animal, depending on the researcher's experience and on whether marking or parasite sampling is carried out.

### ***Marking of animals***

Released animals should be marked to avoid re-counting and re-measuring the same individuals. Marking can be done by different methods: permanent markers (tattooing, toe clipping, ear punching), or temporary markers (paints, powders) (Rudran & Kunz, 1996). When selecting an appropriate marking technique for the survey one should consider the need for individual identification, the period for which the mark should be visible, and number of animals which should be marked. For an inventory temporary markers should be sufficient and should be easy to apply. Therefore we recommend easy techniques such as hair-cutting or nail-clipping. For nail-clipping the same clipping pattern as for toe-clipping can be used (cf. Twigg, 1975, 1978). As toes are needed for pawing and personal hygiene not more than two nails per foot should be cut. Hair trimming can be applied on the back, for which the back is divided into sectors (cf. Twigg, 1978; Gurnell & Flowerdew, 2006). Both markings can be applied while the animal is in the tube (Second option above).

### ***Sampling of parasites***

Captured animals can be sampled for ectoparasites. Ticks, lice and parasitic flies can be removed from the fur and preserved in ethanol. Fleas can be collected after they jump off or have been brushed off a voucher specimen that has been euthanized inside a clean plastic bag or other closed container. It is important to keep detailed notes and cross-reference host numbers on parasite vials, field data sheets and/or field catalogue. For more details on parasite collecting see Gardner (1996).

#### 2.2.4. Primary recording data

For each individual, sex and reproductive status should be recorded. Moreover body mass and selected body measurements (tail, hind foot, ear) should be taken (Fig. 4). Body measurements should have an accuracy of 0.5-1.0 mm. Reliable body measurements can only be taken from dead animals. Body length in particular is impossible to measure in living animals. But also the determination of sex and age is often difficult with animals to be released. Field teams should agree on whether they are using the American or European convention of standards measurements.

##### **Sex determination**

Different sexually dimorphic characters can be used to distinguish males from females, including differences in genitalia, body size, pelage, scent glands, and behaviour. Accurate sexing requires some knowledge of the natural history and morphology of individual species (Kunz *et al.*, 1996c). Primarily males are distinguished by possessing testes and a penis, females by the presence of a vaginal opening and nipples, but the visibility and spacing of the genitalia depends on age, reproductive condition and taxon. For example in many rodents the clitoris superficially resembles the male urinary papilla, but the anal-genital distance is diagnostic, typically being shorter in females than in males. Males with scrotal testes (sometimes only during the breeding season) are easy to identify, but males with non-scrotal (inguinal) testes are common, especially in Soricomorpha (shrews, moles, solenodons). The penis in some species may be retracted into a cloaca (Soricidae) and there may be other anatomical challenges like the pseudo-cloaca in *Ochotona*. Female reproductive activity is represented by gestation and lactation (enlarged nipples). The external condition of the vagina can indicate the reproductively activity in females as well, e.g. due to a perforated vagina or the presence of a vaginal plug (Kunz *et al.*, 1996c).

##### **Age categories**

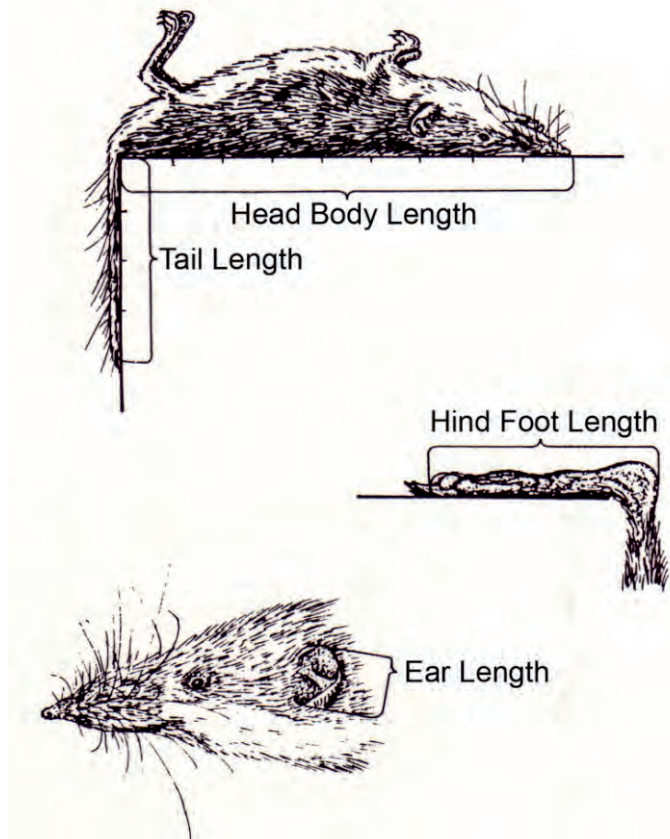
Age categories for mammals generally are listed in Kunz *et al.* (1996c). A combination of body measurements and reproductive criteria offers the best means to determine the age of small mammals in the field. Cranial and dental characteristics are valuable for an accurate age estimation done in the lab (Morris, 1972; Pucek & Lowe, 1975). For fieldwork we generally distinguish just between three age classes:

- **Juvenile:** A small young animal in grey and soft juvenile pelage, smaller than a subadult and not sexually mature.
- **Subadult:** A young animal that is not fully grown and often not in fully adult pelage. May or may not be sexually mature.
- **Adult:** A fully grown animal in adult pelage that is sexually mature.

### **Body measurements**

Please be aware when taking body measurements that the European convention is different from the American one, this concerns in particular the hind foot length and the total length.

- **Body Mass** (BM) is measured to the closest gram by using a spring balance (see subchapter handling) for living animals, for dead animals a digital scale can also be used.
- **Total Length** (ToL) is the distance from the tip of the nose to the end of the fleshy part of the tail not including the tuft of hair at tail's end. Lay the voucher specimen on its back on a ruler, grasp head and tail to straighten the body and take the measurement (American convention).
- **Tail Length** (TL) is the distance from the base of the tail (after the anus) to the tip of the tail. Do not include the tuft of hair at the very end.
- **Head-Body Length** (HBL) is obtained by subtracting TL from ToL.



**Fig. 4.** Measurements of a small mammal (example: shrew). From Boye (1994), modified.



- **Hind Foot Length** (HFL) is the distance from the back of the heel to the end of the fleshy part of the longest toe (*s.u.* = *sine unguis*) or to the end of the largest claw (*c.u.* = *cum unguis*; American convention). Provide both measurements, when in doubt.
- **Ear Length** (EL) is the distance from the bottom of the notch to the furthest edge of the pinna. Before measuring grasp the ear and briefly stretch it out and release it. Hairs or tufts at the tip of the ear should not be included in the measurement.

A template data sheet for recording capture and habitat data can be designed and photocopied prior to the survey. Relevant recording data elements are listed in Appendix 1, but the selection may vary by study.

### 2.2.5. Workflow and personnel

We recommend a trapping period of at least 3 trap-nights with an array of about 100-150 traps and a team size of 2-3 persons (researcher, assistants) or 3-4 persons (2 researchers, 1-2 assistants) if more than 200 traps are used or bat work is planned during the night. Considering the time for set-up and removal of traplines two additional days should be scheduled. Traps can be carried to the trapline in a back-pack or strong cloth bags. One person can transport the traps and mark trap stations while the second person sets, baits and places the traps. Depending on the habitat and the distance to other traplines several hours should be scheduled for these procedures. In the morning after the last trap-night the traps can be collected just after or during the trap inspection.

We recommend at least a 2-day break between back-to-back trapping periods that require several days of camping at remote locations from the base camp. This time is needed for re-organisation, such as data entry, maintenance of field equipment like trap cleaning and mending of bat nets, preparation of vouchers and processing the by-catch (non mammals).

### 2.2.6. Habitat assessment

For understanding the interrelation between small mammal assemblages and their habitats, different environmental features should be assessed at the time of trapping. Habitats provide food supply, nesting sites and other hiding places. Vegetation cover, habitat structure and food availability (vegetation, arthropods) can be recorded along the traplines. More detailed vegetation surveys with species lists should be done by botanists (see Chapter 14). Rainfall and temperature data can be recorded in the microhabitat or requested from the nearest meteorological stations.

#### **Habitat description**

General description of the habitat near each trapline:

- Habitat type: type of forest, savannah, grassland, etc.; possibly list of dominant plant species.

- Altitude (m), exposition (geographic), georeference data.
- Distance to nearest water source (m).
- Existence of rocks, termite mounds, burrows, etc.
- Note: type of land use, availability of seeds and fruits, evidence of past fires, presence of large mammals, etc.

#### Microhabitat recording

Notes on the microhabitat of each captured individual can be recorded on standardized habitat data sheets. This may include estimations of percent canopy cover using a spherical densiometer (Forestry Suppliers Inc.) and estimations of ground cover types in a one-square meter area centred on each trap station. If there is more than one capture at the same trap station the recording of microhabitat data need not be repeated. Averages of these recordings can be used for a general habitat description. Recordings at each capture may include:

- Canopy cover (%).
- Distance and diameter breast height (dbh) of nearest tree.
- Percent ground cover types (in 1 m<sup>2</sup> centred on trap): herbs, grass or sedge, bare soil, leaf litter, rock, water.
- Vegetation height (cm) of ground cover.
- Vegetation density (using a density board).

The inventory of the microhabitat should be done without disturbance of the trapping procedure. The best time would be immediately after releasing or collecting the animal, during the non-activity phase or when the traps are closed. More elaborate microhabitat sampling schemes using 10-meter radius circular vegetation plots have been described by several authors (James & Shugart, 1970; James, 1978; Dueser & Shugart, 1978, 1979; Kitchings & Levy, 1981).

If the study requires the identification of a possible correlation between the diversity and abundance of small mammals and the quantity of epigaeic arthropods as available diet source, arthropod sampling can be done during the trapping period. Suitable pitfall methods are described in Chapters 9 & 15.

### 2.3. Bats

Bats are cryptic and nocturnal animals that are difficult to observe. Therefore, monitoring bat diversity can be a challenging task. In this section we review the most frequently used techniques to capture bats. For a more detailed methodological review we especially recommend the chapter “Methods of Capturing and Handling Bats” by Kunz *et al.* (2009). Here, we will provide a brief hands-on description of how to capture, handle and process bats in the field. Each technique may bear a certain bias in capture success and the combined

use of different methods should warrant the best success. Also, capturing protocols, e.g. time and duration of capture, used capturing devices, mist netting sites etc., should be consistent when comparing bat diversity among sites. Also, given that bats may carry various zoonotic diseases, such as rabies, bat workers should be familiar with health issues, e.g. all persons handling bats should be vaccinated against rabies (no exceptions allowed). Mist netting in or at the entry of caves may also expose people to inhalation of spores from *Histoplasma capsulatum*, a zoonotic fungus (Di Salvo *et al.*, 1969). As a general rule, all people involved in capturing bats should be informed about potential health risks.

### **2.3.1. Nets and traps**

#### ***Ground-based mist nets***

Mist nets set up horizontally and ground-based are the most common and most efficient devices to capture flying bats. Mist nets are made out of a mesh of fine synthetic fibres (monofilament nylon and braided nylon or Dacron polyester). For capturing bats, the net material is usually black and the strength of the net (mono- versus bifilament and thickness of the nylon) is chosen according to the size of the expected bats. In general, most people use mist nets with the following features: 50 denier, 2 ply nylon and 28 mm mesh size. If using thicker mist net material (higher denier value), the net can withstand larger bats, but the net is more easily detectable by the bats. Standard net sizes are 6 m (18'), 12 m (42') or 18 m (60') long and 2.1 m to 2.4 m high when set. Usually, the height is divided by several horizontal shelf strings that form 4 or 5 horizontal loose pockets, which hold the trapped bats once they bounce against the layer of net material and drop into the pockets. Each end of the shelf string has a loop of stronger string material that can be put around supporting poles. These poles, e.g. aluminium tent poles or bamboo culms, should be set up at a distance equal to the net length (Kunz *et al.*, 1996b). For setting up a mist net, the loops are placed around the first pole. The top loop, which is usually white or coloured, and the following loops should be attached in the right order from top to base. The first pole is tied with ropes to either vegetation (e.g. nearby trees) or attached to stakes put into the ground. If no tree is close to the net, two ropes or one twisted around the pole may be used to stabilize the net. With the two ends of the rope/ropes attached to a near-by tree or a stake, an angle of approximately 70° is established between the two ropes. To provide a better support, the base of poles should be pressed slightly into the ground. The net should be held with caution as it will unwrap itself when the carrier slowly walks towards the second pole. It should be taken care not to let the net touch the ground during that process as e.g. leaves might get entangled in the net. After placing the second set of loops around the second pole, with the white (or coloured) loop at the very top, once again ropes are used to tie the pole to trees, branches or stakes. The ropes should be tied in a way that moderate tension is inflicted on the net. In the last step, positioning the loops from top to the base of the poles should unclosethe net. Once the mist net is open, the net material should form a pocket at each shelf string.

Mist nets should be closed during a break of a night capture session or when using a mist net more than one night at the same location. All debris such as leaves should be removed from the net before pushing the shelves together to close the net. The net can be furled by draping the net repeatedly around the gathered shelves and tucking the loose ends of the net pockets into the shelf strings. Gently spinning can also be used to furl a net. Several short strips of cloth or rope should be tied around the net to prevent unwinding.

For dismantling a mist net, the loops of the first pole are gathered at the top and are then removed from the pole, still maintaining the correct top to bottom order and keeping tension on the net to prevent it from touching the ground. The top loop should be used to tie the other loops before folding the net. By doing this, it is easier to maintain the top to base order when unravelling the net the next time. The loops of the second pole are removed in the same way as before. The net should be folded before storing it in a bag, preferably in a cotton bag as plastic bags restrict air circulation and therefore support fungal growth on the net material. Mist nets may become wet after rain or at high humidity. Then, nets should be dried before storing them over a prolonged period of time in bags.

To cover the sub-canopy of the forest, mist nets can also be stacked on top of one another. Freestanding poles with a rigging system (Rautenbach, 1985) optimize this system. The loops can be attached to carabiners on a hoist with strong free-standing net poles, which allows raising the net(s) high above the forest floor.

### ***Canopy mist nets***

Several previous studies have highlighted that bat assemblage composition differs between the ground and canopy level (e.g. Francis, 1994). Some species may not even be captured at ground level, because of their exclusive canopy lifestyle, e.g. molossid bats, large pteropodids or some phyllostomid bat species. Thus, vertical stratification of a bat assemblage is an important aspect when assessing local bat species diversity. In general, two methods are available for capturing bats at canopy height: either suspended horizontal mist nets as described in the previous paragraph or suspended vertical canopy mist nets. Both techniques require some training and sufficient time for preparation. To suspend a canopy mist net, it is necessary to first search for a good spot that provides (1) sufficient open space so that the net does not get entangled with twigs and branches, and (2) some large sturdy branches from which a rope can suspend the net. Horizontal canopy mist nets require two of such branches at distances larger than the length of the canopy mist net, whereas vertical canopy mist nets require only a single branch. The chosen branches should be sufficiently strong to support the weight of poles, ropes and mist nets. To hoist canopy nets to canopy height, it is first necessary to shoot a line with a lead fishing weight at its end over the branch. Use two lines to hoist a horizontal canopy net and one line for a vertical canopy nets. This is best achieved by using a slingshot (Kunz & Kurta, 1988; Nadkarni, 1988; Munn, 1991), a bow and arrow (Greenlaw & Swinebroad, 1967), a crossbow or a line-shooting gun. Since the thin line gets easily entangled during this process, it is best to use an

open-faced spinning reel that can be purchased from a fishing store. The line is then used to hoist a heavier cord (at least 10 mm in diameter and longer than twice the height of the branch) over the branch. Also, it should be noted that protective devices should be worn when using sling shoots or similar devices to prevent accidents. Alternatively, ropes can also be positioned by climbing. Vertical and horizontal canopy nets differ in some main features and in the way they are operated.

Vertical canopy nets require two branches from which the net is suspended and consequently two ropes. Once the rope is put around the branch in the canopy, one end should be attached to the top of the pole and the other end to the base of the same pole. This is repeated with the second rope and the second pole. Care should be taken that sufficiently strong knots are made to support the weight of the poles, ropes and net. Alternatively, carabiners can be permanently attached to the poles to warrant more support. Ideally, two persons are present when hoisting the canopy net by pulling the rope that is attached to the top of the pole. Caution should be taken not to stand right below the net in case the net or branches fall to the ground. Also, people should wear gloves when hoisting and manipulating the net. Once the net is positioned in the canopy, the rope should be attached to a tree trunk or some sturdy branches. Persons operating these nets should attach the rope very tightly to the vegetation structure. Afterwards, the opposite end of the rope that is attached to the base of the pole is manipulated in a way so that the vertical canopy net expands to its full size. The opposite ends of the ropes are also attached to vegetation to stabilize the canopy net. In order to put a canopy mist net down, it is necessary to first unknot both ends attached to the base of the pole. Then, the ends of the rope attached to the top of the pole are unknotted and held firmly with both hands. We recommend laying out a plastic tarp on the ground where the canopy mist net is supposed to stand on the ground to prevent leaves and debris from getting entangled in the net.

Vertical mist nets are designed for the purpose of canopy mist netting and bear the great advantage that they can be hoisted and handled by a single person (see Kunz *et al.*, 2009 for a detailed description). They are made out of the same material, but have a vertical instead of a horizontal rectangular shape. Usually, they are 6 to 9 m high and 3 to 4 m wide. Accordingly they do not have 4 to 5 horizontal shelf strings like a horizontal mist net, but 8 to 10 shelf strings. Three ropes (10 mm diameter) are required to deploy a vertical canopy net. A support rope with a length of at least twice the canopy height and a carabineer attached to its end. This support rope is put around an exposed sturdy branch as described before. A second rope of approximately the length of the pole is then attached from end to the other end of the pole. A third rope is then attached to the second rope at equal distance to the pole's ends. This third rope is guided through the carabineer of the first rope (the one suspending from the branch). Then, the support rope is pulled so that the carabineer at its end is at the desired height. Afterwards, the canopy net is hoisted by pulling the rope, which is attached to the pole rope. A fourth rope can be attached to the base pole to facility the operation of the net in case it gets entangled in branches. Again, a plastic tarp should be placed at the spot where the canopy net is put down (Fig. 5).



**Fig. 5.** Schematic view of a vertical canopy mist net (modified after Kunz *et al.*, 1988). The net is hoisted via a pulley into the canopy. A tarp is put on the floor on which the mist net can be lowered when bats are extracted from the net.

### **Harp traps**

Harp traps have been a successful addition to the tool case of bat biologists, since bats that are never trapped with mist nets are sometimes captured with harp traps. The reason for this is that fishing lines are very difficult to detect for bats based on acoustical (or visual) cues. An additional advantage of harp traps is that bats can be more easily removed from them. Usually, harp traps consist of 2 to 4 parallel rectangular metal frames (usually 2 m x 3 m) at distances of 4 to 6 cm that each carries a layer of vertically oriented monofilament fishing lines at distances of 2-3 cm (Fig. 6).

Normally, lines of the outer layer are separated at somewhat larger distances than the inner layer (in case of three layers). Flying bats usually fall through the first layer by the momentum of their flight or manage to manoeuvre around the lines of the first layer but they will hit the second layer. Then, bats fall to the base of the harp trap into a large canvas or plastic bag. Captured bats can be easily picked out of this bag. Harp traps with four layers of lines have been successful for capturing palaeotropical insectivorous bats (Kingston *et al.*, 2003). The tension of lines, the number of line layers, and the placement of the harp trap greatly affect its capturing success.



**Fig. 6.** Harp trap set up in front of the exit hole of a daytime roost. Two bat species, *Noctilio albiventris* and *Molossus molossus* emerged from the roost, hit the layer of fishing lines and tumbled into the plastic bag from which they were quickly recovered and transferred to linen bag. (Photo by D.K.N. Dechmann).

### **2.3.2. Trapping procedure**

#### ***Optimal sites for mist nets***

Finding the right spot for putting up mist nets is crucial for a successful mist netting night. In general, capture success is enhanced when nets are put at natural flyways, *e.g.* at a perpendicular angle to a forest edge or across forest trails. Distinct objects such as cave entrances, buildings, rocks, water holes, etc. also present good mist netting sites. If bats pass by a certain structure on a daily basis or emerge from known roosts such as cave entrances, a mist net will yield a large number of captured bats within a short time period. It may be worth counting or estimating bats that emerge from a roost before putting up a mist net. The capture success is enhanced when several mist nets are used at the same time, preferably in a T-, Z-, Y or V pattern. Feeding sites are also suitable for capturing specific species. A fruiting or flowering tree will probably attract several bat species in the tropics or subtropics. Some bat species are attracted by artificial volatiles *e.g.* Neotropical nectar-feeding bats by Dimethyldisulphide (Helversen *et al.*, 2000) and fruit-eating bats by essential oils (Mikich *et al.*, 2003) or the pulp of fruits (Rieger & Jakob, 1988). Some species are lured into nets by playback of their prey, conspecific social or echolocation calls.

#### ***Optimal sites for harp traps***

Harp traps are most efficient when set up at natural flyways of bats (see above). Since bats can be removed from harp traps at a faster rate than from mist nets, harp traps should be chosen, when large numbers of bats are expected, *e.g.* in front of daytime roosts, at cave entrances, etc. Like canopy nets, harp traps can also be suspended from large trees or into a canyon.

#### ***Time of capture***

The number of nets depends on the expected number of bats per mist net, the number of field workers available and the duration of mist netting. Usually capturing devices should be set up before sunset, because the 1 to 2 hours time period following sunset is often the most rewarding time in terms of number of bats. Mist nets and harp traps should be controlled on a regular schedule depending on the frequency of captures. In general, a net or trap should be checked at least every 15 minutes during the peak activity time after sunset. Bats will readily bite large holes into mist nets while trying to find their way out of the net. Also, bats may get severely entangled when their presence in the net is overlooked. Thus, regular visual inspection of mist nets is important.

Capture success decreases as night progresses. Mist netting success also drops drastically, when mist nets or harp traps are set up at the same spot during subsequent nights. Comparative studies need to ensure that capturing effort (= total time of mist netting and total length of used mist nets) is about the same for all study areas.



### **2.3.3. Animal handling**

#### ***Removing bats from mist nets***

With some experience, most bats can be removed from a mist net within a short time period. In general, many field workers prefer to wear at least one glove to be protected while holding the bat and then use the other hand to extract it from the net. When a bat is found entangled in the net, first of all it is essential to assess from which direction it has flown into the net. As a general rule, those parts of the bat that entered the net last should be removed first. Therefore, it is important to check whether the legs of the bat can be grabbed directly. During the whole process, it is most important to take care that the bat is not injured. A bat's finger and forearm bones are particularly vulnerable to physical fraction. Difficulties may arise when bats get entangled for a prolonged period of time and when the size of finger bones and forearm matches closely with the mesh size. In that case, one should start to work first at a single wing, extracting the fingers and the forearm carefully from the net. Sometimes it helps to expand the wing moderately. Occasionally, bats get irritated or distressed during the removal process and may start to bite and emit distress calls. Bats should then be held firmly and possibly a linen bag should be put close or around the bat's head to provide something for the bat to bite. In some rare instances it might be helpful to have a pair of small scissors at hand to cut some net strands into which the bat is hopelessly entangled. Sometimes bats may bite into the net, the string or the glove. Never pull the object away from the bat, but instead blow frontally against the bat's head. Eventually the bat will let go. If this is not the case, use forceps, Q-tips or a small stick to gently open the bat's mandibles. Once bats are removed from mist nets, they can be kept in a linen or cotton bag over a short period of time. In case the bat is supposed to be released, a linen bag that is wrapped around the bat's body will also facilitate measurements and species identification.

#### ***Keeping bats temporarily***

Soft linen bags are best for keeping bats temporarily. Some materials are too rough for the skin of bats, especially for the joint of the forearms, which causes irritations and may consequently lead to inflammations. Therefore, bags should have approximately thrice the size of the bat in length and width. Preferably a single bat is put into one bag. Sometimes it may be necessary to put several bats into a single bag. Then, only individuals of the same species should share the same bag. However, we advise researchers to avoid this situation as even individuals of the same species may bite each other when forced to share a bag. Bats that foraged successfully before being trapped in a net can be kept over several hours in a bag. Sometimes bats will enter torpor, *i.e.* they reduce their body temperature, when kept over a prolonged period of time. Some fruit-eating and nectar-feeding bats, in particular small ones, should be fed with diluted honey water before keeping them in a capture bag. They may also benefit from a few droplets of honey water shortly before they are released.

#### 2.3.4. Primary recording data

##### ***Species identification***

When bats are released at the site of capture it is important to identify the species in the field. Identification keys are available for some regions of the world, but not for all. Sometimes, the primary literature needs to be studied prior to the field trip. In many cases it is essential to bring copies of the primary and secondary literature to the field. If animals are collected as museum specimens, identification may be postponed until it is routinely done at the museum. However, to prevent collection of a superfluous large number of specimens of the same species, we advise a rough identification of all captured individuals beforehand.

##### ***Sex determination***

For the determination of sex, it is important to examine primary and secondary sexual characteristics, most importantly the genitalia. In some species, both sexes have well-developed nipples, females may have penis-like clitoris or males may have minute penis. Therefore, a combination of different traits is most useful for assessing the sex of a bat.

The reproductive status of females is checked by gentle palpation of the abdomen. If the reproductive status is important for the study, the reproductive tract has to be examined after dissection and the size of the fetus (if present) has to be measured. The examination of the nipples will provide information about whether a female has lactated or not (Racey, 1988). If the nipples are enlarged and keratinized, the female is lactating or has lactated very recently. Lactating bats should never be kept over a prolonged period of time and be released as soon as possible after processing. In lactating females the area surrounding the nipples is usually lacking fur.

Males should be checked for inguinal (abdominal) or scrotal testes and epididymis. Frequently, testes ascend into the abdominal cavity. To verify the correct sex, testes can be forced to descend by gently pushing the abdomen. We encourage to record scrotal males and whether a female is lactating or not.

##### ***Age categories***

Age is categorized in bats as juvenile, subadult and adult.

- ***Juveniles*** are generally defined as non-volant young individuals that are smaller and weigh less than adult individuals. They are often captured together with their mother. The epiphyses of their bones are not fused, yet, *i.e.* there is a light area of a few mm close to the joints of the finger bones (best examined in the finger bones by shining with a torch light through the wing from underneath the bat), the pelage is often grayer than the adult fur and they have deciduous teeth.

- **Subadults** are volant and fully-grown, but still show the unfused epiphyses (Anthony, 1988).
- **Adults** show mature size, fused epiphyses, pelage and are often reproductively active, *i.e.* males may have large (scrotal or abdominal) testes and females may be either pregnant or lactating.

### **Body measurements**

The standard measurements for bats include head-body length, tail length, hind foot length, ear length, forearm length, and body mass (Handley, 1988).

- **Body Mass** (BM) is recorded in grams with small spring-scales available in a variety of sizes from 10 g up to 2000 g (*e.g.* Pesola) and used accordingly to the animals' size. Especially for small bats, it should be checked whether the bat had ingested food before the capture (a bat caught very early in the night might not have had the chance to ingest a measureable amount of food). Keeping the bat for a period of time and recording the weight after excretion might provide a relatively exact weight of small bats (important for bats with body weights of less than 7 g).
- **Head-Body Length** (HBL) of a bat is the distance between the back of the bat's pelvis and the tip of the snout (European convention) or the distance between the last caudal vertebra (for bats with tails) and the tip of the snout (American convention). It should be measured to the nearest 1 mm.
- **Tail Length** (TL) is the distance from the base of the pelvis to the tip of the tail. The zero end of the ruler is placed at the base of the tail and the tail is straightened on the ruler and measured to the nearest 1 mm.
- **Hind Foot Length** (HFL) is the distance from the base of the calcar or the calcaneum in bats lacking a calcar respectively to the tip of the longest toe (tip of the claw for American convention). The foot is flattened on a ruler and the length is recorded to the nearest 1 mm.
- **Ear Length** (EL) is measured with a ruler placed gently in the notch at the base of the ear. The distance between the base and the tip of the pinna should be measured to the nearest 0.5 mm. In presence of a tragus, form and length are recorded.
- **Forearm Length** (FA) is defined as the distance from the elbow (tip of the ulnar olecranon process) to the wrist. To measure the forearm, the wing has to be folded. A ruler can be used, but a sliding calliper is more convenient to record to the nearest 0.5 mm.

Apart from these 5 standard field measurements, other data may also be relevant for species identification, *e.g.* the colour of the fur and presence/absence of colour patterns (*e.g.* epaulettes, stripes; Handley, 1988).

### **Other field notes**

For each **capturing site**, the GPS and location data (*e.g.* distances to road, river, building, etc.) should be gathered. Additionally, it is important to write

down the number and types of nets used. A brief description of the vegetation and type of habitat (*e.g.* gallery forest, primary forest, savannah) will help future data analysis. In some instances, it may also be helpful to draw a map of the capturing sites. If possible, meteorological data from a close-by weather station should be noted. As a general recommendation, the season (rainy or dry season), the cloud cover and the moon phase should be recorded in the field book.

For each ***captured bat*** it is important to note the site of the net, the net number and type and the height above ground (net shelf). It is essential to record the time of capture and if applicable the type and location of the roost. In addition, notes should be made regarding the number of ectoparasites and whether the captured bat was a recapture. All samples (*e.g.* faecal, pollen, blood, tissue samples) taken from the specimen in the field should be labelled and identification numbers should be added to the field note book. Appendix 2 provides a template data sheet.

### **2.3.5. Acoustic techniques**

Acoustic sampling of bats provides the inventory with important additional information. Bats, which routinely fly beyond the reach of nets and traps, can be sampled. Many different bat detectors are used to identify bat species without the necessity to capture the animals. For more detailed information see Chapter 5.

### **2.3.6. Workflow and personnel**

Mist nets should be set up before sunset. Care should be taken not to open the nets too early, because birds could get trapped as well. We recommend setting up a work station where people can work nearby using chairs and a table when dealing with several mist nets at the same time and when many bats are expected. Once mist nets are opened, they should be checked at regular intervals depending on the frequency of bat captures.

If many different samples (*e.g.* ectoparasites, wing punches, blood samples) are planned to be collected from each captured bat and a high number of bats are expected at one site, it might be helpful to divide the work. One person should check the nets and traps frequently and store the captured bats in bat bags and provide each bag with a small sheet of paper with notes (net number and shelf, time of capture). A second person measures and identifies the bats, while a third person is taking the samples and releases the animals (or keeps the animals in case of specimen preservation).

## **3. Preservation and DNA sampling**

Depending on the project objectives, the collection and preservation of whole voucher specimens, or taking DNA tissue samples from live animals are the best way to identify and document small mammals and bats. For any voucher

preservation, collection and import and export permits need to be obtained well in advance of the field work and special regulations regarding the presence of threatened or endangered species should be known (see also Chapter 3).

For the collection of specimens, the following five conditions should be met:

- Obtain the appropriate training (and practice) in the preparation and preservation of vouchers along with all health & safety precautions and equipment.
- Obtain research methodology clearance from your institutional animal care and use committee (IACUC in the US). See the recent review by Gannon *et al.* (2007) for the US.
- Arrange with a well-curated and officially recognized collection for accession of your specimens upon return from the field. No vouchers should be held in “private” or “personal” collections for extended periods.
- Obtain all necessary collecting and export permits from the host country or state and customs and other import permits from the country where the vouchers are to be housed (USFWS form 3-177 in the US).
- Keep meticulous records. Attach basic field data and/or a unique number to each specimen and cross-reference it in your field catalogue or field journal (Yates *et al.*, 1996). Do not rely solely on electronic records!

The field methods used to kill animals should be quick and as painless as possible for the animal. Humane methods for euthanizing small mammals in the field include the use of inhalants like Isofluran and cervical dislocation (Simmons & Voss, 2009). Lethal injection is another method, but it requires veterinary training. We highly recommend following regulations of the particular country, *e.g.* Veterinary Medical Associations.

Specimens to be kept as vouchers can be killed in a large, tightly closable container (large wide-mouth lab jars, large pickle jar, some plastic buckets with lids) in which a cotton swab soaked with an inhalation anaesthetic such as Isofluran or Enfluran has been placed. The animal should be left in the container for about 20-30 min. To avoid needless stress for larger animal we suggest placing the animal in the trap together with the anaesthetic in a tightly sealing durable plastic bag. Anaesthetics can be difficult to obtain in tropical countries (contact the country’s chief veterinarian office and/or hospital medical supply companies). Most anaesthetics are controlled substances for airline travel and can only be transported by air with special permits and specially labelled packaging. See the 2007 report of the AVMA (American Veterinary Medical Association) Panel on Euthanasia for more details (AVMA, 2007). Gannon *et al.* (2007) also recommend quick mechanical methods like cervical dislocation for mammals of small body size, instead of the extra steps of sedation and anaesthesia that might only add distress to the animals. Field workers should receive the appropriate training and permits for all of these methods!

Depending on the specific study goal, different ways of preservation are possible for voucher specimens: museum dry mounts with skull or skeleton or complete liquid preservation.

Irrespective of the type of preservation, standard measurements should be taken from all specimens before fixation and dead animals should be processed as quickly as possible. All specimens should be tagged. Tags on dry specimens should note the (1) collection date, (2) capture locality, (3) collector, (4) field measurements (Yates *et al.*, 1996). Tags on fluid preserved specimens should only note the specimen's sex, the collector's initials and the field catalogue number.

### ***Fluid preservation***

Fluid preservation is increasingly being used over making dry mounts to save field time for other activities. It is the preferred method for bats to preserve important diagnostic facial features (nose-leaves, etc.). DNA tissue samples from internal organs (*e.g.* liver, spleen, kidneys) are taken before fluid preservation. The skull can be removed immediately or later in the museum. Fur colour should be recorded as exactly as possible before preparing the specimen because it will fade over time in fluid preservation (Simmons & Voss, 2009). Usually specimens are now fixed directly in 75% ethanol without intermediate fixing in formalin (Handley, 1988). In most museum collections the storage media are ethyl alcohol or isopropyl alcohol. If the specimen has not been opened to extract DNA tissue samples or to remove internal organs a certain amount of the storage media should be injected into the specimen's abdomen using a conventional syringe and needle. This is particularly important in large specimens because fermentation of ingesta in the digestive tract will damage the abdominal tissue. All specimens should be preserved in containers that are filled with sufficient amounts of fluid and all containers should be tightly sealed. No pinning or other preparations are required, except for a bit of manual manipulation of the carcass to straighten it out – in cases where the specimen has died in a contorted or curled-up position (Griffin & Kolberg, 2004).

Fluid-submerged labels should be of 100% rag paper and labelled with permanent ink (*e.g.* Pelican fine drawing ink or similar). Test permanence of inks/markers before leaving for the field! Attach the label to the right hind foot of specimens with 100% cotton string (Yates *et al.*, 1996). For field transportation fluid-preserved specimens remain submerged in ethanol in a tightly sealed container carried upright (*e.g.* wide-mouth barrel normally used for water sports: <http://www.curtec.com>; available from *e.g.* <http://www.globetrotter.de>).

For overseas transportation fluid preserved specimens can be temporarily preserved by wrapping them in several layers of cotton cheesecloth soaked in ethanol (moist but not dripping wet!) and packed in a triple layer of zip loc plastic bags inside a sealed container. In this way they can be safely transported for up to three days. However, specimens should not be preserved in such a way over a prolonged period of time.

### ***Dry skins***

Prepared dry skins have the advantage of preserving fur colour variations and of being relatively easy to transport, store, and manage long-term in collections,

as they do not require special fire-safe storage facilities and regular fluid level controls.

In museum dry mounts of small mammals cotton-filled and subsequently thoroughly dried skins of the animals are preserved with tail and feet attached. The skull or entire skeleton are usually dried or temporarily stored in ethanol and later cleaned with the help of dermestid beetle larvae before they are rinsed and dried again for the collection. We do not provide guidelines for making dry mounts here but refer readers to various detailed and well-illustrated sources (Hall, 1962; Setzer, 1968; Nagorsen & Peterson, 1980; Griffin & Kolberg, 2004) and the abbreviated recommendations in Yates *et al.* (1996). All of these sources also discuss standard methods of field catalogue and journal keeping and appropriate tagging of specimens (Fig. 7).



**Fig. 7.** Dry-mounted small mammal (*Hylomyscus allenii*) skin showing “field side” of the specimen tag with sex, field (collector’s) number, locality, field measurements (total length-tail-hind foot-ear-weight) and date. (Photo by Jan Decher)

Dry mounted specimens should remain pinned on a foam board or Styrofoam sheets for several days. In the field these sheets can be cut to fit in shallow plastic (“Tupperware”) containers where the specimens can be stored safely from ants and humidity at night or during rain when not being air-dried. A desiccant (Silicagel-type), which can be recharged by heating over a small fire or camp stove should be placed in cloth bags inside the specimen containers at night and during transport. Air-drying skulls or skeletons should be hung from wire rings to keep away ants or other predators and/or lay them in a little screen cage to protect them from insects. For overseas transport, specimens should be un-pinned from the foam sheets and packed in layers of cotton inside the plastic tubs, which can then placed in expedition boxes or duffle bags padded with clothes. Skulls can be packed with fluid-preserved materials. If specimens cannot be prepared immediately post mortem, they should be stored in ethanol or in plastic bags (to reduce dehydration) and kept frozen until further treatment.

## **DNA samples**

Species identification or the verification of the morphologically identified species in the field can be achieved by DNA analysis. In many cases for the sampling of DNA tissues the animals do not need to be killed.

Taking wing biopsy punches or small amounts of blood are the most common DNA sampling methods for bats. Blood can be obtained from venous puncture of the antebrachial vein running along the anterior edge of the antebrachium or of the major vein in the interfemoral membrane (Kunz & Kurta, 1988; Watt & Fenton, 1995). Small amounts of blood can be collected in heparinised hematocrit tubes and larger samples should be collected by using heparinised syringes (Dessauer *et al.*, 1990, 1996; Prendini *et al.*, 2002). If the project design aims to quantify blood parasites or other pathogens, blood should be collected on a filter paper and/or prepared as a blood smear. Blood smears are usually fixed in methanol and air-dried.

Tissue samples for genetic analyses can be collected from bats by puncturing the wing membrane (chiroptagium) or the tail membrane (uropatagium) using biopsy punches (Worthington-Wilmer & Barratt, 1996). The chiroptagium is easy to access, is less vascularized and bleeds less compared to the tail membrane (uropatagium). A 3 mm diameter biopsy punch will yield sufficient DNA for future analysis. When taking biopsy samples from the wing membrane, care should be taken not to cause damage to larger veins. The results of Faure *et al.* (2009) show that tail wounds healed significantly faster than wing wounds and more DNA from tail biopsies could be extracted than from wing biopsies of the same size. They recommend that tissue biopsy for molecular analyses in bats should be taken from the tail membrane. Biopsies of the wing membrane are useful for marking associated with recapture programmes, because the wound and scar will persist longer (Faure *et al.*, 2009). The hole, which is left in the membrane after puncture, will close and heal within 2 to 4 weeks. If dry mounted or fluid specimens are being collected, small DNA tissues from internal organs (liver, kidney, spleen) can be sampled, before these organs are removed from the specimens.

Tissue samples from live small terrestrial mammals can be collected by tail-clipping or ear punching. The tail-clipping method implies the amputation of a small portion (1-2 mm) of the distal tail using sharp scissors. The ear punch method involves punching a hole or making a notch in the ear. Both methods do not require the use of anaesthesia or analgetics. In case toe-clipping is applied as a marking method (see Section 2.2.3) the amputated phalange can be used for genetic analyses. The tissue is immediately transferred in a vial with preserving solution (see Chapter 7). Afterwards the scissors and biopsies punch should be disinfected by dipping the tool into 95 % ethanol and burning the liquid off with a lighter flame.

The best preservation of fresh tissue samples can be achieved by freezing the samples using either dry ice or liquid nitrogen (Prendini *et al.*, 2002) or preserving in a lysis buffer (Longmire *et al.*, 1997). Placing the tissue sample directly in 95-100% ethanol in leak-proof 5 ml centrifillation vials (or similar small plastic containers) will be more feasible in most situations (Kilpatrick, 2002). For



long-term preservation all tissues should be kept frozen (methods for collecting, storing, and archiving tissue samples: Dessauer *et al.*, 1990, 1996; Longmire *et al.*, 1997; Kilpatrick, 2002; Prendini *et al.*, 2002). For more detailed DNA/tissue collection techniques see Chapter 7. The collection of fur, faecal and pollen samples might be useful for a variety of further studies and we recommend the book edited by Kunz & Parsons (2009) as a reference.

#### 4. Safety precautions

Handling wild animals can always include the possibility of exposure to zoonotic diseases (Childs *et al.*, 1995; Gage *et al.*, 1995; Kunz *et al.*, 1996a; Chomel *et al.*, 2007). Some of these pathogens may have limited health risks others can lead to fatal diseases (*i.e.* rabies). This short section discusses some issues regarding the reduction of health risk during field work. Zoonotic pathogens are transmitted by various routes. Beside the well-known “rabid bite”, where the rabies virus is transmitted into the wound via saliva of an infected animal, all other body fluids can also contain infectious agents. Urine from rodents, for example, may be a source of hantavirus or *Leptospira* spp. (Levett, 2001; Fulhorst *et al.*, 2007; Machado *et al.*, 2009). Even if mice are not handled directly, urine may have contaminated traps or the surrounding soil. Aeroionisation is the typical way of contracting a hantavirus infection (Machado *et al.*, 2009; Olsson *et al.*, 2009). Faeces, blood, and fur can also contain infectious pathogens. Wearing disposable gloves should become routine habit when working in direct contact with wild animals. With some larger or more aggressive species leather gloves are a good protection against bites and scratches. When gloves become soiled by the animal’s excretions, they can be disinfected with a spray solution, which needs to be designed to eliminate viruses, bacteria and fungi on most material surfaces (*e.g.* Pursept-A Xpress® (Merz)) at the end of a day’s/night’s field work. The manufacturer’s instructions should be checked beforehand as not all disinfectants will destroy every pathogen. Many disinfectants are only designed to destroy bacteria and do not contain protective remedies for certain viruses or protozoa. A hypochlorite solution (household bleach) in a 1:10 dilution can also be used. If using a spray, avoid inhaling the aerosol, for example outdoors by monitoring the wind direction. Spray solution can also be used to wipe off all equipment (including traps) and sample containers. For skin disinfection, products are available that are specially made for this particular purpose (for example: Virusept Manorapid Synergy® (Merz)), which are less aggressive and also contain some skin care ingredients.

Another important pathogen vector is dust. Face masks can prevent inhaling aerosols and/or light particulate matter. Surgical masks may be a first precaution, but they cannot be considered pathogen safe as they do not seal. A safe mask needs to cover mouth and nose without any gap and should remain so for some time. However, in a hot and humid environment the mask’s fabric easily gets soaked with moisture allowing particles to enter and should be replaced in time. So-called FFP3 masks (for example manufactured by 3MTR) feature a small breathing filter to allow air to enter at a lower point of resistance simultaneously keeping the rim of the mask better attached to the skin. As

peoples' faces are differently shaped it is advisable to try out differently shaped/sized masks before setting off. Additionally, protective eye gear might have to be included for field work, particularly if there is a possibility to be exposed to contaminated material dropping from ceiling inside caves, etc., or exposure to blood or urine. Mucous membranes can serve as contact points for many pathogens and the eyes are least protected by the immune system. Overalls protect and allow discriminating between contaminated working and everyday clothing.

Carcass dissection increases the risk of exposure to zoonotic pathogens. Beside the described personal protection, dissection should be performed as safe and clean as possible. Covering the worktop with a paper towel for each animal will give cleanliness. A separate disposal bag should be kept ready to collect soiled gloves, paper towels, etc. Sharp items like scalpel blades must be stored in an unbreakable container. In case of accidental cuts sufficient amounts of blood should be pressed out of the wound and the lesion cleaned from pathogenic agents. The wound can be washed with mineral water or safe drinking water. Afterwards a disinfectant like Povidone iodine (spray or ointment) should be applied onto the wound and covered with plaster or clean bandages. If immediate medical support is far away, the body temperature should be monitored to detect an infection, which should be medically treated with antibiotics or likewise as soon as possible.

Above all, it is important to become familiar with zoonotic pathogens that can occur in certain animal species and a particular geographical area, during the planning phase. Medical advice should be sought about vaccinations and a well-equipped first-aid kit, including medication, should be planned well in advance. All scientists who are working with bats should be vaccinated against rabies (Rupprecht *et al.*, 2008) as well as travelling to countries with high rabies prevalence should imply rabies vaccination (Briggs & Hanlon, 2007). Vaccination against tetanus should be considered obligatory for anybody working in the field. To list all zoonotic agents would exceed the given space, but the following website (<http://www.merckvetmanual.com/mvm/htm/bc/tzns01.htm>) provides a summary of pathogens, their animal source and means of transmission. As the distribution of diseases varies by the different geographic regions information on specific pathogens can also be retrieved from web pages of OIE, WHO or CDC.

Further information about disease risks for mammalogists can also be found in the following publications: Cox (1979); Krebs *et al.* (1995); Mills *et al.* (1995); Kunz *et al.* (1996a); Hafner (2007).

## **5. Practical notes**

### **5.1. Checklist "Before launching"**

Before launching a mammal biodiversity inventory, the investigator must clearly define the objective(s) of the study. The objective(s) guide the survey through all stages of planning and execution (Rudran & Foster, 1996). Fund raising, establishing contacts with other experts, review of scientific literature, purchase

of equipment, recruitment of personnel and organisation of the travel itself (flight, visa, permits, transport of equipment, vaccinations, etc.) usually need more time than estimated in the beginning, according to experience.

The first step in preparing for a survey is to review the scientific literature for mammal studies conducted in the project area, at nearby sites or in comparable habitats in the region. The information obtained is used to compile a preliminary list of species that may be encountered at the study site. Identification keys relevant to the study area and other guide books should be obtained. For example, in the case of West Africa, the only published field key for shrews is *The Shrews of Nigeria* (Hutterer & Happold, 1988) and for rodents we still frequently use *The Mammals of Africa*, an identification manual (Meester & Setzer, 1971). If no identification keys are available for the specific study area, a preliminary survey and/or visits to museum mammal collections to become familiar with the species which might be expected, should be considered. Knowledge of the natural history (physiology, behaviour, distribution) of the target species is important for choosing the right techniques. The choice of appropriate techniques depends on the available budget and on the specifics of the field situation. Purchase of equipment and recruitment of personnel should commence as their need is really identified.

During the planning phase it is important to contact other experts and/or project coordinators to gain access to information about the region, habitat and fauna. Information about on-site logistics, e.g. accessibility of field sites (foot/vehicle), storage options for vouchers (esp. in the tropics), lab space (if needed), availability of drinking water, medical care, maps, etc. are helpful for planning. Please inform and prepare yourself also about human health concerns and disease risks. It might also be useful to coordinate your survey with other scientists, for example with botanists, who would provide habitat descriptions.

## **5.2. Field equipment**

A list of field equipment for an inventory of terrestrial small mammals and bats is provided in Appendix 3. It covers: trapping, netting, treatment of the animals, tissue taking in the field, specimen preservation, habitat assessment and others. This list is not exhaustive and has to be adapted to the particular inventory. The set of equipment of course depends on e.g. the selected methods, selected sites, local conditions and others.

## **5.3. Simultaneous inventory of small mammals and bats**

If nocturnal bat work is planned (see Section 2.3) it can be helpful to split the team into a bat and a terrestrial small mammal group allowing the bat group to sleep in the morning and not participate in early morning trap checks after long hours of nocturnal netting and processing of bats (Tab. 2).

	Bat Group	Small Mammal Group
0400-0600h	resting (alternative: early morning bat netting, depending on team size)	resting
0600-0900h	resting	early morning trap inspection
0900-1000h	resting / breakfast	breakfast
1000-1300h	Bat voucher processing	Small Mammal voucher processing
1300-1400h	lunch	lunch
1400-1500h	afternoon rest / field note writing	afternoon rest / field note writing
1500-1800h	re-setting of bat nets / harp trap. Mending of nets	voucher preparation followed by replacing/opening of traps
1800-1900h	dinner & opening of nets	dinner
1900-2300h	bat netting	assist with bat netting, also nocturnal surveys for galagos, civets, pottos etc. with flash light and camera / tape recorder
2300-0100h	bat netting	resting
0100-0400h	resting	resting

**Table 2.** “Idealized” 2-group field team schedule for bat and small mammal work.

#### 5.4. By-catch

Quite frequently non-target species are captured during small mammal and bat surveys. Apart from non-target mammal species a diverse suite of species belonging to invertebrates (insects, snails, etc.) and other vertebrates, e.g. lizards, snakes, birds are sometimes captured coincidentally.

See the corresponding Chapters in this manual for handling/preservation of these species.

- Nocturnal/crepuscular birds in mist nets; during night and day birds in tomahawk traps, large Sherman traps, snap traps.
- Snakes, lizards, amphibians in Pitfall traps, Sherman traps, Snap traps.
- Insects in mist nets (e.g. Coleoptera, Lepidoptera), Sherman traps, Pitfall traps.

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## 7. Appendices

### Appendix 1. List of recording elements of capture for small terrestrial mammals.

Abbreviations: ID = identification

Recording capture data	(Micro-) Habitat description
<ul style="list-style-type: none"> <li>• Field number (consecutive)</li> <li>• Individual ID (in case of recapture)</li> <li>• Date</li> <li>• Collector(s)</li> <li>• Site ID</li> <li>• Check time / Control</li> <li>• Trapline ID</li> <li>• Trap ID</li> <li>• Trap type</li> <li>• Bait used</li> <li>• GPS</li> <li>• Species (Field ID)</li> <li>• Sex: Male, Female, unknown</li> <li>• Age: Adult, Subadult, Juvenile</li> <li>• Reproductive Status:               <ul style="list-style-type: none"> <li>Male: Testes descended or non descended</li> <li>Female: Pregnant, Lactating, Vagina perforated or non-perforated or plugged</li> </ul> </li> <li>• Body mass (g), BM</li> <li>• Head-body length (mm), HBL</li> <li>• Tail length (mm), TL</li> <li>• Hind foot length (mm), HFL</li> <li>• Ear length (mm), EL</li> <li>• Tissue sample</li> <li>• Parasite sample</li> <li>• Marking</li> <li>• Remarks: <i>e.g.</i> fate (released/recapture/voucher/dead/kept/marked/ etc.)</li> </ul>	<p><b>Description of trap location/station</b></p> <ul style="list-style-type: none"> <li>• Trap height</li> <li>• Canopy density</li> <li>• Nearest tree/dbh</li> <li>• Nearest log/stump &amp; diameter</li> <li>• Distance to water</li> <li>• Groundcover (percent herbs, grass, soil, leaf litter, wood, rocks, debris)</li> <li>• Nearest termite mound (in tropical environments)</li> </ul> <p><b>Inventory of environmental features</b></p> <ul style="list-style-type: none"> <li>• Elevation</li> <li>• Rainfall (last 12/24 hours if rain gauge has been installed)</li> <li>• Temperature (min/max if recording thermometer has been installed)</li> <li>• Humidity (min/max if recording hygrometer has been installed)</li> <li>• Vegetation: ground cover, plant height, plant diversity, stage of maturity, canopy density</li> <li>• Habitat structures (rocks, burrows, soil, logs, termite mounds etc)</li> <li>• Abundance of epigeaic arthropods</li> </ul>



**Appendix 3.** List of field equipment for an inventory of small terrestrial mammals and bats.

This list is not exhaustive.

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<b>Small Mammals</b>	<b>Bats</b>
<b>Trapping</b> <ul style="list-style-type: none"><li>• Traps: Sherman, Tomahawk, (Snap traps?)</li><li>• Bait: Peanut butter (unsalted), oats</li><li>• Insulation material: e.g. 2 litre milk cartons (tetrapaks)</li><li>• Bedding material for traps</li><li>• Pitfall traps (buckets 5 litre)</li><li>• Funnel (custom-made)</li><li>• Drift fence (e.g. roll of green nylon cord)</li><li>• Staple gun and staples</li><li>• Poles</li><li>• Tape measure (30 m)</li><li>• Marker tape (biodegradable, non-polluting/brightly coloured / reflective)</li><li>• Marking pen (water resistant)</li><li>• Aluminium tags</li></ul>	<b>Netting</b> <ul style="list-style-type: none"><li>• Mist nets (different sizes)</li><li>• 3 meter poles for standard ground nets (e.g. sectional aluminium or PVC, if they can not be cut in the field)</li><li>• Stakes</li><li>• Roles of string</li><li>• Canopy net unit, freestanding (sectional aluminium poles, ropes, pulley carabiners, large stakes) or: Canopy unit hanging</li><li>• Sling shot, bow and arrow, crossbow or a line-shooting gun to attach hanging canopy unit</li><li>• Harp trap kit (additional fishing lines)</li><li>• Marker tape (biodegradable, non-polluting/brightly coloured/ reflective)</li></ul>
<b>Treatment of animals</b> <ul style="list-style-type: none"><li>• Gloves (firm to bites)</li><li>• Disposable gloves</li><li>• Plastic bags (3 litre size) or cloth</li><li>• Measurement tools (ruler, calliper)</li><li>• Spring balances (10 g, 30 g, 100 g and 300 g); or larger ones (1 kg, 5 kg) for animals caught in Tomahawk traps</li><li>• Tubes 5-6 (15-50 mm diameter, length 15-25 cm) from acrylic glass</li><li>• Cotton wool</li><li>• Marking tools (if requested)</li><li>• Cage</li><li>• Field book</li><li>• Identification keys</li></ul>	<b>Treatment of animals</b> <ul style="list-style-type: none"><li>• Gloves (firm to bites)</li><li>• Linen or cotton capture bags</li><li>• Measurement tools (ruler, calliper)</li><li>• Spring balances (10 g, 30 g, 100 g and 300 g); or larger ones (1 kg ) for Megachiroptera</li><li>• Field book</li><li>• Identification keys</li></ul>
<b>Tissue taking in the field</b> <ul style="list-style-type: none"><li>• Scissors, forceps</li><li>• DNA tools: vials ...</li><li>• 95% Ethanol for DNA tissue (or DMSO)</li></ul>	<b>Tissue taking in the field</b> <ul style="list-style-type: none"><li>• Forceps</li><li>• Syringes and needles</li><li>• Heparinised hematocrit tubes</li><li>• Microscope slides</li><li>• Biopsy punches &amp; 2 ml vials</li><li>• Filter paper</li><li>• Lighter (for forceps sterilization)</li><li>• Methanol (fixation of blood smears)</li><li>• 95% Ethanol for DNA tissue (or DMSO)</li></ul>

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**Specimen preservation dry/wet**

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- Container/jar (large, tightly sealing) for killing
  - Shallow plastic containers (“Tupperware”)
  - Wide-mouth barrel (CurTec wide neck kegs)
  - Disposable gloves
  - Plastic bags (3 litre size) or cloth
  - Inhalation anaesthetic (e.g. Isofluran)
  - Formalin
  - 95% (75%) Ethanol for whole body preservation
  - Cintillation vials (leak-proof 5 ml)
  - Scissors, scalpel
  - Labels (fluid-submerged) of 100% rag paper
  - Tags
  - Thread or twine for tags
  - Permanent ink (e.g. Pelican fine drawing ink or similar)
  - Board or styrofoam sheets
  - Pins, wire rings, needle
  - Wires of differing thickness, wire cutters
  - Cotton wool, quilting cotton, long fibre cotton
  - Maize meal
  - Desiccants (Silica Gel-type)
  - Screen cage
- 

**Other**

- Hand-held GPS unit
  - Digital camera/ ideally SLR with Macro lens & flash
  - Binocular
  - Headlamps, additional flash lights
  - Spare batteries of all needed sizes (D cell, AA, AAA etc.)
  - Buckets, bowls, strainer, measuring pitcher and funnel
  - Equipment case (waterproof, firm, well-arranged) e.g. light toolbox
  - Rucksacks/bags for transport of material (e.g. traps)
  - Tools: spade, pliers, shovel, hammer or small hatchet for stakes, hoe or pickaxe in tough soils
  - Disinfectant
- 

**Optional**

- Bat detector (Anabat, Pettersson etc.) & cassette recorder or CF cards
  - Folding table/ Folding chairs
  - Warm clothes (it can get quite cold at night; even in tropical areas)
  - Bug repellent
- 

**Habitat assessment**

- Altimeter (not necessary if GPS available)
  - Spherical densiometer
  - Fiber glass diameter tape measure (width 16 mm, length 10 m)
  - Folding rule
-



## **Chapter 20**

### **Sampling Amphibians and Reptiles**

by

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## **Abstract**

Amphibians and reptiles rank among the most charismatic vertebrates and have received a lot of taxonomic attention over time. Nevertheless, to improve the speed and quality of inventory and monitoring of these animals, we here provide an overview of the different sampling techniques used per taxon and detail the different ways of handling amphibians and reptiles. Also, we indicate the different types of data that are to be collected from specimens. Throughout the chapter tips and tricks for safety are also provided.

**Key words:** amphibians, reptiles, sampling methods, handling methods, safety

## **1. Introduction**

Amphibians and reptiles are important components of ecosystems. Recently, there is an increasing awareness that, like for many other taxa, herpetological diversity is threatened, and the growing list of declining populations suggests a worldwide crisis (Blaustein *et al.*, 1994). Efforts to collect baseline data about occurrence, distribution, and status of populations are relatively well advanced for amphibians, but much less so for reptiles for which recent data suggest that turtles and crocodilians are as threatened as anurans (Gibbons *et al.*, 2000).

In this chapter, we review field techniques for sampling reptiles and amphibians. However, as good body of literature that addresses in detail different topics of the sampling of reptiles and amphibians (see Heyer *et al.*, 1994; Dodd, 2009; Graeter *et al.*, 2008; Kok & Kalamandeen, 2008 and the references there contained) already exists, this work is only a general manual useful in ATBI initiatives. As such, it does not go into detail of all the aspects that need to be considered when inventorying and monitoring reptiles and amphibians.

Inventories may range from quick short-term surveys to extensive long-term monitoring programs. Inventories ideally involve the use of many collecting and observational techniques, in addition to the compilation of data from past surveys obtained from the literature and museum specimens. Monitoring programs ideally should use standardised techniques so that statistical analysis of data becomes possible.

## **2. Inventory and monitoring techniques for amphibians and reptiles**

This is a condensed review of the most common techniques used in inventories and monitoring of amphibians and reptiles whereby we focus on the possibilities, limitations and materials needed.

### **2.1. Standardising the sampling effort**

In monitoring, the importance of having standardised sampling procedures cannot be overemphasised. The methods mentioned hereunder are suitable for both terrestrial and aquatic habitats, and may be an efficient way of standardising monitoring surveys (*e.g.*, Brown 2001; Meik *et al.*, 2002). Next to recording biological data, the environmental conditions must also be scored and this again in a standardised way.

Standardisation of sampling can be achieved through: (i) time-constrained searches; (ii) area-constrained searches; (iii) quadrat sampling; (iv) transect surveys.

### **2.1.1. Time-constrained searches**

The premise behind this technique is to actively search for animals in a given area for a pre-defined amount of time. If additional information will be collected from the animals found (*e.g.*, body measurements or marking individuals), then the time invested in these activities should not be considered as part of the search. Time-constrained searches are mostly applied during terrestrial surveys, although they can also be used in aquatic habitats, particularly for amphibians.

The main limitation of a time-constrained search is the long periods that the survey participants must commit to it. Furthermore, it must consider that the results of time-constrained searches are highly influenced by environmental factors such as time of the day, season, and weather (*e.g.*, it is well known that amphibian activity increases very much after rainfall). Another factor that will heavily influence time-constrained searches is the level of experience of the surveyors. Experts are likely to find more animals than inexperienced workers. It is vital to keep these factors in mind when designing a study.

For an inventory it is advisable to repeat the sampling to include several days with different weather conditions and to always follow the same previously planned search routine (*e.g.*, if the first search included turning stones, then that should also be included when repeating searches).

### **2.1.2. Area-constrained searches**

With area-constrained searches the search is focused on a certain area and not on an amount of time. Area-constrained searches will give information in terms of absence or presence of species, and potentially some data on life history of the species such as time of reproduction, activity patterns, and habitat use. The size of the area to be searched might vary but it will depend either on the habitat type (*e.g.*, pond, creek, meadow, etc.) or on the focal species.

The main limitation of this technique rests with the effect of environmental conditions, the experience of the workers, and the planned search routine. As with time-constrained searches, the searches should be done during several days with different weather conditions or even different seasons to maximize the chance of encountering all species present in the area.

### **2.1.3. Quadrat sampling**

In this technique, sampling arrays in a study area must be randomly distributed and the absence or presence of animals in these arrays verified. The sampling areas are usually squares (quadrats) that are thoroughly searched (Jaeger & Inger, 1994).

The main drawback of quadrat sampling is that the setup can be very time-consuming.

Within quadrat sampling, we can differentiate point sampling where small squares are used, and broad sampling where larger quadrats are used (Kok & Kalamandeen, 2008). Point sampling is preferred when studying single species

in which the individuals are relatively small and densely distributed, while broad sampling is applied to species that are widely dispersed, large bodied or both, as well as for multispecies assemblages. In both cases, all quadrats need to be of the same size within each study area. A modification of this technique is called 'patch sampling', in which the sampling arrays are normally specific microhabitats (*e.g.*, logs, bushes, etc.). Patch sampling is applied when looking for specific target species, which we know or suspect that are confined to specific microhabitats within a larger habitat (Jaeger, 1994a). For both techniques some pre-requisites have to be met.

For quadrat sampling:

- Animals may not leave the quadrat before being observed.
- The quadrats are randomly distributed.

For patch sampling:

- Each patch must be defined precisely and in an operational way.
- All patches must be equally locatable by the observer without any bias.
- Animals may not leave the patch before being observed.

If these criteria are met, then quadrats and patches can be distributed randomly within the study area. Each of them then represents an independent sample, allowing statistical analysis of the obtained data if at least 25 to 30 quadrats were scored (Jaeger & Inger, 1994).

Quadrat sampling has proved to be particularly useful in forests when searching for ground-dwelling amphibians and reptiles (Rodda & Dean-Braleay, 2002). For best results in this methodology of quadrat (or patch) sampling, it will be important to apply the most appropriate searching technique within each of the quadrats (*e.g.*, using a rakes over leaf litter).

#### **2.1.4. Transect surveys**

A linear transect is established and the whole narrow strip (and nearby areas) is searched for animals. This is usually utilised for surveying herpetofauna across environmental gradients but can also be used within a single habitat (Jaeger, 1994a). However, for homogeneous study areas, quadrat sampling is recommended. If the design is properly randomized this method will provide a good representation of the occurring fauna over all habitat types. Depending on how the transects are set regarding the gradient, different information will be obtained. If transects are set in parallel to the gradient studied, then these surveys may be used to compare species across habitats. If on the other hands, transects are set perpendicularly to a gradient (*e.g.*, along a river), then one will be able to study changes in parameters of a given species along the gradient. The most common scale used in transect surveys is at the habitat level, but it is possible to work on a larger scale (ecosystem or landscape) by using, for example, aerial surveys across a large transect (Mourão *et al.*, 2000).

Ideally, transect surveys have to meet the following assumptions:

- Specimens are randomly distributed throughout the transects.

- Transect lines are randomly chosen.
- All the specimens in the transect will be observed.
- Animals will not be counted twice within a transect and among transects.

When preparing transect surveys it is important to consider that some species will not meet all the method's assumptions. For example, cryptic species will not be observed or will flee from the observer without any notice, or many species do not have a random distribution, as they are associated to specific microhabitats.

## **2.2. Sampling Techniques**

For selecting the most suitable sampling technique, it is necessary to evaluate :

- The objective of the study.
- The conspicuousness of the species of interest (their activity and habitat).
- The cost, time, and resources needed.

By and large, the methods that are more time- and resource-intensive will yield most information, and will allow more powerful statistical analyses. However, depending on the goal of the study, such intensive methods might provide data that are not needed (*e.g.*, obtain detailed ecological data when presence or absence of species would suffice). Furthermore, it is also more productive to use a combination of techniques instead of applying a single one, but again this will require more resources. Therefore, one must strike a balance between available resources for research and desired results before starting fieldwork.

The most common techniques used for sampling reptiles and amphibians can be divided into active and passive sampling, each with a number of specific techniques.

## **2.3. Active sampling**

### **2.3.1. Visual encounter surveys (VES)**

VES is by definition a time-constrained method in which observers sample for species richness and abundance along a survey path (Crump & Scott, 1994). The time spent in the field and the numbers of observers are taken into account. This technique is appropriate for both inventory and monitoring. VES might be particularly useful for detecting rare species that seldom fall into traps, and thus by using VES in combination with a passive sampling technique, it is potentially possible to obtain the complete species composition of the sampled area. Nevertheless, the efficiency of VES will vary much depending on the type of habitat (*e.g.* low vs. high vegetation) and the species biology (*e.g.* fossorial vs. arboreal). As a matter of fact, visual encounter surveys have a number of assumptions that in many occasions cannot be fully met :

- Each individual of every species must have the same probability of being encountered. This will not be met for example in species with a large sexual dimorphism were one of the sexes is much more visible than the other;

- Each individual is only recorded once during the survey. For this the use of individual marking may be the solution, but it implies a higher time investment;
- Each observer doing the survey must have similar experience and be able to potentially obtain the same results. The best approach to this problem is by training the workers in advance to ensure a similar level of experience.

Road cruising and aerial surveys could be cited as visual encounter surveys, although these are done at a different scale and have specific characteristics. In the case of road cruising, a road is used as a survey transect that is methodically driven through looking for both alive and roadkill specimens (Andrews, 2008). Aerial surveys are mostly used for estimating population size and distributions of large-bodied reptiles such as crocodylians or sea turtles (Glaudas, 2008).

### **2.3.2. Dipnetting and kick sampling**

We refer to dipnetting when a dipnet is swept through an aquatic habitat to capture herpetofauna. When the dipnetting process is semi-standardised – the number of sweeps is recorded and compared among habitats – one may call it sweep sampling (Dodd, 2003).

Sweep sampling is used for sampling herpetofauna in small aquatic habitats (treeholes, springs, puddles, and ponds) where it is more efficient. However, sweep sampling may be used in larger aquatic habitats such as lakes as well with the aid of seines and nets. The main targets of this technique are amphibian larvae. It is important to consider that specific differences in animal positioning in the water column may result in differences in the ability to catch different species. Also not all species can be caught with the same net, so the type of net and its mesh size must be carefully selected depending on the ecology and size of the targeted species. Moreover, dipnetting should be scheduled in the season when the species are most likely to be found in the water. When these factors are taken into consideration sweep sampling may be a very effective sampling method that allows for comparisons among aquatic habitats that are somewhat homogeneous.

Kick sampling is a technique that is especially fit for aquatic habitats of small to intermediate size and with fast flowing current. It is predominantly used when looking for stream dwelling amphibians. It consists of lifting and removing all loose substrate from a stream bottom, kicking loose pebbles or even hand raking everything into a net. Typically, the most common nets used are those with a D-frame whose flat side may rest on the bottom of the stream. The most efficient way to kick-sample is with two-person teams, where one worker loosens the woody debris, rocks and other substrate, while the other holds the net in place. It is very important when sampling for herpetofauna to check the nets very often (every 5 minutes or less) to decrease animal stress and mortality. As before, mesh size has to be considered based on the target species size. The smaller the mesh size, the more species will be captured, but small mesh nets tend to clog up faster with debris, and thus require more frequent maintenance to maintain efficiency.

The main limitations of kick sampling are that it is very labour intensive and that it can cause habitat disruption. For the latter reason, it is very important to redeposit the habitat items (e.g. large stones, wood debris, etc.) that had been moved.

### **2.3.3. Stovepipe sampling**

Stovepipe sampling is a quantitative method in which aquatic animals are trapped within an enclosure and later removed from it with a net (Shaffer *et al.*, 1994). The enclosures or samplers are typically pipe-like (one may use air conditioning ducts, culverts, stove pipes, and PVC pipes) (e.g., Alford, 1986 or Skelly, 1996) or a rectangular box (e.g., Harris *et al.*, 1988). These samplers are placed in the water, firmly set against the substrate, but with enough care as to not disturb the environment and cause the animals to flee. Once the sampler is in place, a net is swept within the enclosure to collect the animals. This technique is especially suitable for obtaining quantitative estimates of larval densities that can be used to estimate population size. Samplers should be placed randomly across the habitat, and their dimensions and the water depth recorded to obtain values of captured animals per volume. The best habitats to apply stovepipe sampling are shallow waters with sandy or mucky substrates, which allow to easily install the samplers. In habitats with water vegetation, pipe enclosures are easier to install than rectangular ones. This technique can be time intensive to use, so in case of large habitats or if we only want to determine the presence of a particular species, other methods such as dipnetting will be more useful.

### **2.3.4. Egg mass and nest counts**

This is a method that can be used during breeding periods to monitor the reproductive activity in reptile and amphibian populations. In amphibians, egg masses are counted around a pond perimeter or within the pond and it is particularly useful for explosive breeders and those that reproduce in communal aggregations. For identification purposes it is recommended to photograph the egg masses or at least use detailed language to describe it. Mitchell (2000) recommends making the following observations:

- Is the mass globular or round?
- Are the eggs clumped, separated or on a string?
- What colour and shape are the embryos?
- Is jelly surrounding the eggs firm or loose?
- Is there a film on the surface of the mass?
- To what type of vegetation is the mass attached?

In the case of reptile egg nest counts, this technique is most useful for turtles and crocodylians. Normally a relatively large area must be checked and there is need of having some previous knowledge of nesting grounds, and sometimes the recognition of tracks can be very useful, as well as the leftover from predation over the nests or the remnants materials after the babies hatch (e.g., broken egg shells). Egg mass and nests counts is a relatively simple and powerful method for determining the presence of species, and especially in the case of species



that lay a single clutch per year it can be a reliable indicator of population size. This technique is nevertheless useless for amphibians that lay eggs in the land and for most squamata reptiles. Finally, it is important to consider that the lack of egg masses or nests cannot rule out the possibility of a species being present, but not reproducing.

### 2.3.5. Auditory surveys

Auditory surveys are very useful for estimating species richness of anurans. Male anurans in particular tend to be fairly conspicuous during breeding season when they use their mating calls for attracting females. These calls are species specific, so during the breeding season listening stations can be randomly selected along the breeding site to identify species presence and their relative abundance. This technique has the advantage of easily covering rather large areas while being hardly non-invasive.

Not all anurans are equally easy to detect, but with some training even non-expert workers can obtain good results. In inventory, regular auditory surveys are very helpful for determining species composition, but there are some limits when it comes to monitoring changes in a population because there is always a bias towards only observing declines in calling activity and it is difficult to evaluate if these are due to natural fluctuations. If the aim is monitoring, acoustical surveys should always be coupled with other sampling techniques. In the chapter on bioacoustics more information can be found.



Fig.1. Calling frog. (Photo by author).

### 2.3.6. Basking surveys and basking traps

Sampling techniques based on the animal's basking activity are applied in aquatic habitats, especially rivers where the observer can advance in parallel to the river bank while scanning basking sites with binoculars. The studied animals are normally turtles (Buhlmann & Vaughan, 1991; Lindeman, 1998), although it has also been applied on water snakes (Mills *et al.*, 1995).

Apart from species presence, basking surveys can also give information on sex ratios and juvenile recruitment, but when further information is needed, this technique is to be complemented with basking traps. These are wire traps that

are attached to the underside of the basking log so when the animal instinctively jumps into the water, dives to the bottom of the trap giving the observer time to retrieve it. It is important to remember that basking traps must allow the animal to ultimately climb out of the trap if they fall in and the researcher is not present. The effectiveness of the basking surveys will depend on the amount of basking surface available, the time of the day or season when it is done and the animal's basking behaviour. The main limitation of this technique is that it depends on amount of basking surface available. If there are no basking sites, then no animals are observed, but it does not mean that the species is absent. For using basking traps it is absolutely necessary to identify first favourite basking sites, so a basking survey will always precede the setup of basking traps. In monitoring initiatives basking surveys and basking traps should always be made in conjunction with mark-recapture studies.

## **2.4. Passive sampling**

### **2.4.1. Artificial cover**

Many reptiles and amphibians use covers in the wild for hiding. Logs, rocks and even human debris provide refuge to many species, which implies that sampling these covers many times is an effective method. The problem with these "natural" covers is that quantifying their effectiveness is difficult.

By using artificial coverboards we can standardize the sampling effort maintaining the natural habitat and limit biases. The materials most commonly used for coverboards are solid wood boards, plywood boards, corrugated metal strips, tarpaper and horticultural plastic sheeting. These coverboards are set in array designs as linear transects, rectangular grids or webs, depending on the species and/or habitat sampled. Artificial coverboards have been used to sample many species of reptiles and amphibians (Parmelee & Fitch, 1995; Sutton *et al.*, 1999; Houze & Chandler, 2002; Ryan *et al.*, 2002; Smith *et al.*, 2006). An additional benefit when using coverboards is that as they do not restrict movement, it does not require continuous surveillance as for example pitfall traps. Their maintenance is also easy and inexpensive when compared to pitfall traps. In studies where coverboards and pitfall trap arrays have been used, pitfall always captured more species and more individuals (Sutton *et al.*, 1999; Ryan *et al.*, 2002), although coverboards detect species that are not found in pitfalls. In this sense this technique has proved to be particularly useful for small secretive snake species (Fitch, 1992).

When checking coverboards it is advisable to use tool such as snake hooks to avoid accidental bites. It is also advisable to flip the coverboards always towards the researcher to avoid the animals to escape. Finally, when sampling the coverboards it is also advisable to record environmental data such as the weather conditions, time of the day or the temperature. Sampling encompassing as many environmental conditions as possible will always yield better results.

### **2.4.2. Polyvinyl chloride (PVC) pipe surveys**

PVC pipes are an easy and inexpensive technique for sampling hylid tree frogs. These PVC pipes can be placed in the ground or mounted on trees following a grid or transect setup. The ground-placed PVC pipes can be used nearby the breeding areas of the hylids or as a complement to pitfall traps and drift fences, which are normally easy to avoid for the tree frogs. The tree-mounted PVC pipes on the other hand are suitable for sampling the tree frogs even outside their breeding season (Dodd, 2003). For ground-placed pipes a good length is 1 m vertical pipe with around 60 cm sticking out of the surface, while tree-mounted pipes can be of around 60 cm with the bottom part set at a height of 2-4 m. These pipes should have the bottom sealed with a cap to retain some water, but holes should be made in the pipe at about 15 cm to allow draining the excess of water. A good average diameter for the pipes in both cases is about 2-5 cm. Nevertheless, tree frogs can be of many different sizes, so it might be necessary to try out pipes with different sizes and diameters until finding the most successful design for a given species. An important benefit of PVC pipes is that it causes no mortality on the sampled animals, so the frequency and timing of the checks can be very flexible. This allows accommodating this technique easily with other activities and also makes it suitable for using in remote field sites. The main limitation it has is that it is very specific (only for tree frogs) and that PVC pipes are rather conspicuous, so they can be subject of theft or unwanted manipulation. This technique is most useful for detecting presence/absence of species, and even for determining timing and dispersal from breeding grounds. On the other hand it is very tricky for comparing between sites because its results will depend very much on species assemblage and on the availability of other natural hiding sites. If the aim is monitoring through time in a same site, PVC pipes in conjunction with marking individuals can give much information.

### **2.4.3. Leaf-litterbag surveys**

Leaf-litterbag surveys are specific for salamanders, which can be difficult to monitor due to their cryptic and fossorial nature. Litterbags have been commonly used for many years to estimate leaf litter breakdown in streams (*e.g.*, Peterson & Cummins, 1974), but it has been adapted for sampling stream-dwelling salamanders (Pauley & Little, 1998). This technique was successfully applied in the Great Smokey Mountains National Park to inventory various streams (Waldron *et al.*, 2003). Their basic design consists of a square (50-90 cm per side, with 70 cm x 70 cm being the optimal size) piece of plastic netting with 1.9 cm mesh. Small rocks are placed on the netting in the field and covered with leaves before the corners are brought together and bound with cable ties to form the litterbag. Finished bags are placed in the stream at regular intervals and after an acclimation period of a couple of weeks, each bag is checked by placing a dip net underneath and lifting the bag into a bucket of water. Then, to extract the salamanders from the bag, dip the bag repeatedly in the bucket and then pour the water through the dip net. The salamanders are then processed and the bags are placed back into the stream.

Although this technique has proven to be successful for detecting the presence of salamander species, it is not capable of indexing populations sizes, so it cannot be applied on its own in monitoring programs.

#### **2.4.4. Aquatic and terrestrial funnel trapping**

Funnel trapping is a standard method for trapping many groups of animals including reptiles and amphibians. The principle behind these traps is pretty simple: animals are directed through a small opening in the trap via a funnel or ramp, and once inside, are unable to find their way out. This is a technique especially useful for capturing rare cryptic species and has the advantage of being suitable for standardizing. In addition as traps are used during a lapse of time, this technique is also less sensitive to biases resulting from temporal variations. On the other hand funnel trapping requires a substantial investment of time and equipment. The traps themselves can be expensive, and should be checked often to avoid mortality of the trapped animals. When applying funnel traps in inventories, the effort should focus on habitats and times when the target animals are more likely to be active, and the more different habitats sampled the more species we will likely detect. Nevertheless, funnel traps have generally a low capture rate, so for successful inventories, a high intensity sampling is recommended (several hundred trap-nights spread across the season).

Funnel traps are also very useful in long term monitoring programs as the trapping scheme can be easily replicated allowing comparisons. For this, traps can either be set in systematic or random arrays. Based on the capture rates detected we will be able to infer population status, but always with some reserves, as capture rates will depend not only in population size, but also in level of activity and the propensity of the species to enter and remain in the traps. The ideal situation is when traps are complemented by mark-recapture data.

Funnel traps can be used in aquatic and terrestrial habitats, and can be of different sizes, materials and shapes. The use of one or other will normally depend on the target species:

- **Small aquatic funnel traps:** These can be either cylindrical or rectangular and are normally used for trapping water snakes and aquatic amphibians. The traps that are commercially available are designed for capturing crawfish or eels, but these can also be used for amphibians. They are typically double ended and built of steel hardware cloth, plastic or nylon mesh. The plastic traps are normally the most suitable for trapping the smaller species. As an alternative, small and inexpensive traps can be made by inverting the top of a plastic soda bottle and anchoring it to the substrate with a stake (Willson & Dorcas, 2003).
- **Hoop-nets:** These are large funnel traps used primarily for trapping highly aquatic carnivorous turtles, although it is potentially useful for trapping any aquatic turtle. These traps are also commercially available in different sizes and made of twine or mesh. In their setting, the traps normally have a part above the surface allowing the captured turtles access to air. Normally hoop nets are baited to increase success and should be checked at least daily. In

occasions hoop nets can also capture large aquatic salamanders and large snakes.

- **Interruption traps and fake nets:** These traps are suitable for complementing the hoop nets. In this case the trap is unbaited, but uses nets or natural channels to draw the turtle towards the funnel. Essentially they work like drift fences but on the water. The design of the trap can include unbaited hoop-nets, swing door traps or pressure plate traps at the end of the channels of nets. As with the hoop-nets, although these traps are mainly for turtles, they can capture other species such as large amphibians or large snakes (Vogt, 1980).
- **Terrestrial funnel trapping:** Terrestrial funnel traps are typically used in conjunction with pitfall traps along drift fences. The design of the trap can be very variable, although the most common variation consists of a wire hardware cloth cylinder with inverted hardware cloth funnels pinned into each side (Fitch, 1987). It is advisable to set the traps in the shade or cover them with a board to make them more attractive and to protect the captured animals from the rain and the heat. In the case of amphibians it is also advisable to use some kind method to moisture the inside of the trap (*e.g.*, a moist sponge). Terrestrial funnel traps can also be constructed of wood boxes, which makes their building more complex and time-consuming, but in different studies have proved to capture almost any snake, reptile or amphibian possible (*e.g.*, Burgdorf *et al.*, 2005; Enge, 2001; Greenberg *et al.*, 1994).

#### 2.4.5. Terrestrial drift fences and pitfall traps

Drift fences have proven to be effective for sampling most amphibians and squamata reptiles (Nelson & Gibbons, 1972; Semlitsch *et al.*, 1981; Hanlin *et al.*, 2000; Enge, 2001; Russell *et al.*, 2002; Ryan *et al.*, 2002, Todd *et al.*, 2008). The basic design of a drift fence is a straight fence buried slightly below ground, and standing up to 50 cm high. Pitfall traps are then buried at floor level and placed at a certain interval alongside the fence. The spatial arrangement of the fence can vary, and we can separate drift fence arrays into:

- **Straight-line drift fences:** These can be set up in X or Y-shaped arrays and are normally used for sampling upland habitat (Corn, 1994).
- **Continuous or partial drift fences:** This setting is commonly used to circle partially or completely wetlands (Dodd & Scott, 1994).

The capture rates and effectiveness of this technique may differ very much between sites, but it is clear that this technique is particularly useful for determining species richness and relative abundance (see Ryan *et al.*, 2002 for comparisons with coverboards and time-constrained visual surveys). The main limitations are as follows:

- Expensive and hard to set up. After installing, the traps should be visited at least once in a day.
- Capture biases. Some species may show trap avoidance or even attraction towards the pitfall traps.

- Many species such as large snakes or tree frogs can escape from the pitfall traps. This can be somewhat avoided with putting plastic collars on top of the pitfall traps or using double-pit systems.
- Species associated to certain microhabitats might not be sampled.

The best way to improve the success of drift fence arrays is to combine pitfall traps with funnel traps. This technique is normally used on long-term monitoring programmes due to the relatively high amount of time and funding needed to install them.

### 3. Capturing and handling animals

When sampling animals they should be handled in a way that allows further study (vouchering, photographing, marking, etc.). Handling is generally done by hand, but several tools and utensils can ease the task and increase the safety of both the sampler and the specimen.

#### 3.1. Snakes

Prior to identification, all snakes should be considered potentially venomous. When identified as venomous, **ONLY EXPERIENCED AND TRAINED PROFESSIONALS SHOULD EVER ATTEMPT TO CAPTURE AND HANDLE THEM.**

The most common tools used for capturing snakes are hooks and tongs that are used to immobilize the snake and keep it at a safe distance from the researcher. The usual procedure for manipulating a snake is using the hook or tong for lifting up the animal gently from the mid-front body while keeping hold of the snake's tail to avoid it from turning around.

For hand-catching snakes, we should set it in an open area and press its head gently, but firmly against the floor, using for example the bottom of the hook, so we can safely manipulate it. We can secure the head between the thumb and fingers of one hand, and use the other hand to sustain the rest of the body weight to make sure the snake does not suffer spinal injuries. Although giant snakes (boas and pythons) are not poisonous we should never underrate their strength. They should never be handled by only one person and special care must always be paid to their heads. These animals need to bite in order to strangle and their bites can easily infect due to the bacteria in their mouth.



**Fig. 2.** Handling a small snake. (Photo by author).

For smaller harmless and fast moving colubrids, hooks and tongs might not be appropriate and collecting directly by hand with thick protection gloves is recommended.

Hooks can easily be handcrafted, but tongs are more difficult to manufacture and are normally purchased from supply companies. Currently both hooks and tongs from different brands are readily available through the Internet. They should be made of a light but resistant materials such as anodized aluminium or titanium. The size of the hook and tongs will depend on the size of snake we target. The handles of both tongs and hooks should be made of a material that will not slip during the manipulations, such as rubber. Finally the material that will be in contact with the snake should minimize the chance of injuring the snake while manipulating it (e.g., rubber coated).

### **3.2. Lizards**

Lizards on average can be quite difficult to capture by hand due to their size and fast movements, so to assist on their capture we can use a small noose. The noose can be built with a long, slender pole such as a bamboo stick or a telescopic fishing pole where a thread of dental floss or fishing line can be attached. It is common lizard behaviour to flee upon sensing something approaching and then freeze shortly, and it is then when the noose can be placed over the head to trap the animal from a certain distance (see Marcellini & Jenssen, 1991). In the case of large lizards, caution must be taken when handling as they can cause injuries with their claws, and deliver powerful bites that can easily become infected. It is recommended to manipulate these animals wearing heavy-duty gloves to prevent any possible wounds. It is very important to avoid capturing lizards by the tail as it will break off in many occasions.

### **3.3. Aquatic turtles and tortoises**

Aquatic turtles can sometime be captured by hand and with the aid of a dip net, although the usual way of capturing turtles is using traps (see survey methods). In the case of turtles or tortoises they should always be handled with care as they can deliver powerful bites, but this is easily avoided by keeping your hands away from their head. Normally turtles can easily be held at mid- or back-body, although additional attention should also be paid for some species' claws that can be elongated and inflict deep wounds. As with large lizards the use of thick gloves to manipulate the animals is also recommended.



**Fig. 3.** Handling turtles. (Photo by author).

### **3.4. Crocodylians**

Due to their size and dangerous bites, crocodylians should exclusively be handled by experts. Normally their capture is done by several people and with the aid of a noose. While small and young animals can be grabbed from behind the head with one hand, using the other hand to support their weight (as you would do with a large lizard), larger animals have to be handled by several people. It is important to make sure that the jaws are closed, for example by wrapping duct tape around them, before doing any measuring, and extreme caution must be paid to the tail which can deliver powerful strokes. It is highly advisable to cover the animals' eyes to reduce their stress.





**Fig. 4.** Handling large animals. A. Small crocodilian; B. Large lizard. (Photos by author).

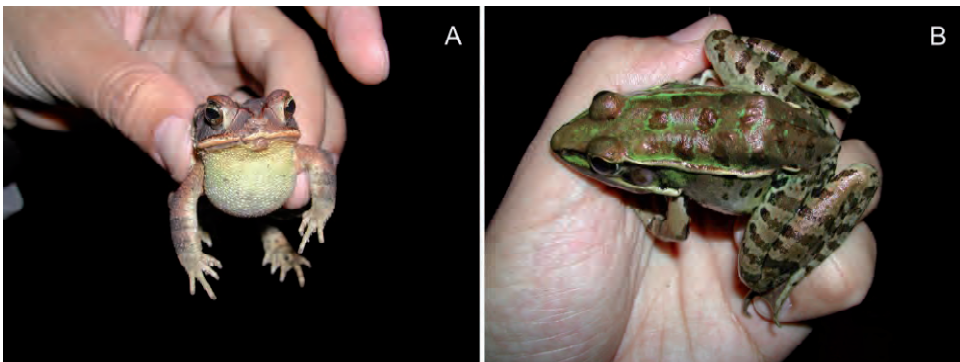
### **3.5. Amphibians (frogs, toads, newts, salamanders and caecilians)**

Aquatic amphibians can be captured by hand and with the aid of a dip net before they jump into the water or while floating in shallow waters. Most amphibians are nocturnal, so a flashlight can also be used to temporarily blind them and get close enough to them. In the case of terrestrial amphibians the challenge is locating them, as on average capturing them by hand is not difficult. Nevertheless we should have in mind that all amphibians have some degree of toxicity in their skins. Cutaneous glands are a shared character of all adult amphibians and they are normally the main source of biological active compounds found in the amphibians skin. The level of toxicity depends on the exact components of these substances and can range from noxious to highly toxic depending on the animals. The highest toxicity is due to the presence of alkaloids that in most cases derive from the arthropods the animals eat in the wild. Alkaloids have been found in some salamanders, but especially in Dendrobatidae and Mantellidae (Daly, 1998). The secretion of these compounds will be increased when the animals are stressed due to handling so the use of latex gloves or an inside out Ziploc bag is recommended to avoid direct contact with the skin. If none of these are available, and we must necessarily have direct contact with the animal, hands should always be thoroughly washed after manipulating them, making sure we avoid contact with our eyes or mouth. In the same way, any surface that has been in contact with the animals should be thoroughly rinsed and cleaned with water.



**Fig. 5.** Stressed frog. (Photo by author).

For safely handling frogs and toads, they should be held between the fingers and thumbs around the waist of the animal. For some specific measurements or for photographing the frogs should be grabbed from one of the front legs between the thumb and index finger while sitting on top of the hand. The grab should be firm enough to avoid the animal from escaping using their strong back legs, but with much care to avoid any damage to the front limbs. In the case of salamanders and newts, we should hold them in the entire hand gently restraining the animal between the thumbs and fingers just behind the head, in a similar way as it is done with medium and small-sized lizards.



**Fig. 6.** Handling frogs. A. Holding the animal safely; B. Handling position for measurements. (Photos by author).

Finally, it is important to consider that when handling different amphibian specimens in the field, a researcher can involuntarily become a vector for transmitting pathogens such as chytrid fungi. The chytrid fungus *Batrachochytridium dendrobatidis* is behind the disappearance of entire populations of amphibians around the world, so if you are going to handle amphibians in the wild, there are a number of rules you should strictly respect to avoid the transmission of chytrid fungi between populations or sites:

- Never move individuals of adult amphibians, larvae or egg between distinct places even if they are very close since this could contribute to the dispersion of the pathogens;
- Never introduce animals, plants or any other organism in the environment, because, besides interfering with native species, they may carry pathogens. We know that fish can transmit viruses that affect amphibians, and in many countries the native amphibians are infected by introduced amphibian species that carry the chytrid fungus. If you detect introduced (allochthonous) organisms in your area, get in contact with an expert;
- Avoid accidentally transporting the pathogens yourself. The chytrid fungus does not have a stage that is resistant to desiccation but it can survive in whatever type of organic material that maintains humidity. As such, after a trip to the field wash well at the site all the objects that have been in touch with the environment (e.g., boots and sample nets). After submerging them in bleach (a bath of 30 seconds is sufficient if you use domestic bleach with at least 4% sodium hypochlorite) or in other suitable disinfectants put them out in the sun for as long as possible;
- If you do not want to use bleach to clean your field material, you can use commercial products specifically sold in veterinary stores. Some suitable commercial products are: Halamid® ([www.alpharmaanimalhealth.co.uk](http://www.alpharmaanimalhealth.co.uk)) and Virkon® ([www.antechh.com](http://www.antechh.com));
- If you hold amphibians use disposable gloves or if it is necessary to keep them for some time use disposable containers or ones that have been previously sterilised. Do not put them in touch with specimens from other areas if you are going to return them to the natural environment. Remember that you must sterilise all equipment before using it;
- Inform when possible about the problem of emerging diseases in amphibians and how it is possible to avoid contributing to its spread.

#### **4. Transporting and housing captured animals**

If the captured animals must be transported to the lab and housed for some time it is necessary to use appropriate containers.

In the case of amphibians it is most important to keep them in moist substrate in containers or sealed plastic bags. It is a good practice to include some leaves or leafy branches to prevent squashing and maintain humidity. A moist paper towel or standing water in the container usually is effective depending on the needs of the species in question. For tadpoles, plastic containers filled in with water from the capture site can be used, and these containers should be transported in lightly chilled coolers to keep the tadpoles with a relatively low metabolic rate.

Small containers with ventilation are useful for holding small snakes, small turtles, and most lizards. Cloth bags of all sizes, including pillow-cases, are useful for temporarily holding even the largest lizards, turtles, snakes, and small crocodylians. One must be careful not to allow the animals to suffocate or drown while transporting them, and avoid placing them in direct sunlight where any container can rapidly overheat and the animals inside die.

Once in the lab, the setting prepared for short term housing the animals can be very simple. One must make sure that the temperature is suitable for the animals, that natural photoperiods are respected and that the containers are clean and have sufficient water and food.

## **5. Collecting information from captured animals**

### **5.1. Measurements**

All amphibians, squamata reptiles (lizards and snakes) and crocodylians the standardized measure used is the snout-vent length (SVL) that is defined as the distance between the tip of the head and the end of the cloaca. In addition, the tail length can also be recorded to have the total length of the animal, but salamanders and squamata reptiles have the ability to loose their tails as a defensive mechanism upon being attacked by a predator.

Together with the measurements of the body length, the typical measurement is weight. Most herpetofauna can be weighted with either a spring scale or an electronic scale, but for larger species (giant snakes, crocodylians, large turtles) a truck scale will be necessary.

Due to the ectotherm nature of reptiles and amphibians, in many occasions, it will also be of interest to obtain the cloacal temperature of the animals. Ambient temperature can be used as an approximation if it is not possible to measure body temperature, but it must be remembered that there can be significant differences between both measurements due to fluctuations that the animals metabolism can produce in their body temperature. The body temperature of the animals will affect their activity, so this information can be relevant for comparing between sampling periods in a monitoring activity. For measuring the body temperature we can use cloacal thermometers or digital thermometers with a probe. Take into consideration that, especially for smaller specimens, contact with our hands will affect their body temperature, so the measuring of temperature should be done immediately upon capturing the animal.

After collecting the animals it can sometimes be necessary to preserve them as vouchers. The preservation of specimens is a key element for taxonomic identification and when accompanied by properly compiled field notes, it becomes an excellent resource for scientific research in many branches of biology. For example, historical data from museum specimens can allow researchers to detect and assess changes in biodiversity in an area over time. For the preparation of vouchers it will be necessary to kill the animals, although in some cases it is possible to use animals that are already dead due to traps or road mortality. We should collect the minimum number of specimens possible

depending on the aims of our study. Although it can depend on how common the animal in question is, it would be advisable to preserve around 20-30 animals for scientific studies and a minimum of 4 for voucher specimens (Graeter *et al.*, 2008). It is mandatory to follow any institutional guidelines that may apply or to request the necessary permits. The procedure to euthanize the sampled animals should be humane and should preserve the condition of the animal. The most preferred techniques for killing reptiles and amphibians are by injecting or submerging the animals in lethal doses of one of the following:

- Sodium pentobarbital
- Hydrous chlorobutanol
- Tricaine methenesulfonate
- Cloretone
- Ethanol
- Other anesthetics

In the case of amphibians, due to their permeable skin, immersion in anaesthetic solutions is the most frequent way of humanely killing them. The most common products used are chlorobutanol and tricaine methanesulfonate, also called MS-222 (Andreone *et al.*, 2008). The minimum concentration should be 250 mg/l (concentrations >500 mg/l must be buffered with an equal weight of sodium bicarbonate as it is an acidic product).

In the case of reptiles, sodium pentobarbital has traditionally been used injected intravenously, intra-abdominally or intrapleuropitoneally (Cooper *et al.*, 1989), but recently the use of MS-222 has also been recommended through intracoleomic injections of 250 to 500 mg/kg at 1% solution (Conroy *et al.*, 2009).

The fixation of the specimens should only begin once we are sure that the animals are dead. As chemical fixation affects the proteins in the tissue of the animals, we should attempt to fix them in positions that preserve their morphology and that allows for the observation of key identification characters. The fixation in 10% formalin (obtained by diluting 40% formol) allows a better preservation of morphology so it is ideal for the animals that will be used for formal taxonomic description or for exhibit. Formalin is carcinogenic, flammable and dangerous if fumes are inhaled, so the appropriate cautions must be taken when working with it. In addition it will not allow to use the specimens for posterior DNA analysis so it is advisable to collect tissue samples before fixing the entire specimen, and fix these in pure ethanol. In case formalin is not available, 70% ethyl alcohol can be used, but other alcohols are not recommended (McDiarmid, 1994).

Once the specimen is fixed, it is extremely important to attach each specimen with data such as the field number and any information recorded from the field (GPS coordinates, time, habitat, initial identification, collector, sampling method or weather conditions). It is advisable to use acronyms in the field number referring to the collector, followed by a progressive number and keep the same structure within sampling efforts. This should be printed in hard paper resistant to ethanol and formalin; either hand-written or printed with water resistant ink as there is a risk of losing the information during transport or long-term storage.

Dependent of the aim of the study it could only be necessary to take a tissue sample or biopsy from the captured animals instead of preserving the whole specimen.

Blood samples are the most common procedures as when it is correctly done it may be less invasive than taking other tissues. In the case of DNA analysis rather small amounts of blood will be necessary, although the amount will be larger for physiological studies. Turtle blood can be obtained from a femoral or jugular vein, a carotid artery, the retrorbital space or the paired cervical sinuses (Dessauer, 1970). In medium and large sized lizards blood is typically collected from orbital sinuses (*e.g.*, Haenel *et al.*, 2003), and in crocodylians blood is normally taken from internal jugular or caudal veins. In the case of amphibians, only the larger species can endure blood sampling and this can be done through the midline abdominal vein. Finally for most relatively large reptiles and amphibians heart puncture can also be a viable way to extract blood although this can cause mortality if done by inexperienced workers. In the case of smaller animals, heart puncture will be the only way to take blood samples and will necessarily be fatal. The blood samples can be collected through heparinized capillary tubes.

Alternative tissue samples that can be collected in reptiles and amphibians are tail clips from salamanders, lizards, turtles or snakes. Toe clips may be used as well in salamanders, frogs and lizards, while clipping scutes of the tail of crocodylians and ventral scales from snakes are also common practice. These sampling techniques have the additional benefit of potentially being very useful to researchers who need to mark animals for individual identification.

Finally for DNA studies there is the possibility of using other non traditional sources of tissue which are not aggressive but can later prove difficult to analyze due to the low molecular weight and concentration of DNA in the samples. The most relevant of these sources in amphibians and reptiles are feces, although orifice swabs and shell or scale remnants can also be useful (Poschadel & Moller, 2004). For methods to better preserve tissues for future DNA analyses, we refer to the chapter of Gemeinholzer *et al.* (this volume) on organizing specimen and tissue preservation techniques in the field for subsequent molecular analyses

## **5.2. Photo-vouchering**

Photo-vouchering entails using photographs to document the occurrence of encountered wildlife. This is particularly useful in herpetology as it is very possible to make photographs of the animals accenting the key features that allow for a doubtless identification. These photo-vouchers, if correctly complemented with additional information will provide long-term evidence that those species exist or existed in a given geographical location. A literature record complemented with a photograph will make the report reliable without the shadow of a doubt. In addition photo-vouchers can be the alternative to traditional vouchers in the case of rare, threatened and endangered species or the alternative to the records of animals difficult to capture, such as basking water turtles. In the cases when the preparation of vouchers specimens is

absolutely unavoidable, photographs of the living animal will also be of much help as after fixation specimens tend to lose their colours and even some patterns. The ideal situation of documenting the occurrence of a certain species is having the voucher specimen for detailed analysis complemented with photographs of the specimen before fixating.

Currently the use of digital cameras has made photographing cheaper. It is possible to quickly review the photographs taken and make as many pictures as necessary, although we should remember that digital files can also become corrupted and the information lost. Some recommendations for preparing photo-vouchers are:

- If the photographs are going to eventually be deposited in a natural history museum or other repositories we should obtain information on the format, size and resolution needed;
- Include some kind of scale in the photograph to have information on the size of the animal photographed;
- Make the photographs of the animals as soon as possible after capturing, as especially some amphibians tend to change colours and patterns after being captured;
- If the animals are very active, it can be useful to lightly chill them in a refrigerator, but never in the freezer. The amount of time should never be over a few minutes depending on the size, and if the animals are later going to be released back to the wild, first make sure that it has returned to normal temperature before doing so.

## **6. Field notes and data collection**

Most serious shortfalls in gathering and managing descriptive data on amphibians and reptiles can be avoided through planning and preparation prior to collecting data. The list below compiled by Greene (2008) includes common issues and problems that need to be addressed when implementing an inventory or monitoring program:

- Research and study goals and the specific data to be gathered must be clear to all parties involved (*e.g.*, funding agency representatives, researchers, and technicians);
- Data must be gathered in an organized, consistent manner. Design a datasheet that is objective and simple to use, and which includes all relevant information in sufficient detail. If funds and expertise allow it, invest in personal digital assistants (PDAs) or electronic laboratory notebooks which can be programmed with customized forms for direct data entry in the field (this can help minimize data entry and data transfer errors);
- All personnel involved must be trained to gather data in the same manner. Attention to detail and consistency are paramount. Handwriting must be legible;
- Store data routinely in one place until the data can be entered into a database. Keep electronic backups or photocopies of the originals in a different secure location. More than one person should be familiar with the procedure and storage locations;

- Consider how the data will be used and then enter the data into an appropriately designed database. A spreadsheet such as Microsoft-Excel is adequate for many straightforward datasets. Microsoft-Access may be a better option if the data are a subset of a bigger relational database. Copy the data on a weekly basis at minimum to a portable storage medium and keep the files in a separate location;
- Review the data and the data management system early in the process and then periodically on a regular basis. This will allow early detection of errors and inconsistencies, which can be identified and corrected before valuable information is lost;
- One competent, detail-oriented person should oversee the entire process from data collection to data entry to data storage.

For some examples of datasheets that can be used during inventories and monitoring, I refer to Graeter (2008).

## 7. Acknowledgements

I would like to thank Franco Andreone and José Pedro Sousa do Amaral who reviewed parts of the text. Andreas Schlüter kindly read the whole manuscript and gave comments and suggestions to improve it. I am also in debt to an anonymous referee that helped to greatly improve the final structure and contents of the chapter. Finally I would like to thank Abc Taxa editor Yves Samyn and Jutta Eymann for their enormous help throughout the editorial process.

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