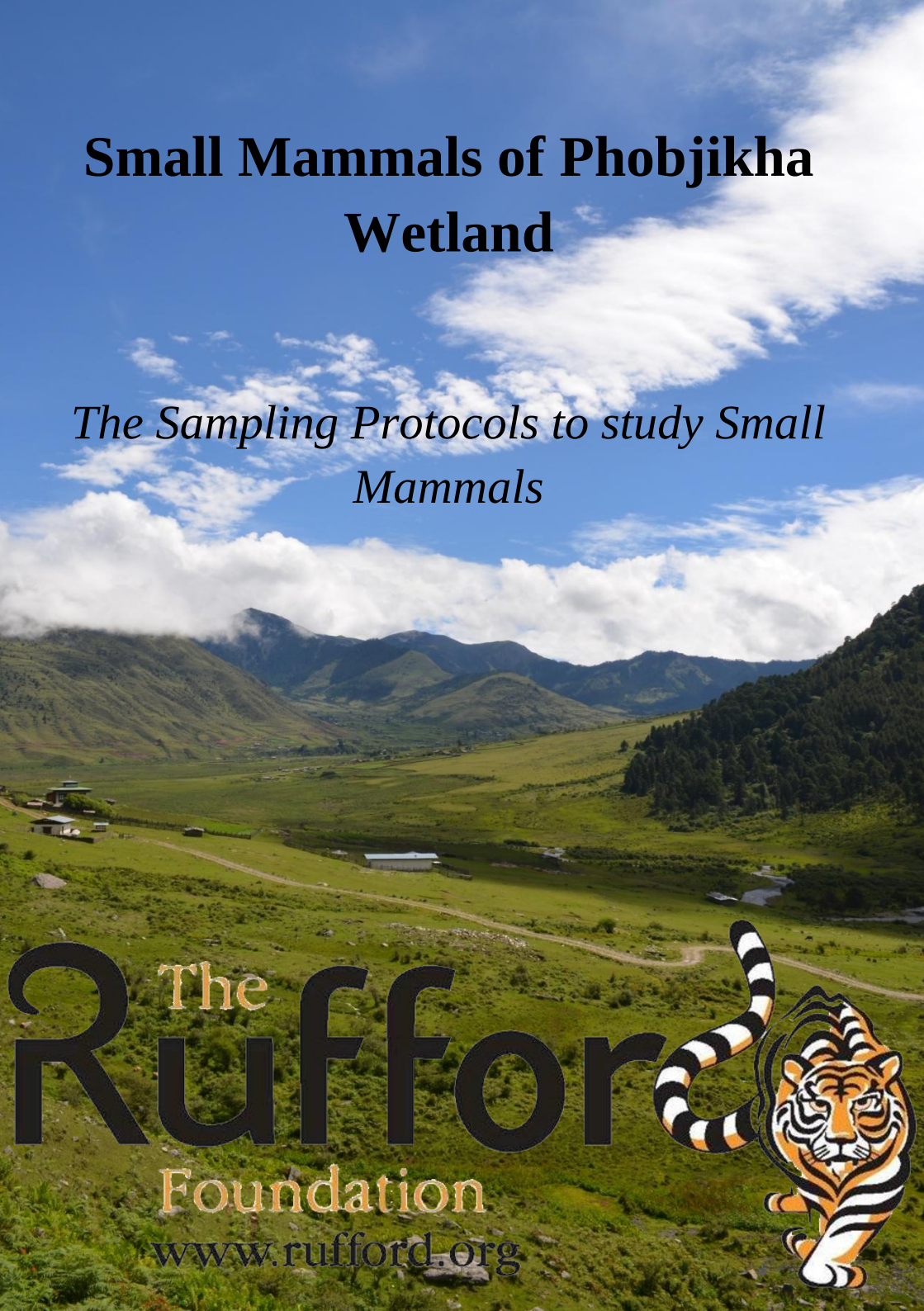
Small Mammals of Phobjikha Wetland

The Sampling Protocols to study Small Mammals



ACKNOWLEDGEMENT

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Background

Within the mammalogy, small mammals have highest diversity comprising of 3821 species of order Rodentia ,Chiroptera and Soricomorpha (Anke *et al.*,2010). Srinivasulu *et al*.,(2012) recorded 332 species of small mammals belonging to the orders Rodentia (rodents), Chiroptera (bats), Soricomorpha(shrews), Erinaceomorpha (hedgehogs), Lagomorpha (pikas and hares), Scandentia (tree shrews), and Pholidota (pangolins) in South Asia. Currently India has 103 species and 89 subspecies of small mammals under 46 genera and it represents 66 % of Indian mammals records (Nameer *et al.*, 2001).

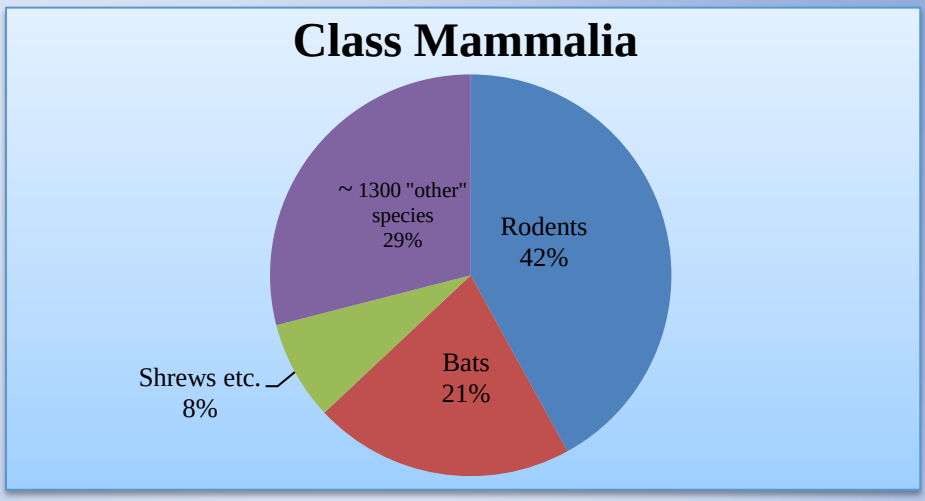
Small mammals refers to species which weighs less than 500 g, the upper size limit that can easily be caught in commercially produced live traps (Anke *et al.*, 2010). Small mammals are terrestrial and volant in nature and smaller than the largest rodents or lagomorphs. Shrews, moles, mice, voles, gophers and ground squirrels are some of the small mammals (Barnett & Dutton, 1995). Taylor *et al.*, (2007) in their studies in South Africa reserved the term "small mammals" for mammals weighing less than some arbitrary threshold (e.g. 2 kg or 5 kg) and limited to rodents, shrews and bats. Despite few studies with scanty information on small mammals in Bhutan (RGoB, 2009), Bhutan has a record of 44 species of small mammals, constituting 20 percent of mammals record of the country (Wangchuk, 2009). Small mammals have significant influences on vegetation and soils, exert predatory pressure on other animals and provide food for predators (Sieg, 1987).

Small mammals have ability to indicate the health and state of wetlands due to their rapid turnover rate, high biotic potential, ability to invade reclaimed areas and sensitivity to environmental disturbance (Griffen et al., 2010). Wetland functions as a habitat for animals as well as people (IWWR, 2010). Many species of mammals depend on wetland for food resources and their survival (Institute, 2004). Wetlands supports high small mammals communities and represent area of considerable conservation importance (Erasmus, 1992). However, across the globe, high altitude wetlands are facing an array of threats, including the rapid melting of glaciers due to climate change, overgrazing of livestock, water diversions for agricultural and human use, and increased pollution.

Definitions

Small mammals are animal which weighs less than 500 g and are terrestrial and arboreal in nature (Anke *et al.,* 2010). Small mammals have highest diversity comprising of 3821 species (ibid). Wetlands support high small mammal communities and represent area of considerable conservation importance (Erasmus, 1992).

Small' mammals are <500 g and include shrews, moles, most rats, lemmings, gerbils, and many squirrels and ‘medium-sized' mammals are those >500 g and smaller than or similar in size to a fox. Almost 3 of every 4 mammals are 'small' mammals, e.g., rodents, bats, shrews, etc.



Sampling Design (Applicable for small mammal survey in other areas)

Habitat Stratification

Phobjikha Conservation Area is defined by seven major land use: forest, agriculture, scrub, marsh, water bodies, pasture and settlements(RSPN, 2010). Habitat was classified into four types based on vegetation and land use types. Habitat stratification was necessary to compare species diversity and relative abundance of small mammals among different habitat types. The ArcGIS 9.3 was used to stratify the habitat of PCA using land use 2010 into four types viz. Forest, open grass (pasture,shrubs and meadows), agricultural land (agriculture land and settlements) and riparian(water bodies, marsh) as shown in figure 3.1. Of these, forest covered 63% of habitat, open grass and shrubs is 28%, agriculture is 6% and riparian makes nearly 3% of total habitats. The marshy area and open shrub with water were excluded from the sampling.

Table .1. Area of land under major habitat types

Habitat Types	ea (km²)
Forest	101.67
Open Grass Land	45.4
Agricultural Land	9.44
Riparian	5.49
Total	**162**

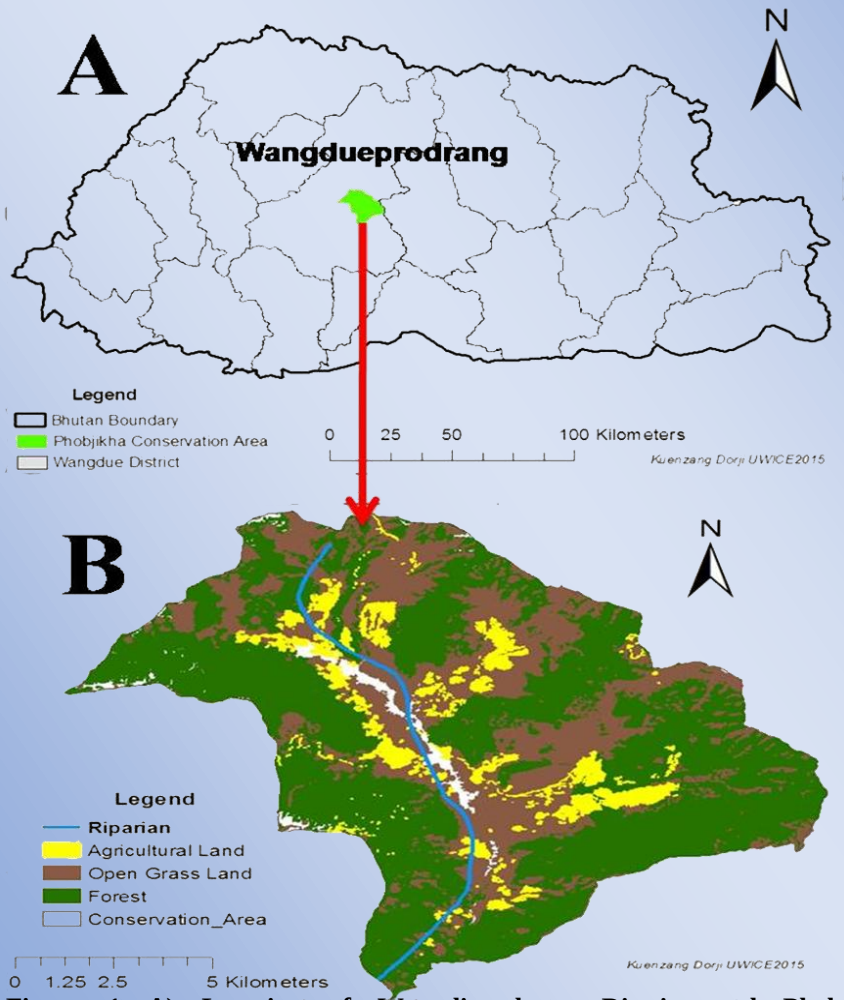


Figure 1 A) Location of Wangdiprodrang District and Phobjikha Conservation Area. B) Four habitat types of study site (PCA)

Sampling Procedure

To characterize abundance or species richness across an entire area of multiple habitat types, stratified sampling to place random trapping grids or transect was used (UWICE, 2011). A stratified random sampling method was adopted for the study because the sampling units are heterogeneous. Transects has advantage over grids or webs because transects have been shown to yield greater numbers of captures, individuals captured, and species captured(Fricke *et al.*, 2009). Transects also sample a greater area (Pearson & Ruggiero 2003).

The starting point of four transects in forest was generated by using Geographic Information System (ArcGIS 9.3) and the point was navigated by Global Positioning System (Garmin GPS eTrex Vista HCX) to avoid unbiased estimate of population parameters. Subsequent transects in open land and agriculture are located following random direction (0°-360°) and laid at 500 meters from each habitat type to eased the logistics mainly the monitoring of traps.

For the riparian ecosystem, start point for two transects along the Nake Chu River and one random start point along two main streams (Lolephage Chu and Gau Chu) was generated using Geographic Information System (GIS). Transect were placed within 30 meters following the course of the river and streams.

Live trapping

Preferably the transect line can be straight or meandering (Environment, 1998) and the spacing among traps usually ranges from 10-25 meters (Gray *et al.*,1996). Straight transect were laid in terrestrial habitats and meandering transect for the riparian habitat. Sherman live trap is used as it captures the most animals and appeared to be most effective traps for small sized mammal (Hoffmann *et al* 2010). Four 300 meters length transects in four different habitats (forest, agriculture land, open grass and riparian) were laid. 20 sherman live traps of size 23 x 9 x 8 cm was set on the ground at 15 meters interval to capture mouse/rat/squirrel sized animals. Colored ribbon (red, orange, pink and blue) were tied on twigs and branches of shrubs for ease locating. The traps were baited with mixture of oats, apple, peanut butter and puffed rice.

As adopted by Borchert, (2014) to study response of small mammals to wild fire in Mixed Conifer Forest, traps were placed under vegetative cover or close proximity to rocks, tree bases or coarse woody debris. The traps were covered with leaves or litter for camouflage (Mulungu *et al.*,2008) and cotton batting was placed at back of each trap for insulation (Borchert, 2014). During this survey, pine needles, *Pteridum sp*, dry grasses, old and torn clothes were used to cover the traps and cotton was also placed to provide insulation.

The Ministry of Environment (1998) had suggested using pitfall traps at minimum of 60 meters spacing to capture smaller species like shrews. Similarly, six pitfall traps were deployed at every 60 meters to capture shrews. A mixture of flour and canned fish was baited with slices carrots for moisture and to increase animal survival. As recommended by (Jones *et al*, 1996) cleaning of the traps after trapping session is essential to increase the consistency in trapping success. The traps were soaked in water for more than 12 hours and cleaned before start of live trapping in other new location.

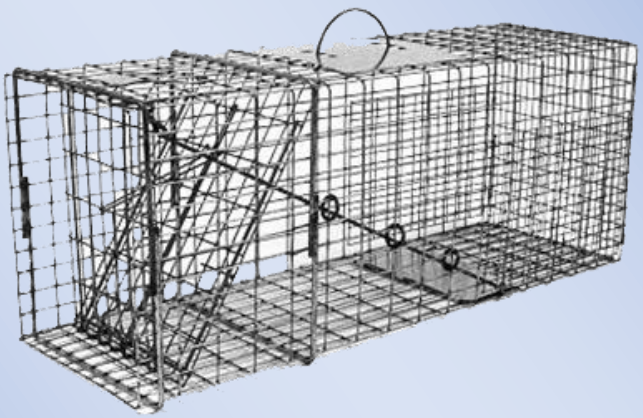
Live trap methods (Foresman, 2009)

Sherman® live traps -

The collapsible model is the most convenient to use since a large number can be carried, collapsed, in to the field. These traps are primarily used to capture small species such as mice, voles, chipmunks, and smaller tree squirrel species. For these species the most common bait would be a small handful of grain such as oats.

Tomahawk® live traps –

These traps are larger than Sherman traps (they actually come in many sizes) and are commonly used for species of marten, red pandas, badgers, civets, mongoose, flying squirrels etc.; Often if larger-sized animals are captured it may be necessary to administer anesthetics via injection before they can be handled.



Pitfall traps -

These traps are used for the smallest species, particularly shrews. Small cans (~ 6 inches in diameter and 6.5-10 inches in height) are buried flush with the ground. A small amount of bait can be placed in the can to feed the animal before it is checked. The animal is so small that it falls in to the can and cannot jump out. Then animal can be simply transferred from the can to a handling bag for study. Often a "drift fence" can be built next to cans to encourage the animal to move to the can. The walls of the drift fences are constructed out of plastic sheeting stapled to wooden stakes forming a wall that the shrew bumps in to and then runs down to the can. One pitfall design is to have one can in the middle and three drift fences radiating out with cans at the end of each. Animals can be coming from any direction and will be funneled in to a can. This is actually a very good trap and design for small amphibians and reptiles.

Mist nets and Harp traps for bats –

Bats can be trapped using either mist nets as is commonly done with birds or "Harp" traps as shown here. The harp traps are made up of two sets of monofilament lines running vertically spaced approximately 1 inch apart (b). A collection bag is placed below the lines (c). The principle is that flying bats are able to turn sideways and fly through the first set of lines but they do not have enough time to adjust their flight to miss the second set of lines that is offset from the first by ½ inch. They collide with the second set of lines and fall in to the bag where they will stay until released (a). The collection bag has a plastic flap inside which allows the bats to climb up under it protecting them from rain. A large number of bats can be captured in this manner if the trap is placed at a cave entrance. Bats can be picked out of the bag and handled directly for identification and measurements. For the smaller species light weight cotton gloves should be worn; heavier gloves should be worn when handling larger species. Bats are known to carry rabies so they should only be handled if you have taken the prophylactic rabies vaccination series. An excellent reference source for conducting bat research is: **"Ecological and Behavioral Methods for the Study of Bats."** (2009) by Tom Kunz and Stuart Parsons.

General Guidelines for transect layout

- Place transects in the habitat types of interest. When comparing habitats, best to have equal survey effort in each habitat type.
- A typical transect length is ~100 to 150 m
- Traps should be equally spaced (usually 10-20 m apart) along the transect
- At each trap station, place the trap at locations most likely to capture small mammals (but near the station point)
- Use multiple trap types per transect
- Replicate transects at a site should be a specified distance (e.g., 100 m) apart

Setting up of Sherman Traps

1. Check the trap for any holes—discard if the trap has been chewed through. Make sure the trap works properly.
2. Find a favorable location for the trap within 2 m of a trap station
    - along downed logs, near trees, rocks, bushes, or woody debris
    - along a small mammal runway or near small burrow holes
3. Place bait (apple, fishball, peanut butter pack) and bedding material at the back of the trap, away from the treadle. Place a tiny bit of bait just outside trap entrance as well.
4. Place the trap flat on the ground so it is stable (you may need to clear some ground by hand).

5. Clear obstructions away from the trap entrance

6. Place a coverboard, tree branch or other woody debris over the trap for extra protection against weather and predator detection

7. Just before leaving the trap, check treadle sensitivity to make sure the trap door closes with a small tap on the trap

8. Place flagging near the trap (with station # written) for easy finding

Setting up of pitfall traps-*for shrews & other mammals < 10g, and herps*

1. Make sure the pitfall trap has drainage holes so water doesn't accumulate if it rains

2. Find a favorable location for the trap within 2 m of a trap station

3. Dig a hole at the trap location. Place some rocks (for water drainage) in the bottom of the hole. Then place the pitfall trap in the hole so the rim is flush with the surrounding ground.

4. Place bait, bedding, some plants and small woody debris (for shelter) in the bottom of the trap. Don't put so much that an animal can climb out.

5. Set up drift fences (5-m long in each of 3 directions) to direct small mammals to the pitfall trap

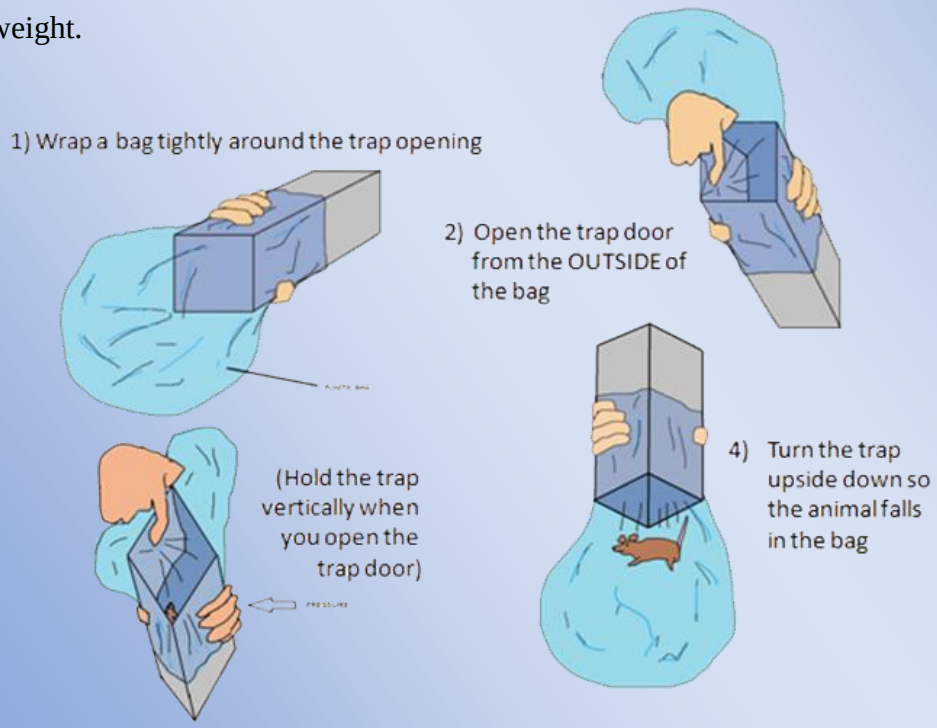
6. Place flagging near the trap (with station # written) for easy finding

General Guideline for trapping

- Pre-baiting traps can increase trap success
- Trapping at a site should run 3-5 nights (not more, due to trap stress/deaths)
- Traps should be placed near habitat features (e.g., downed logs, trees, rocks, bushes, small mammal runways or burrow holes) but within 2 m of a trap station
- If traps become saturated (>80% of traps capture animals in a night), then place 2 traps per station to increase likelihood of capturing rarer species
- If transects cross near water bodies, make sure some traps are placed near the water
- If shrews are common at the site, checking traps every several hrs will minimize shrew mortalities
- Conduct daytime trapping at least 2 days per site, to check for diurnal species
- At least 400 ‘trap-nights’ is recommended for preliminary inventory of a habitat

General Handling Procedures

Small mammals can be easily handled without anesthesia though if tissue samples are to be taken anesthesia should be used. Larger species (and most carnivores) are commonly anesthetized. The animal is transferred from the trap to a handling bag by (a) opening the door of the trap through the bag, and (b) shaking the animal in to the bag. Once the animal is in the bag you can (c) grasp it through the bag on the nape of the neck and, while holding it securely, (d) evert the bag so that you can identify it. The animal can be weighed in the bag (e), using a Pesola spring scale and then by subtracting the weight of the bag you can obtain the animal's final weight.



Sexing small mammals: **[From Cait McKeown - http://www.fancymice.info/sexingcait.htm]**

Females (does) - Female mice are known as does. They have eight to twelve nipples that they use to feed their litter until it is fully weaned at around four weeks. These nipples can sometimes be seen and are a good way to identify female mice since males do not have nipples. However, nipples are usually most visible in young mice or in does that have had a litter, and cannot always be seen through the mouse's fur. If the nipples are not obvious then you must rely on checking the mouse's genitals alone to sex it. A female mouse will have a tear shaped vagina close to its anus, with little space in between the two.

- Look for nipples
- Check distance between anus and reproductive organ – if they are close together you probably have a female.

Males (bucks) - Male mice are called bucks. They do not have nipples, but just because you cannot see a mouse's nipples does not mean they are not there. If a buck is old enough to leave its mother (four weeks or older) then it will probably have fairly obvious testicles though many wild rodent species are seasonal so testes are drawn inside the body during the non-reproductive period. Mice have large testicles in comparison to their body – each testicle is the size of a peanut minus the shell. These testicles will 'drop' as the mouse matures into adolescence and can be seen as early as two and a half weeks in very early developers.

- Look for testicles
- Look for the scrotum
- Check the distance between the anus and reproductive organ – if the distance is quite large you are probably looking at a male.

Cream buck - note the distance between and the separation of the anus and genitals in males, whereas does' may appear to be 'connected' and closer together. 15

Below are additional pictures of 2 adult mice, one buck and one doe. You will notice that there is a larger gap between the anus and penile shaft of the buck than that between the anus and vagina of the doe. The distance between the anus and genitals is called the "anogenital distance." You will also notice that the female has a Vaginal Orifice, whereas the buck does not.

White-bellied Rat

Species : Niviventer niviventer

Status : Least Concern (Version 3.1)

Author : Hodgson, 1836

Morphometric Measurement

Weight : 38-40 grams

Tail Length : 15- 18 Centimeters

Body Length : 13-16 centimeters

Ear Length : 1.6 Centimeters

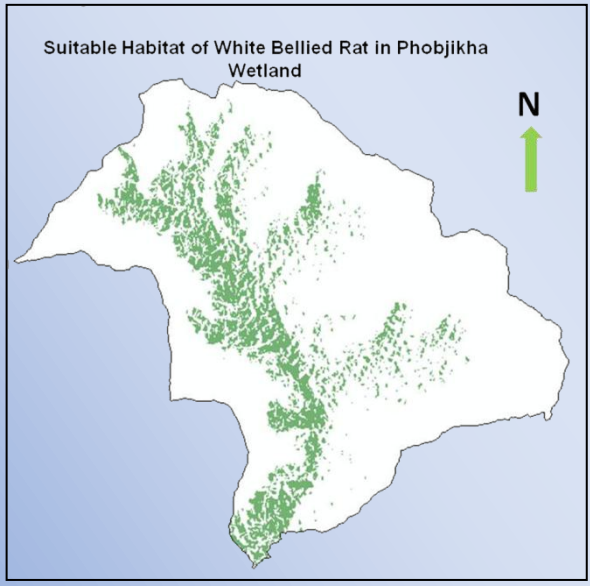
Hind Feet :2.78 Centimeters

The measurements are solely site specific. The measurement may differ from one site to other.

Habitat : Forests, Riparian, Grassland and Agricultural fields.

Threats : Road construction, Timber and Boulder Extraction, and Grazing

Distribution Map:



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Common House Rat

Species : Rattus Rattus

Status : Least Concern (Version 3.1)

Author : Linnaeus, 1758

Morphometric Measurement

Weight : 17 grams

Tail Length : 7 Centimeters

Body Length : 9 centimeters

Ear Length : 1.4 Centimeters

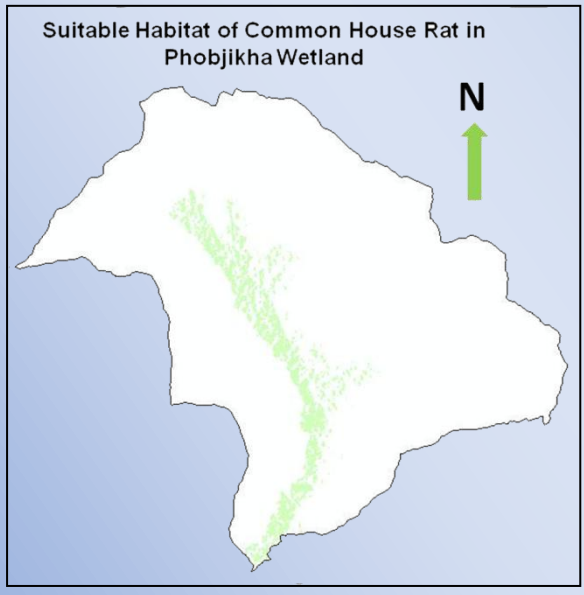
Hind Feet : 1.8 Centimeters

The measurements are solely site specific. The measurement may differ from one site to other.

Habitat : Forests, Riparian and Agricultural fields.

Threats : Road construction, Timber and Boulder Extraction, and Grazing

Distribution Map:



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Little Himalayan Rat

Species : Niviventer eha

Status : Least Concern (Version 3.1)

Author : Wroughton, 1916

Morphometric Measurement

Weight : 22 grams

Tail Length : 11 Centimeters

Body Length : 14.6 centimeters

Ear Length : 2.6 Centimeters

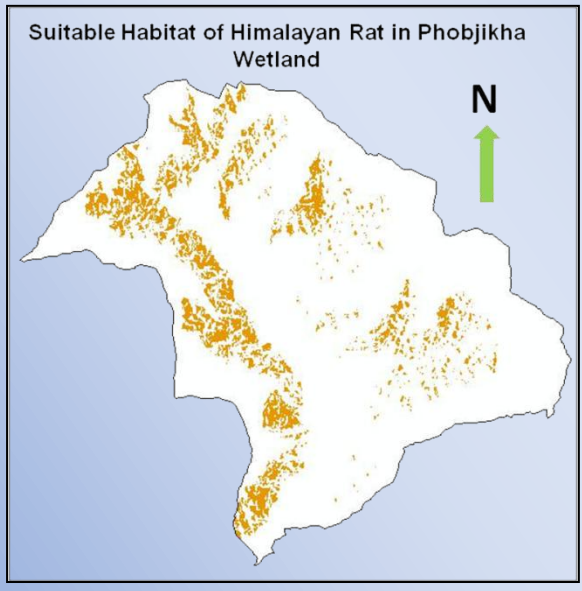
Hind Feet : 1.5 Centimeters

The measurements are solely site specific. The measurement may differ from one site to other.

Habitat : Forests

Threats : Road construction, Timber and Boulder Extraction, and Grazing

Distribution Map:



Large Eared Pika

Species : Ochotona macrotis

Status : Least Concern (Version 3.1)

Author : Günther, 1875

Morphometric Measurement

Weight : 50 grams

Tail Length : NA

Body Length : 19 centimeters

Ear Length : 2 Centimeters

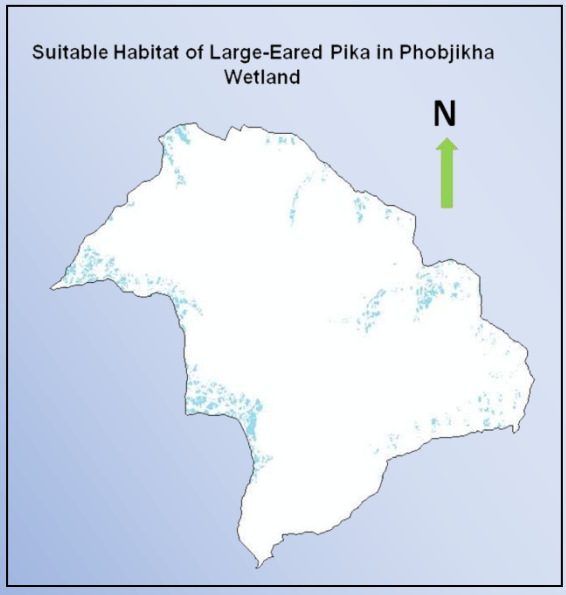
Hind Feet : 3.5 Centimeters

The measurements are solely site specific. The measurement may differ from one site to other.

Habitat : Forests

Threats : Road construction, Timber and Boulder Extraction, and Grazing

Distribution Map:



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Orange-Bellied Himalayan Squirrel

Species : Dremomys lokriah

Status : Least Concern (Version 3.1)

Author : Hodgson, 1836

Morphometric Measurement

Weight : 160 grams

Tail Length : 16 Centimeters

Body Length : 18 centimeters

Ear Length : 1.2 Centimeters

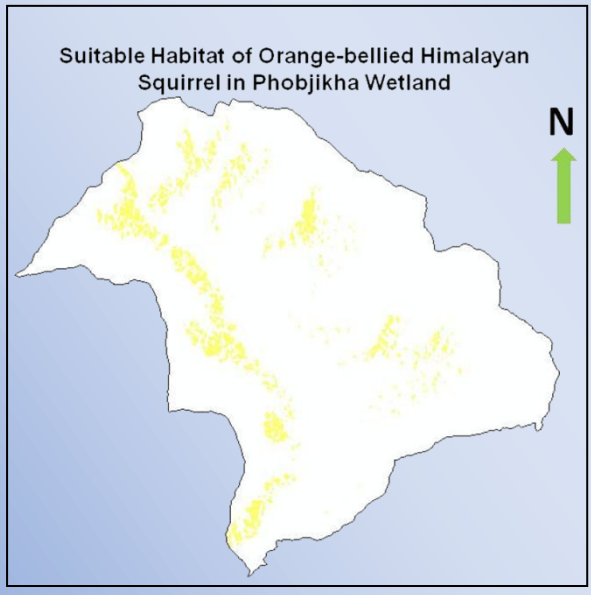
Hind Feet : 4 Centimeters

The measurements are solely site specific. The measurement may differ from one site to other.

Habitat : Forests

Threats : Road construction, Timber and Boulder Extraction, and Grazing

Distribution Map:



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Forest Rat

Species : ???

Status : Least Concern (Version 3.1)

Author : Hodgson, 1836

Morphometric Measurement

Weight : 160 grams

Tail Length : 16 Centimeters

Body Length : 18 centimeters

Ear Length : 1.2 Centimeters

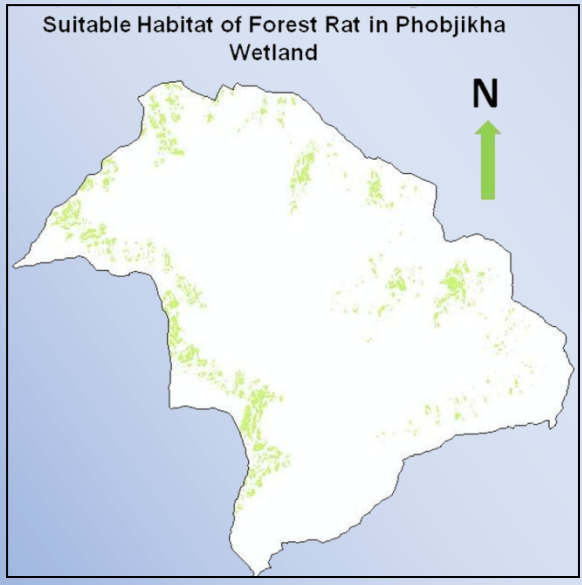
Hind Feet : 4 Centimeters

The measurements are solely site specific. The measurement may differ from one site to other.

Habitat : Forests

Threats : Road construction, Timber and Boulder Extraction, and Grazing

Distribution Map:



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Sikkim Mountain Vole

Species : Neodon sikimensis

Status : Least Concern (Version 3.1)

Author : Horsfield, 1841

Morphometric Measurement

Weight : 33 grams

Tail Length : 6 Centimeters

Body Length : 12 centimeters

Ear Length : 1.28 Centimeters

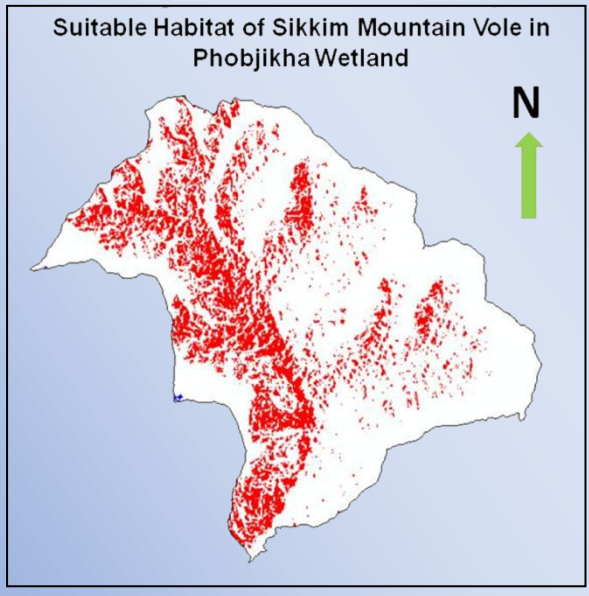
Hind Feet : 2.31 Centimeters

The measurements are solely site specific. The measurement may differ from one site to other.

Habitat : Forests, Riparian and Agricultural fields.

Threats : Road construction, Timber and Boulder Extraction, and Grazing

Distribution Map:



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Pygmy Shrew

Species : Sorex minutus

Status : Least Concern (Version 3.1)

Author : Linnaeus, 1758

Morphometric Measurement

Weight : 9 grams

Tail Length : 7 Centimeters

Body Length : 4.3 centimeters

Ear Length : 0.4 Centimeters

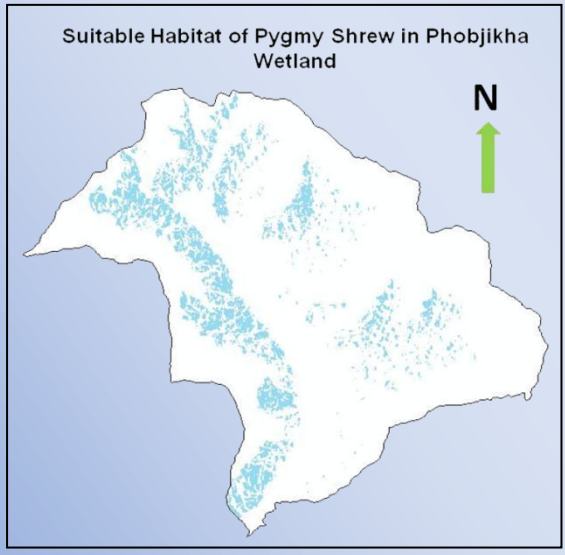
Hind Feet : 1.4 Centimeters

The measurements are solely site specific. The measurement may differ from one site to other.

Habitat : Forests, Riparian and Agricultural fields.

Threats : Road construction, Timber and Boulder Extraction, and Grazing

Distribution Map:



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Materials Used

Figure 4: A. Sherman traps; B. Pitfall traps; C. Ruler; D. Pesola Spring; E. GPS; F. Measuring tape; G. Ribbon; H. Compass; I. Datasheet; J. Gloves; K. Clinometer; L. Scoops; and M. Crowbar

Animal Baits

Figure 5: A. Oats, B. Peanut Butter, C. Can fish, D. Apple, E. Carrot, F. Biscuits and G. Flour

Safety Equipments

Figure 6: A. Hand sanitizer, B. Gloves, C. Soap, and D. Mask

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Annexure I: **Common trapping supplies:**

1- Traps- Sherman, Tomahawk, cans for pitfall traps

2- Hair snares

3- Bait – grain for herbivorous species, meat (e.g., sardines) for carnivorous species

4- Scales – Persola 10g, 50g, 100g, 300g (3-4 sets)

5- Handling bags (3-4); flagging

6- Isoflurane – (see data sheet on drug and its usage)

7- Data sheets; 3-ringed binders; clipboards

8- Vials for tissue samples; ectoparasites

9- 95% EtOH

10- Boxes for organizing samples

11- FTA Elute blotting cards and Nobuto strips for blood samples (see additional information)

12- Ear punch for collecting tissue for DNA analyses

13- Small coin envelopes for hair samples and Nobuto strips

14- Museum specimen instruments (Scissors, scalpels, blades, forceps, thread (black,white, brown), needles, cotton, wire of different sizes, pliers, syringes, styrofoam pin boards, pins, booklet); Small toolkit to store instruments;

15- Rulers – 6”, 12”, small tape measure, Vernier calipers

16- Bottles for skulls

17- Mammal keys (India, China)

18- Handheld GPS units; Maps

19- Reconyx cameras

20- Write-in-Rain notebooks

21- Cameras for specimen and habitat photos

22- Date and Site Board to include in photos – make out of heavy clear plastic laminated white card; erasable marker pens

23- Heavy duty zip lock bags; small and large

24- Labels

25- Film canisters or cardboard tubes to make isoflurane containers

26- Antibacterial hand soap

27- Disposable gloves used when collecting blood

28- Handling gloves: lightweight cotton, leather

(List Courtesy: Kerry R. Foresman, University of Montana, USA)

Annexure II: Specimen Collection Sheet

Date of collection:_____ Collection No.:

Species:_____ Photos of Animal: Dorsal__ Ventral__ Lateral__

Collector: _____ Photo of Skull: Dorsal__ Ventral__ Lateral__

General Locality: Dzongkhag (District)_____ Dungkhag (subdistrict)_____

GPS coordinates:

Survey grid cell - Lat:_____ Long:_____

Elev.:_____

Trap location of capture - Lat:_____ Long:_____

Elev.:_____

Habitat: (Photo #s)

Body wt._____gms; **Length:** Total_____ Tail_____ Hindfoot_____ Ear_____

Males: Testes _____ x _____; Descended____ Non-descended____

Females:

Embryos: ___R ___L; Size in utero: ___ x ___mm;

Placental scars: ___R ___L; Mammary Development: Yes ____ No ____

No. Prominent Nipples: _____

Pelage: ____Juvenile ____ Subadult ____Adult

Material Saved:

Skin___ Skull___ Skeleton____ Tissues____ FTA card____ Nobuto strip____ Ecotoparasites____ Hair samples_____

Notes:

STUDY SITE		HABITAT TYPE		SLOPE	
DATE		TRANSECT ID		ASPECT	
TRAP TYPE (Sherman,, Pit, Snap)		TIME AT START OF CHECK		TEMPERATURE	MAX
OBSERVERS		TIME AT END OF CHECK			MIN
GRID NAME/UTM COORDINATES		WEATHER			

TRAP ID	COORDINATES		ELEVATION	SPP	RECAP	MARKS	SEX	REPRO	WEIGHT			STANDARD MEASUREMENT IN CM				MICROHABITAT
	N	E							TOTAL	BAG	ANIMAL	TAIL LENGTH	HIND FOOT	EAR	BODY	

REPRODUCTION STATUS: MALE (DECENDED TESTES; NOT DECENDED) FEMALE: PROMINENT NIPPLE; NOT PROMINENT
MARK : (RED, BLUE, GREEN, EAR PUNCH)

PELAGE: ADULT, SUBADULT, JUVENILE

RECAP: YES/NO

COMMENT: CLOSED BUT EMPTY (CBE)

MICROHABITAT (ROCK, GULLIES, NO. OF DOWN LOGS)

WEATHER(SUNNY, RAINY, CLOUDY, PARTLY SUNNY, SNOW)

TRAP ID (OPEN GRASSLAND(OG1); AGRICULTURE (AG1); OPEN SHRUBS(OSH1); FOREST (FR1); RIPARIAN (RR1)

Annexure IV. Cover estimation and Vegetation sheet

VEGETATION								
Plot	Over storey	Cover Class			1 2 3 4 5			
	Species	DBH	Bearing			Height	Comments	
			Base	Tip	Dist			
	Under storey	Cover Class			1 2 3 4 5			
	Ground storey	Cover Class			1 2 3 4 5			

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