

PROTOCOL FOR RAPID BIODIVERSITY ASSESSMENT



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Developing Ecosystem-based Solutions for Managing Biodiversity Landscapes in Bhutan

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The project focuses on developing ecosystem-based solutions for managing biodiversity landscapes, with a special focus on establishing approaches and tools for protecting and managing White-bellied Heron (WBH) habitats along Punatsangchhu and Mangdechhu basins in Bhutan.

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1.1 General concepts and principles

The rapid biodiversity assessment approach is a methodology to collect biological information to inform conservation decision-making (Alonso *et al.* 2011). This method is particularly designed to rapidly assess the biodiversity of highly diverse areas, identify the threats, and prioritize areas for the conservation of this biodiversity. The approach also helps to strategize to strengthen community involvement and participation in conservation management, train local stakeholders in biodiversity survey techniques, and develop management policies and designing the sustainability options. Some of the criteria used to identify priority areas for conservation include: overall species richness, presence of local endemics, rare species, threatened species, and habitat condition.

1.2 Types of biodiversity assessments

Rapid biodiversity assessment - is an important technique for terrestrial, and freshwater, (also including marine, and estuarine system management, in case of coastal countries), assessments especially in areas where the information is sparsely known or unknown at all. Rapid assessments of biodiversity require, a conceptual framework for the design and implementation of the assessment, and a clear definition of the scope of the assessment.

In general, there are five types of assessment which can be applied based on the requirement (SCBD, 2005). The assessment types are:

- a. Baseline inventory – focuses on overall biological diversity rather than extensive or detailed information about specific taxa or habitats.
- b. Species-specific assessment – provides a rapid appraisal of the status of a particular species or taxonomic group in a given area.
- c. Change assessment – is undertaken to determine the effects of human activities or natural disturbances on the ecological integrity and associated biodiversity of an area.
- d. Indicator assessment – assumes that biological diversity, in terms of species and community diversity can inform us about water quality and the overall health of particular ecosystems.
- e. Resource assessment – aims to determine the potential for sustainable use of biological resources in a given area.

In the case of ESRAM (*Developing Ecosystem-based Solutions for Managing Biodiversity Landscapes in Bhutan*), the priority of this guideline is essentially for reconnaissance, a preliminary baseline inventory.

1.3 Designing rapid assessments

Since the inventory is vital that provides the basis as guidance to undertake the appropriate assessment of areas and future monitoring. Therefore, while designing the rapid assessments, taking into account the following element is crucial:

- a. Resources available, including time, money and expertise;
- b. Scope, including the taxonomic and geographic scope and site selection;

Sampling data and analysis, including identification of what data are required, how to collect it, how much to collect, how to enter it into a database, analysis, and integrate it into a report;

However, prior to undertaking the assessment, the following steps should be also be considered which is useful in setting objectives and scopes of the assessments (Maragos and Cook 1995):

- a. Define the purpose and objectives of the rapid assessment;
- b. Define geographic scope, based upon the objectives and constraints;
- c. Select survey team and assign responsibilities;
- d. Undertake a review of literature, maps, and aerial photographs;
- e. Select field sites, relying heavily on the above inputs and steps;
- f. Schedule and accomplish field work;
- g. Finalise and publish technical reports.

1.4 Background

The Royal Society for Protection of Nature (RSPN) has received the Federal Ministry of Environment, Nature Conservation and Nuclear Safety (BMU) funding through the International Climate Initiative (IKI) to implement the project '*Developing Ecosystem-based Solutions for Managing Biodiversity Landscapes in Bhutan*'. The project is being implemented over a period of five years beginning July 2021. The primary objective of the project is to establish approaches and tools for protection and managing White-bellied Heron (WBH) habitats along Punatsangchhu and Mangdechhu basins in Bhutan.

The outcome of the project is effective adaptive ecosystem solutions for WBH conservation in Bhutan created through habitat restoration and livelihood enhancement. The outputs are:

- a. Ecosystem-based biodiversity survey and community engagement strategies for WBH conservation based on the ESRAM approach designed and applied.
- b. Degraded and new potential WBH habitats where rehabilitated and created (feeding, roosting and nesting).

- c. Capacity of all stakeholders in planning and implementation of ESRAM study, ecosystem-based adaptation and livelihood options strengthened.
- d. Ecosystem-based adaptation and livelihood options in the WBH habitat areas where applied and established.
- e. Knowledge base on ecosystem-based biodiversity conservation, WBH habitat, and population and climate adaptation, established and disseminated.
- f. Through the successful implementation of the ecosystem-based conservation measures, the expected project impact is *stable or the increasing WBH population in Bhutan and other Himalayan countries*.

Therefore, the study site to undertake the biodiversity assessment was already defined. The timeline for the assessment was estimated as a total of 4 months from August to November 2021. The implementation of the project time was relatively short however the protocol is developed so that it can be used as reference guide to undertake similar assessment irrespective of time constraints to carry out reliable assessment in other WBH habitats. This protocol covers the following biodiversity component(s):

- » Terrestrial vegetation (flora)
- » Wildlife (Mammals)
- » Birds
- » Aquatic biodiversity (Fishes & Microvertebrates)
- » Herpetofauna
- » Terrestrial invertebrates

The methods suggested align with place-based protection in achieving conservation goals and enhancing ecosystem services rather than focusing on keystone species protection (Sharma et al. 2013). Also, the biodiversity assessment approach used in this project aligns with the Biodiversity Monitoring and Social Survey Protocol of Bhutan 2020 (DoFPS 2020a) and the Field Manual - National Forest Inventory of Bhutan 2020 (DoFPS 2020b).

2.1 Floral assessment: why study vegetation?

The use of vegetation description is in the recognition and definition of “different vegetation types and plant communities, which is known as the science of phytosociology, the mapping of vegetation communities and types, the study of relationships between plant species distributions, environmental controls and their interactions with humans and animals, and the study of vegetation as a habitat for animals, birds and insects” (Kent, 2012). Information on vegetation helps us understand the ecological problems, for biological conservation and management purposes, monitor management practices, and predict the future changes in plant distributions and the effect it can have on the soil and human wellbeing and vice versa.

Floral assessment includes identification and monitoring of ecosystems and habitats followed by inventory, identification, assessment, and monitoring of plant species in an area. For the purpose of this manual, this section will focus on the assessment of the terrestrial ecosystem (floral assessment). Accuracy of floral assessment depends on the sampling design and intensity or the proportion of area subjected to an inventory. However, it is nearly impossible to assess the whole of the ecosystem to have the highest accuracy level assessment. It is, therefore, necessary to do sampling. The sampling should be such that the sample should be representative of the whole area of interest.

The Department of Forests and Park Services (DoFPS) uses grids approximated at 4 x 4 km distance in assessing and monitoring biodiversity at the national level (DoFPS 2020a). However, these grids are useful in locating the nearest sampling sites only and may have limitations of accessibility. In case of the ESRAM site, there are records of WBH nesting and sightings so the focus is to cover the WBH habitat as much as possible and also make use of the national standard as per DoFPS. In such a case, the site map can be generated, by overlaying the grids and the WBH habitat by using GIS. Since in ESRAM site, there are terrain and relatively not accessible therefore, an approximate buffer distance of 100 m from the river can be taken into consideration. Also, the 4 km X 4 km distance appeared to be large so with consideration of past occurrence of WBH as observation points, 1 Km x 1 Km grid over 100 m buffer from the rivers and major tributaries can be taken into consideration (See Figure 1).

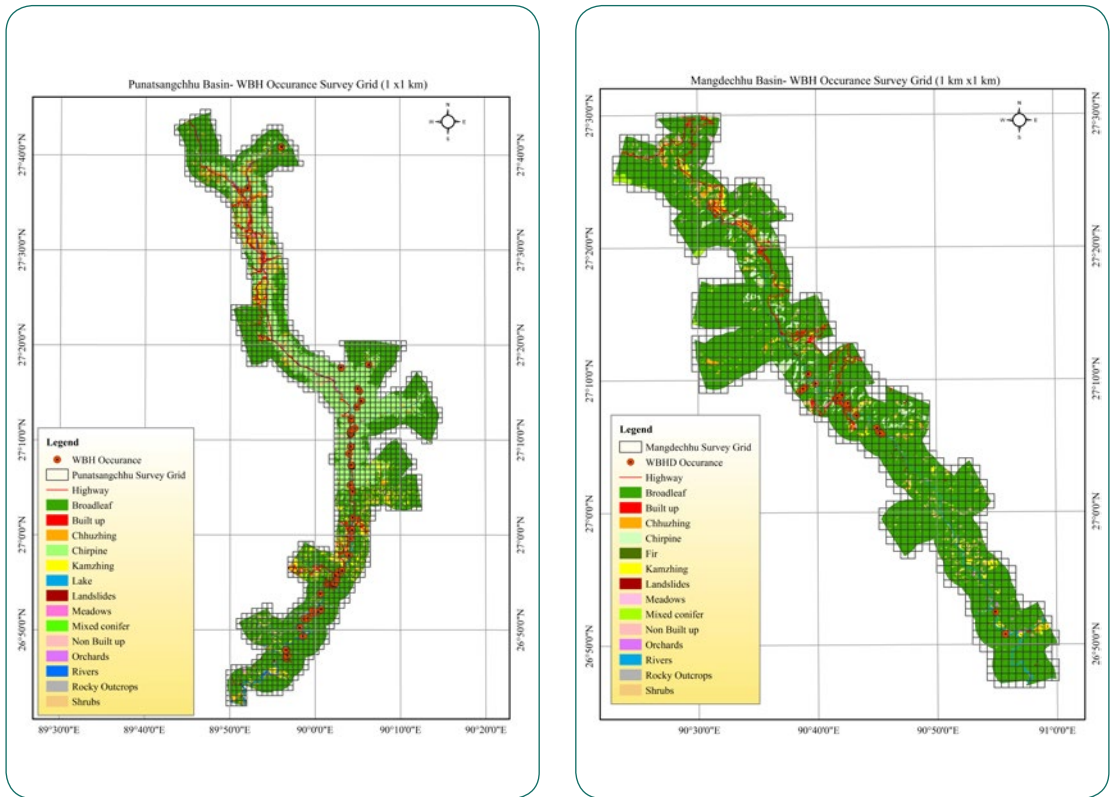


Figure 1: Proposed biodiversity assessment sites showing 100m buffer areas from the main rivers and tributaries in Puntasangchhu and Mangdechhu basins.

To ensure proper representation of the different vegetation and forest formation, it is recommended that the sampling sites should be well stratified. Also the stratification doesn't always need to have proportionate sampling efforts for each vegetation type. However and important consideration for sampling intensity should be given based on the ecosystem's diversity and complexity.

2.2 Data collation and field survey preparation

Before starting the field survey, it is highly recommended to:

- » Obtain colour aerial photography, geological maps, and topographical maps for the survey area.
- » Obtain all relevant literature regarding vegetation and flora for the site, including species lists, herbarium records, etc.
- » Compile a brief history of previous botanical exploration (if any), and check if there are any threatened plant species.

- » Prepare maps that overlay the locations for threatened species on topographical maps, and if available.
- » Prepare images of threatened plant species, ideally colour photographs and/or photographs of herbarium specimens.

Furthermore, it is also recommended to identify the major environmental/ecological conditions based on the drainage and soils, climate and altitude.

For example, an area to be surveyed might comprise different forest types/ecological zones, etc. Areas with rainfall, and its influence and with a major division of habitats occurring between high rainfall and low rainfall areas. It is stated that rare ecosystem types are those that have always been very limited in extent, and are characterised by the presence of rare or endemic species (Williams *et al.* 2007). It is therefore, highly recommended to identify any unusual features that exhibit rare ecosystems or endemic species, cloud forests, etc. Also, identify the location of natural areas. Natural areas can be defined as “habitats within which indigenous plant species are dominant (equal or greater than 50% cover), and may include an exotic component” (Patrick *et al.*, 2014).

In case, if the survey area is extensive and is constrained by time limitations for survey, the survey of all areas would not be possible therefore, it is recommended to assign higher priority to:

- » Larger, more contiguous natural areas.
- » Areas that will ensure that natural areas within each region are included.
- » Areas that may contain historically rare ecosystem types.
- » Areas that are likely to support rare or endemic species.

2.3 Local community engagement

Prior to the execution of the field survey, it is highly recommended to explain the objectives of the vegetation and flora survey to interested local people and seek information for informed decision making for carrying out the reliable field survey. Community engagement will help record any additional known locations or potential sightings of target species. Also, engaging the local community can help to make a correct interpretation of current vegetation types and patterns, such as forest clearings, fires, etc. involvement of the local community can also be useful in the development of a dictionary of local plant names (Hawthorne 2012).

2.4 Equipment requirements

- » Laminated colour aerial photographs of the survey area.
- » Laminated geological and topographical maps of the survey area (if available).
- » Laminated images (preferably colour) of threatened or endemic plant species.

- » Fine-tipped indelible marker pens.
- » Water-proof paper and pencils.
- » Two GPS units.
- » Binoculars (8-10´ minimum).
- » Compass.
- » Secateurs.
- » Bush knife.
- » Strong catapult (for collecting specimens from tall trees).
- » Plastic clip-seal bags (for collection of fleshy specimens such as ripe fruit).
- » Field plant press.
- » Digital camera (plus spare memory cards, and backup).
- » Spare batteries for camera and GPS.

2.5 Reconnaissance survey

A brief reconnaissance survey at the commencement of field work is critical to success. The reconnaissance survey can be done by viewing from a roadside or watching the site from a point of birds eye view or taking a brief walk around the site. The main objective of the reconnaissance survey is to:

- » Identify and map the site not identified during the desktop phase.
- » Ground truth is the location of specific areas of interest such as natural areas.
- » Familiarize with the main vegetation types present in the survey area, if necessary, and identify canopy species.

Once the reconnaissance survey is completed, the field work can be divided into the following:

- » Mapping and description of vegetation types;
- » Compilation of a checklist of the flora, and preparation of voucher specimens, if necessary;
- » Surveys of habitats of threatened or endemic species.

2.6 Sampling intensity and sample size

The total number of potential sites determined by the use of maps can be used in determining the sample size following the Proportionate Probability Sampling (PPS) method (a stratified sampling method). For the ESRAM site, along the two river basins i.e. Punatsangchhu and Mangdechhu, a total of 80 sampling plots are accounted. This is based on the WBH habitats (based on nesting and sighting data). These 20 sample sites

will be allocated to the two basins in proportion to the WBH siting and nesting information for each basin. It may be noted that there are stretches of Punatsangchhu that are devoid of vegetation on either side but are known to be frequently visited by WBH. In such cases, in addition to the PPS method, a purposive sampling of vegetation areas further away from such areas is also considered.

For sampling purposes, all the potential sampling sites generated using maps can be given identity number, put in a bag and the sampling sites drawn randomly – ensuring the rule of randomness and independence; the proportion of samples to be drawn can be determined proportionately as per the Dzongkhags, main rivers and their tributaries. As an alternative option, if the calculated sampling size is disproportionately large and ambitious, since the time and resources required also need to be taken into consideration, sampling sites can be maintained at 30 each for Punatsangchhu and Mangdechhu basins following a thumb rule of a minimum 30 sample size. In the sampled grid of 4x4 km, two plots of 20x20m can be established 0.5 km apart. The sampling plots should be at least 200m away from the vehicular roads and 100m from the footpath. However this manual highlights the general techniques for vegetation sampling based on the requirement as project outcome and also field situation which is detailed in the following section.

2.6.1 Sampling design for vegetation description and analysis

The primary determinant of the sampling design depends on the aims and objectives of the project. Therefore, specific factors such as the time and resources available for study, the type of habitat and proposed methods of data analysis and presentation must also be considered prior to project execution (Maher *et al.*, 1994).

2.6.2 Spatially stratified sampling

This method employs the principle of stratification in which the vegetation of the “area under study is divided up before samples are chosen on the basis of major and usually very obvious variations within it” (Kent, 2012). Stratification should be normally carried out during the initial reconnaissance survey and the criteria to divide or stratify the area would be differences in growth form, physiognomy, and structure of the vegetation, areas of vegetation subject to different management regimes, and important environmental differences, such as aspect, geology or slope form, or areas that are known to have had differing time periods since they were last disturbed and will have thus undergone different degrees of successional change.

The sampling design should be based on “the assumption that the greater the diversity and the rate of change of the vegetation cover over a given distance, the more intensively it should be sampled” (ibid). Once the area is stratified, a second stage sampling called random sampling can be applied within the 4km X 4km grid following the national standard.

In certain cases, **Systematic sampling** can be applied where the location of sampling points at regular or systematic intervals where the vegetation is influenced haphazardly by

furrow, ridges and/or different management regimes. While in the case of mountainous/hilly environments, a **Transects** approach can be used. A transect is a line along which samples of vegetation are taken and are usually set up deliberately across areas where there are rapid changes in vegetation and marked spatial environmental gradients. The main purpose of using transects in vegetation description in varying environmental condition is to describe maximum variation over the shortest distance in the minimum time. When there is a requirement for detailed studies, the transect approach can be broadened out into a grid, where a large number of quadrats are placed adjacent to each other in the grid and species abundances and environmental factors are recorded in each quadrat. Furthermore, in cases where the forest and woodland ecosystems and where vegetation is sparsely distributed, the use of conventional quadrat analysis and sampling may be limited. In the case of woodlands, sampling of the tree cover would require very large quadrats and often the ground flora may be totally impenetrable. In sparse communities, a simple sample strategy known as plotless sampling can be used. It is also known as 'nearest individual method', which involves the location of random sampling points throughout the area.

2.6.3 Sample size

The sample size very much depends primarily on the research objectives, and the availability of resources at hand and others. To overcome this situation, one commonly suggested approach to sample number determination is the species accumulation curve (Barker, 2001). The numbers of species found in quadrats are recorded and the results are plotted against the number of samples. The projection of the curve would indicate whether the slope is already leveling off. The other decision, researchers make is by following the thumb rule that a minimum sample size of around 30 is suggested. However, to employ this decision, other factors affecting the occurrence of vegetation should be clearly understood.

2.7 Description of vegetation in the field

Methods of vegetation description fall into two categories; i.e. (a) Physiognomic or structural – where the description is based upon external morphology, life-form, stratification and size of the species present, and (b) Floristic – where the species present in the study are identified and their presence/absence or abundance is recorded.

2.7.1 Physiognomic or structural description

Physiognomic and structural methods are used “primarily for the classification of vegetation at small scales (over large areas), such as world vegetation formations” while floristic analyses are applied at the large scale (over small areas) particularly at the level of the plant community. Physiognomic and structural methods are useful in habitat classification

One of the physiognomic techniques of vegetation description is known as the life-form

method of Raunkaier (1934, 1937). This is based on the biological spectrum such as the height above ground of the perennating buds of each species, which are the parts of the plant from which growth commences in the next favorable growing season. It is based on the assumption that species morphology is closely related to climatic controls, for example, humid tropics represent the most favorable conditions for species in terms of solar radiation, temperature and precipitation, while species deficient in moisture, solar radiation or temperature, show varying degrees of growth due to varying adaptation and response in the positioning of their perennating buds (Kent, 2012). Five main categories are recognised (see Figure 2).

Group 1 PHANEROPHYTES

Species with perennating buds emerging from aerial parts of the plant:

- (a) evergreen phanerophytes without bud scales
- (b) evergreen phanerophytes with bud scales
- (c) deciduous phanerophytes with bud scales

Each of these types may be classified according to height:

- Megaphanerophytes (> 30 m)
- Mesophanerophytes (8–30 m)
- Microphanerophytes (2–8 m)
- Nanophanerophytes (<2 m)

Group 2 CHAMAEPHYTES

Species with perennating buds borne on aerial parts close to the ground (below 2 m)

They may be woody or herbaceous:

- (a) Suffruticose chamaephytes – after the main growth period, upper shoots die so that only the lower parts of the plant remain in the unfavourable period
- (b) Passive chamaephytes – at the onset of adverse conditions, shoots weaken and fall to the ground, becoming procumbent. They get some protection from environmental stress
- (c) Active chamaephytes – shoots are only produced along the ground and remain so in the unfavourable season
- (d) Cushion chamaephytes – a modification of passive types, where shoots are arranged so close together that they cannot fall over and the close packing of all shoots forms a cushion

Group 3 HEMICRYPTOPHYTES

All above-ground parts of the plant die back in unfavourable conditions and buds are borne at ground level:

- (a) Protohemicyptophytes – leaves become better developed up the stem of the plant. Poorly developed leaves protect the bud in early stages of growth
- (b) Partial rosette plants – the developed leaves form a rosette at the base of the plant in the first year of growth. The following year an elongated aerial shoot may form
- (c) Rosette plants – leaves are restricted to a basal rosette with an elongated aerial shoot, which is exclusively flower-bearing

Group 4 CRYPTOPHYTES

Plant species with buds or shoot apices that survive the unfavourable period below ground or under water

- (a) Geophytes – plants with subterranean organs such as bulbs, rhizomes and tubers from which shoots emerge in the next growing season
- (b) Helophytes – plants with their perennating buds in soil or mud below water and which produce shoots reaching above water
- (c) Hydrophytes – species with buds that lie under water and survive the unfavourable season by budding from rhizomes under water or from detached vegetative buds which sink to the bottom

Group 5 THEROPHYTES

Plants that survive the unfavourable period as seeds. Species are thus annuals and complete their life cycle from seed to seed in the favourable summer months.

Figure 2: The major categories of the Raunkaier life-form classification system - 1937 (Source: Kent, 2012)

2.7.2 Habitat classification (Elton and Miller, 1954; Elton, 1966)

The habitat description and classification system was originally devised by Elton and Miller (1954) and also described in Elton (1966). This method serves as a rapid method of habitat survey and also for general surveys of ecosystems and habitats for rural planning purposes as ecological evaluation. This method assumes that different layers or degrees of layering as stratification of vegetation serve as the diversity of habitat. Three major types of habitat can be identified such as terrestrial, aquatic, and the aquatic-terrestrial transition. The terrestrial habitat system is further divided into four categories such as open ground, field layer, scrub and woodland, on the basis of the height of the dominant species (see Figure 3).

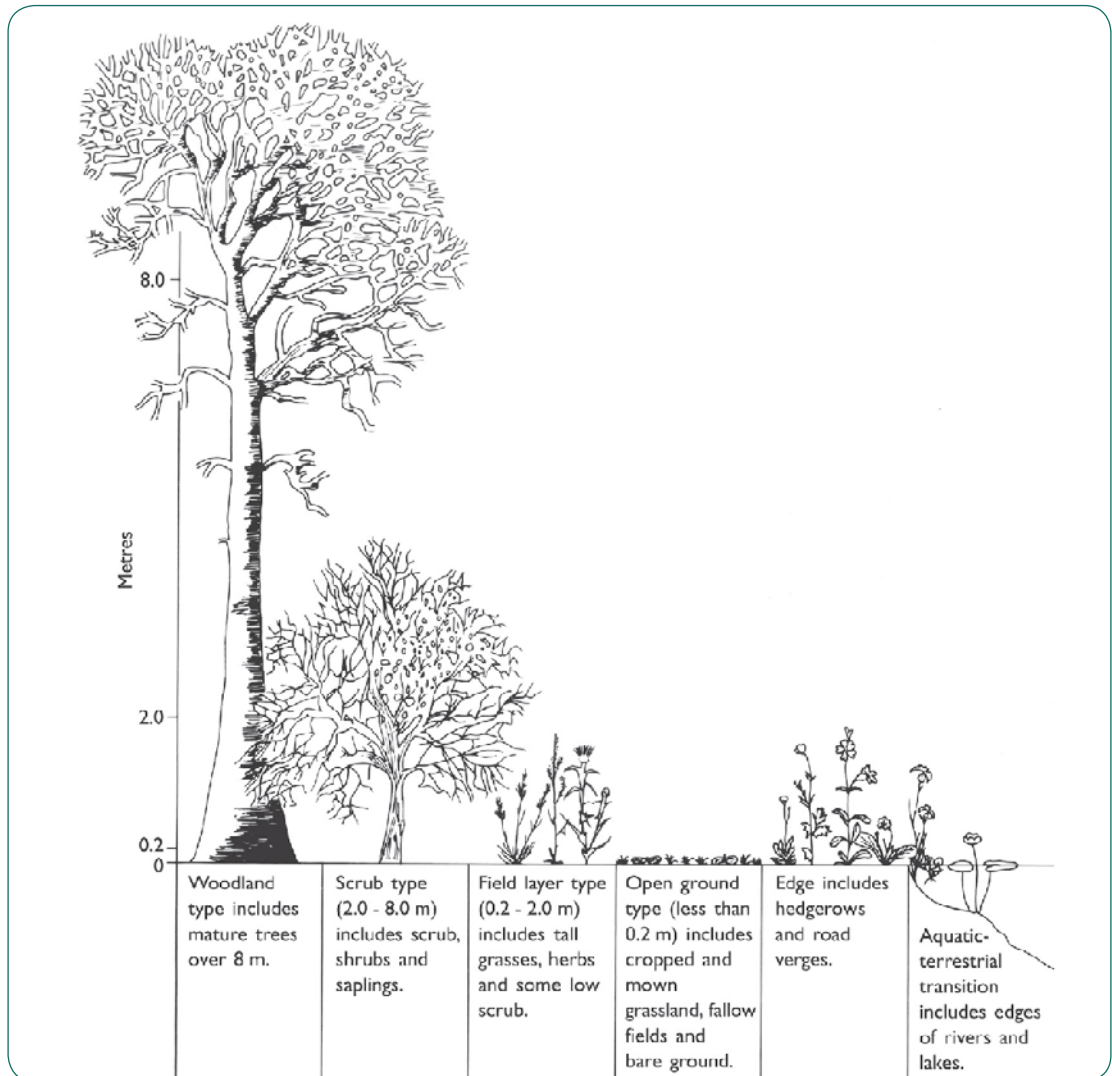


Figure 3: Habitat description approach of Elton and Miller 1954 (Source: Kent, 2012)

2.7.3 Vegetation description based floristics

In this method, the individual species within the community being studied requires identification and documentation for quantitative analysis. In order to carry out the floristic approach of vegetation description, four points need to be understood clearly:

- » Identification of plant species.
- » Whether or not to collect data on the abundance of each species and if so, the scale of measure of abundance.
- » Whether other forms of description than species identification are the best way to describe the vegetation.
- » Whether to sample or sub-sample.

2.8 Sampling frame for recording plant species

The quadrat is the usual means of sampling vegetation for floristic description (Kent 2012). Quadrats can be square, rectangular and even circular and the purpose of the quadrat is to establish a standard area for examining the vegetation. The size of the quadrat to be considered is crucial which depends from one vegetation type to the other (See Table 1). The size of the quadrat also depends on the concepts of minimal area and species–area curves. The most frequently used approach is the Braun-Blanquet school of vegetation classification and phytosociology.

Table 1: Suggested quadrat size (Source: Kent, 2012)

Vegetation type	Quarat size
Bryophyte and lichen communities	0.5m x 0.5m
Grasslands, dwarf health	1.1m - 2x2m
Shrubby healths, tall herbs and grassland communities	2 x 2m - 4 x 4m
Scrub, woodland shrubs, small trees	10m x 10m
Woodland canopies	20m x 20m - 50 x 50m (or use plotless sampling)

For Bhutanese landscape, the standard quadrat size is suggested as 20m X 20m for woodland canopies, 5m X 5m for shrubs and 2m X 2m for herbs or ground cover (DoFPS, 2020).

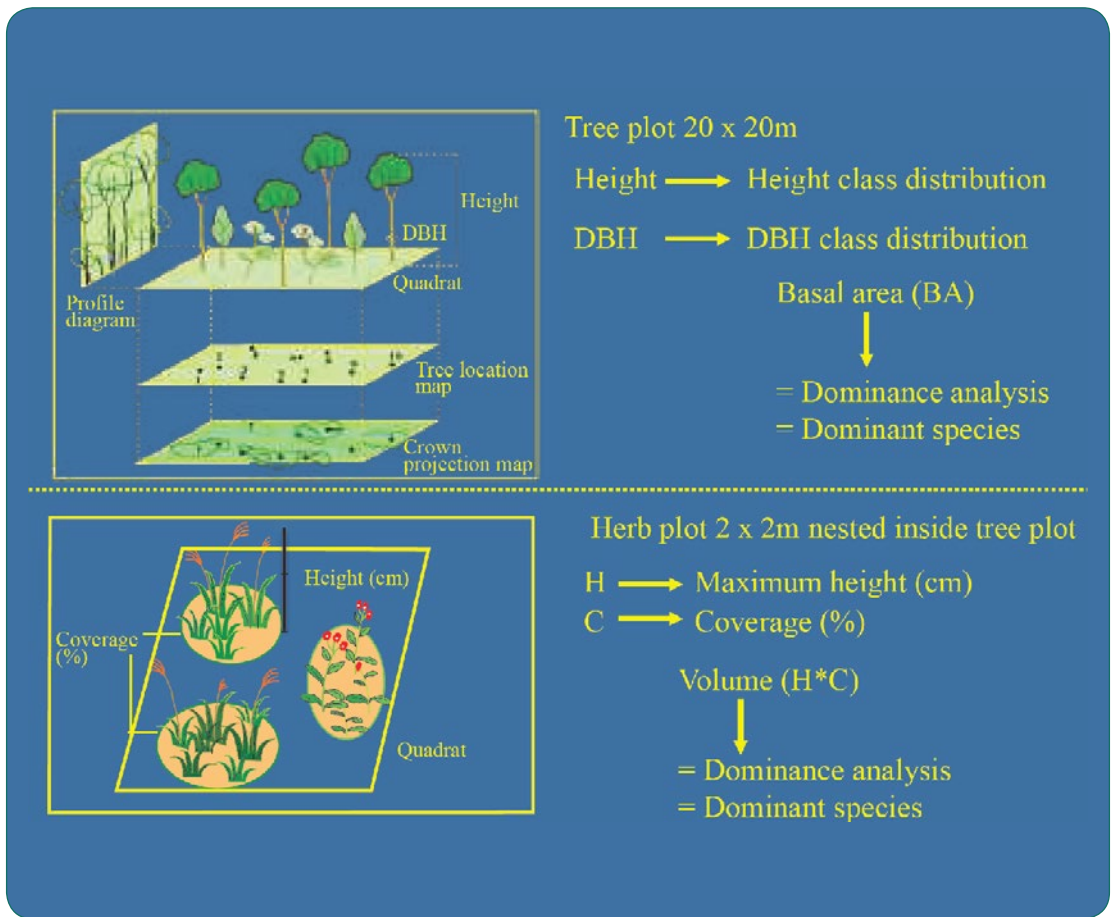


Figure 4: Vegetation data collection profile (Source: Wangda and Ohsawa, 2006)

In order to make sure that data collection is representative, a technique of minimal area or a species curve can be applied. The Braun-Blanquet school of vegetation classification and phytosociology “involves starting with the area that is considered to be the smallest feasible quadrat size, usually just containing one or two species, then doubling the size of the quadrat, counting the number of species present again, doubling the size of quadrat again, counting the number of species and so on, until no new species are recorded at a doubling of quadrat size.

The resulting graph of species numbers against quadrat size is known as the minimal area or species–area curve” (Figure 5a&b). If a homogeneous area of vegetation is taken, then the curve of species numbers levels off and the point at which this occurs is taken as the minimal area for sampling that community. The recommended quadrat size should then be a little larger than the minimal area (Kent 2012).

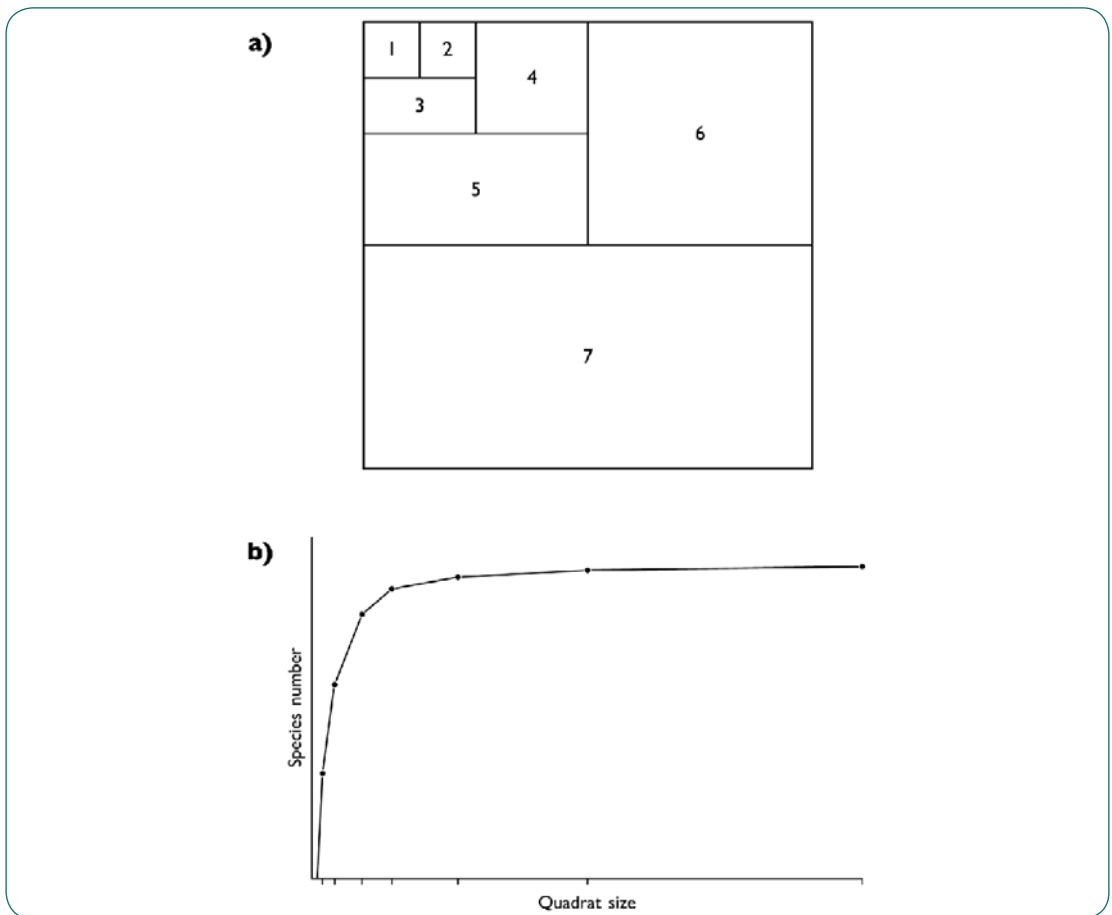


Figure 5 (a & b): Progressive doubling of quadrat size for minimal area and derivation of species area curves (Kent, 2012)

2.8.1 Data collection procedure for trees and shrubs

While collecting data, the following information is required using the appropriate method:

- » Record the plot id, species name (both local and scientific) on the same data form or use different forms for shrubs as shown in Annexure 1.
- » Collect the voucher specimen in case the species is unidentified
- » Start measurement from the North direction (clockwise) till completion
- » Always measure the DBH from uphill and avoid buttressed etc.
- » Measure the DBH of all the individual species using DBH (Diameter at Breast Height) tape above > 1.3m height from the ground floor.
- » Measure the height of the trees using the hypsometer or clinometer.
- » Consider forking above DBH as 2 trees.

- » Multiple stems emerging from the single root system should be considered as one tree, although the measurement should be done for all the sprouts. For calculating the species diversity only one main stem data is taken into consideration.
- » In the case of the woody liana and climber species, only DBH is recorded, height/length may not be relevant.

2.8.2 Vegetation pattern

The manner in which the individuals of a given species are distributed within a plant community is known as vegetation pattern and the species exhibit clustered, random or regular patterns (Figure 6).

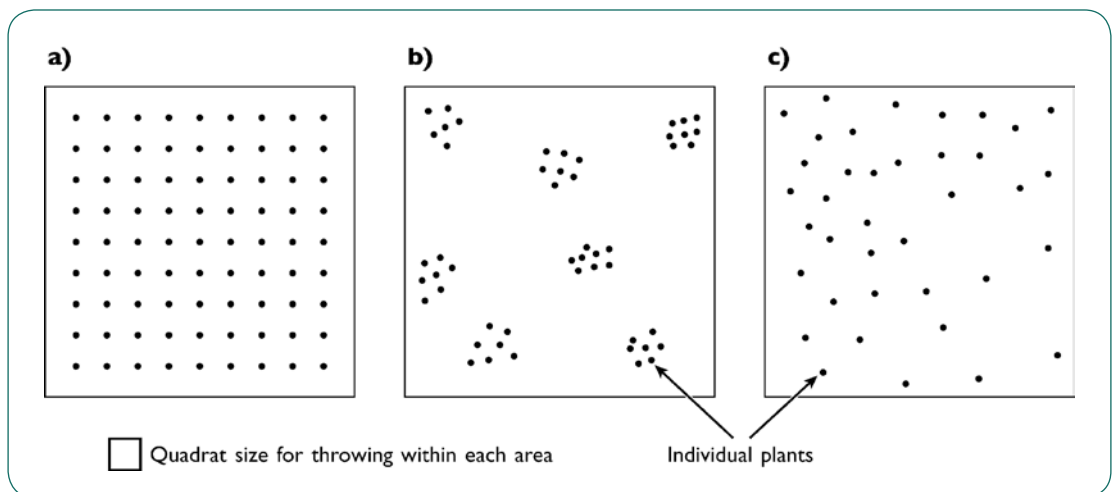


Figure 6: Pattern in vegetation (species distribution) (a) regular, (b) clustered, and (c) random patterns

2.9 Measurement of species abundance

Species abundance should be calculated keeping in mind that a plant species are clearly identifiable as individuals, while for plant species that cannot be identified as a single individual such as grasses and bryophytes or species growing with a network of connected shoots or ramets under or on the surface of the ground as counting individuals and then comparing results for different-sized species can become meaningless (Kent 2012).

2.9.1 Presence/absence or qualitative data

In the measurement of abundance, a distinction is made between presence/absence and abundance data as presence/absence data, only the occurrence of a species within a quadrat is noted and there is no measurement of the amount of each species. Presence/absence methods are extremely rapid to use and represent the vegetation data in simplest form. Hence it is qualitative.

2.9.2 Abundance measures or quantitative data

While the abundance data contain additional information in terms of the number of individuals and abundance measures can be categorised into two types:

1. *Subjective* – these are estimated by eye and thus values will vary from one recorder to another.
2. *Objective* – more accurate and precise measures are taken which should not vary from one recorder to another.

2.9.3 Subjective measures

In subjective measure, the two types of approaches are used such as frequency symbols and cover estimated by eye. Frequency symbols include the most subjective and descriptive method of vegetation description using frequency symbols such as 'DAFOR' scale for each species were dominant (D), abundant (A), frequent (F), occasional (O) and rare (R). Such type of classification is useful while enumerating plant species in grassland or a small wood with or without quadrats.

While the cover is estimated by eye approach, the cover is usually estimated visually as a percentage. The cover is defined as the "area of ground within a quadrat that is occupied by the above-ground parts of each species when viewed from above" (Kent, 2012). Sometimes the use of Domin scale and the Braun-Blanquet scale, where the range 0–100% is partitioned into five or ten classes with smaller graduations nearer to the bottom of the scale are used (Table 2). The use of cover estimates is essential when describing grassland species where individuals of a species cannot be identified.

Table 2: Braun-Blanquet and Domin scale (Source: Kent, 2012)

Value	Braun Blanquet	Domin
+	< 1% cover	A single individual - no measurable cover
1	1 - 5% cover	1 - 2 individuals - no measurable cover-individuals with normal vigour
2	6 - 25% cover	Several individuals but less than 1% cover
3	26 - 50% cover	1 - 4% cover - many individuals
4	51 - 75% cover	5 - 10% cover
5	76 - 100% cover	11 - 25% cover
6		26 - 33% cover
7		34 - 50% cover
8		51 - 75% cover
9		76 - 90% cover
10		91 - 100% cover

Limitations: Since estimation is done by eye, there is certain degree of error in recording and overestimate species that are conspicuous.

2.9.4 Objective measures

The objective measure includes the computation of the density and frequency of species. Density is a count of the number of individuals of a species within the quadrat. Density is most frequently measured in studies of herb species or tree saplings and is rarely used in the description of whole communities. This method however is time consuming where there are large numbers of individuals, individual plants will have to be tagged to avoid double counting. The second method is by computing frequency. It is defined as the probability or chance of finding a species in a given sample area or quadrat. For example, if a species occurs in 63 of those 100 quadrats, it has a frequency of 63%.

Limitations: Computing frequency also depends on the quadrat size and there tends to be a bias towards abundant small species, as opposed to patchy dominants.

2.10 Vegetation data analysis

There are many ways that vegetation data can be analysed but the most common ones that the ecologists or plant scientists use are the measurement of similarity or dissimilarity and species richness and diversity.

2.10.1 Measurement of similarity and dissimilarity

Similarity coefficients measure the degree to which the species composition of quadrats or samples is similar while dissimilarity coefficients assess the degree to which two quadrats or samples differ in composition. Dissimilarity is the complement of similarity. Sometimes the similarity is also known as resemblance, and similarity coefficients are sometimes called resemblance functions (Kent, 2012).

The most useful coefficient to employ similarity is the Jaccard coefficient, which is generally applied to qualitative data. The formula is:

$$S_j = \frac{a}{a + b + c}$$

where a is the number of species common to both quadrats/samples, b is the number of species in quadrat/sample 1 only, and c is the number of species in quadrat/sample 2 only. Often the coefficient is multiplied by 100 to give a percentage similarity figure. Similarly, the dissimilarity (D_j) is computed as:

$$D_j = \frac{b + c}{a + b + c}$$

or $1.0 - S_j$.

A very similar coefficient was introduced by Czekanowski (1909, 1913) which is defined using the same symbolism as:

$$S_j = \frac{2a}{2a + b + c}$$

Generally, Czekanowski coefficient is preferred to the Jaccard coefficient, because it gives weight to the species that are common to both the quadrats and samples, rather than to those which are unique to either sample (Kent 2012). An example is copied for clear understanding while using the formula (see Table 3)

Table 3: Presence/absence data sets (Source: Kent, 2012)

Species	Quadrat 11	Quadrat 12
<i>Atriplex patula</i>	1	1
<i>Distichlis spicata</i>	1	0
<i>Iva frutescens</i>	1	1
<i>Juncus gerardii</i>	0	0
<i>Phragmites communis</i>	1	1
<i>Salicornia europaea</i>	0	0
<i>Salicornia virginica</i>	0	0
<i>Scirpus olneyi</i>	1	0
<i>Solidago sempervirens</i>	1	1
<i>Spartina alterniflora</i>	0	0
<i>Spartina patens</i>	1	1
<i>Sueda maritima</i>	0	0
Total occurrences	7 (b)	5 (c)
Number of joint occurrences	5 (a)	

$$S_j = \frac{2a}{2a + b + c} = S_j = \frac{2 \times 5}{2 \times 5 + 7 + 5} = S_j = \frac{10}{10 + 7 + 5} = 0.45 \text{ or } 45\% \quad D_j = 1 - S_j = 1 - 0.45 = 0.55 \text{ or } 55\%$$

2.10.2 Diversity and richness

Species richness refers to a count of the number of plant species in a quadrat, area or community, and is also referred to as diversity. Thus, sometimes, a high diversity is also referred to as a community containing a large number of different species. However, measuring diversity comprises two components such as species richness, and the relative abundance (evenness) of species within the sample or community (Magurran, 2004). An even community distribution (perfect evenness) would mean that in a total cover of

100%, species distribution as 20, 20, 20, 20, 20. Diversity is thus measured by recording the number of species and their relative abundances. The two components may then be examined separately or combined into some form of an index.

- » The most frequently used diversity is the simple totalling of species numbers to give species richness (Magurran, 2004) and most commonly used are the Simpson index (D) and the Shannon index (H).

Simpson's indices is the method of quantification of communities in terms of number of different types of species in a community and their relative distribution in the community. It is the probability that any two individuals randomly selected from an infinitely large community will belong to the same species, i.e.,

$$D = \sum p_i^2,$$

where p_i is the proportion of individuals in the i th species while for a finite size community, the formula for calculating Simpson's index (D) is:

$$D = \frac{\sum(n_i * (n_i - 1))}{(N * (N - 1))},$$

where:

n_i — Number of individuals in the i th species; and

N — Total number of individuals in the community.

Table 4: Example of Simpson Index calculation (Source: Kent, 2012)

Quadrat 1 - 6 species present

Species	% cover	Proportion of total cover (P_i)	P_i^2
<i>Carex nigra</i>	15	0.0955	0.0091
<i>Drosera rotundifolia</i>	2	0.0127	0.0001
<i>Juncus effusus</i>	5	0.0318	0.0010
<i>Molinia caerulea</i>	35	0.2229	0.0496
<i>Narthecium ossifragum</i>	20	0.1274	0.0162
<i>Sphagnum</i> sp.	80	0.5096	0.2596
Total cover (%)	157		$\sum p_i^2 = 0.3359$

$$D = 1 - 0.3359 = 0.6641$$

Quadrat 25 - 8 species present

Quadrat 1 - 6 species present

Species	% cover	Proportion of total cover (P _i)	P _i ²
<i>Calluna vulgaris</i>	2	0.0139	0.0002
<i>Carex nigra</i>	10	0.0694	0.0048
<i>Dorsera rotundifolia</i>	2	0.0139	0.0002
<i>Juncus effusus</i>	20	0.1389	0.0193
<i>Molinia caerulea</i>	10	0.0694	0.0048
<i>Narthecium ossifragum</i>	5	0.0347	0.0012
<i>Sphagnum</i> sp.	90	0.6250	0.3906
<i>Trichophorum cespitosum</i>	5	0.0347	0.0012
Total cover (%)	144		Σp _i ² = 0.4224

$$D = 1 - 0.4224 = 0.5776$$

An evenness measure can also be obtained by dividing the reciprocal form of the index by the number of species in the sample (S) (Kent, 2012).

$E \frac{1}{D} = \frac{1/D}{S}$, thus from the above example the evenness can be computed as

$$E \frac{1}{D} = \frac{1/0.6641}{6} = 0.2510 \text{ and } E \frac{1}{D} = \frac{1/0.5776}{8} = 0.2164$$

Values of E1/D lie between 0 and 1 and the lower the index value, the more evenly distributed are the species.

The Shannon index (H') is sometimes correctly called the Shannon-Wiener index but elsewhere is referred to wrongly as the Shannon-Weaver index and the index assumes that individuals are randomly sampled from an 'infinitely large' population and that all the species from a community are included in the sample.

The Shannon diversity index is calculated from the formula: $Diversity (H') = \sum_{i=1}^s pi \ln pi$ where s is the number of species, pi is the proportion of individuals or the abundance of the ith species, and ln = log base n (See example Table 5).

Table 5: example on how Shannon Index can be calculated (Source: Kent, 2012).

Quadrat 1 - 6 species present

Species	% cover	Proportion of total cover (P_i)	$\ln P_i$	$P_i \ln P_i$
<i>Carex nigra</i>	15	0.0955	-2.3482	-0.2243
<i>Drosera rotundifolia</i>	2	0.0127	-4.3631	-0.0556
<i>Juncus effusus</i>	5	0.0318	-3.4468	-0.1098
<i>Molinia caerulea</i>	35	0.2229	-1.5009	0.3346
<i>Narthecium ossifragum</i>	20	0.1274	-2.0605	-0.2625
<i>Sphagnum</i> sp.	80	0.5096	-0.6742	-0.3436
Total cover (%)	157	$H' = 1.330$		

The Simpson, and Shannon indices are based upon both species richness and evenness of species abundances. The resulting index is a function of not only species richness (s) but also the overall abundance (N). For this reason, the Simpson and Shannon indices are often preferred because the species abundances are standardised to proportions.

3.1 Wildlife (mammal) survey techniques

The objective of this manual is to provide the basic guidelines to conduct wildlife research both to generate baseline data and to perform periodic monitoring especially of mammals. This manual, therefore, would cover procedures on:

- » Procedures to establish baseline information on distribution and abundance of mammals, and
- » Generate quality information concerning changes in abundance, composition, and distribution with relation to biotic and abiotic changes.

3.2 Scope and limitations

This manual would serve as a guide to undertake research either for publication or for monitoring of species for protection. While collecting data, for specific wildlife species, their habitats and locations can be different and therefore the same protocol and techniques may not be applicable. In such a case the data should be collected on a case by case basis thus the protocol prescribed here can deviate from a general standard or even national standard.

3.3 Overview of study design

Different study designs are available for wildlife research including mammals and the design may depend on the two criteria: (a) How certain are the conclusions reached and how widely applicable are the conclusions? (Silvy, 2012). It does not make sense all the time to follow a single procedure or design. Researchers need to apply the most applicable design based on the available time and resources. In this manual, the most prominent designs are provided such as experiments, Impact assessments, Models, field studies and integrative process (Silvy, 2012). The experiments are further categorised into laboratory experiments, in which most extraneous factors are controlled, Natural experiments, in which large scale perturbations affect populations and landscape naturally; and field experiments, in which manipulative treatments are applied in the field, combine some of the advantages of laboratory and natural experiments.

Impact assessment design has no replication as the impact may occur at one site and at the particular time. This study design is useful for collecting baseline data where the type, time, and place of the impact are known. Normally impact assessments are planned for before-and-after measurements. Furthermore, the versatile way to address a wide range of research questions emphasizing the conclusions following estimates, relationships, and assumptions uses models. Study design that employ models is useful to understand

sensitivity analysis. Modeling can be useful as a deductive tool to synthesize theoretical understanding incorporating potential solutions to a problem or question.

Field studies design are employed to test hypotheses, where the treatments are not assigned at random. For example, in a field study of dietary selection, the plots may be randomly selected plots where the target species of interest has fed and sites where they have not fed to examine whether the species is feeding in the areas with specific variables.

The integrated design builds on a solid base of natural history observations, field observations and conceptual models including field and laboratory experiments (Silvy, 2012). See Figure 7 for a brief overview.

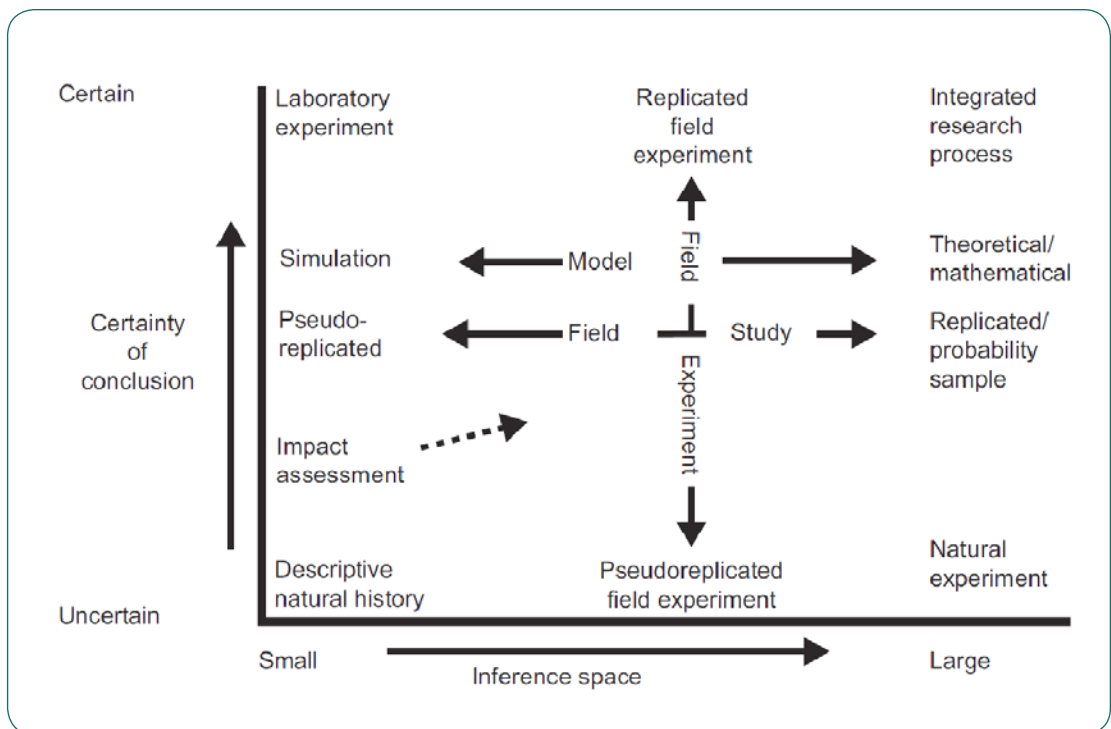


Figure 7: Potential for wildlife study designs

3.4 Sampling for wildlife (mammal) study

It is vital to obtain the best estimates of wildlife species and for that reason varieties of sampling strategies can be used such as (a) Simple random sample, (b) systematic sample, (c) stratified random sample, (d) cluster sample, (e) adaptive cluster sample, (f) point sampling, (g) plots along transects, (h) line transect, and (i) road sampling (see Figure 8).

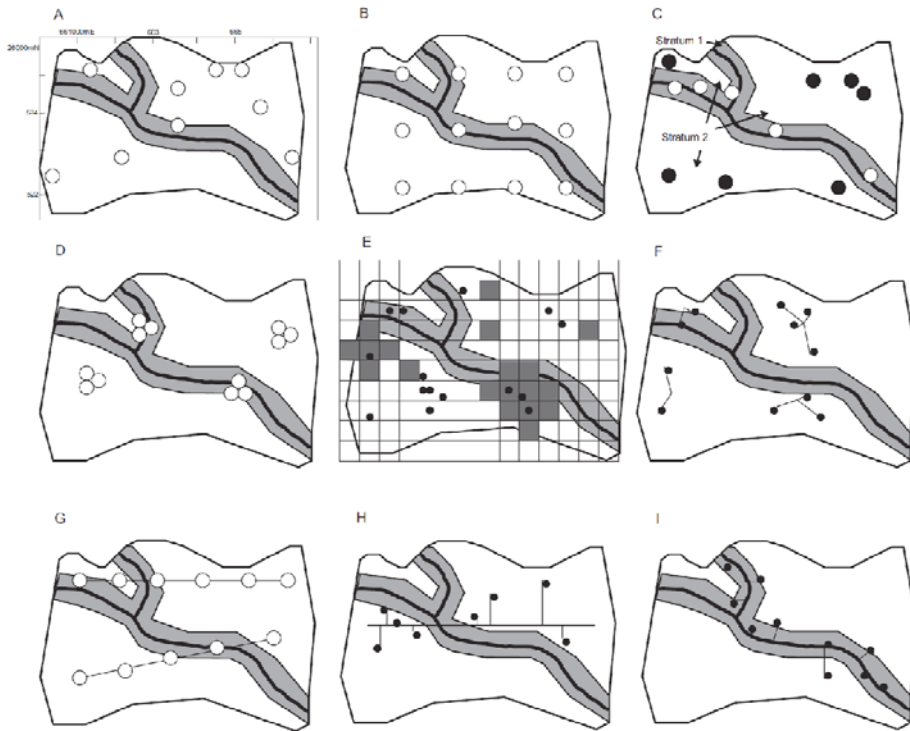


Figure 8: Examples of Sampling methods: (A) simple random sample, (B) systematic sample, (C) stratified random sample, (D) cluster sample, (E) adaptive cluster sample, (F) point sampling, (G) plots along transects, (H) line transect, (I) road sampling.

In simple random techniques, the sample and the procedure for selecting units are truly random while in a systematic sample sampling units are established at regular intervals as they are encountered. In many species, obvious subpopulations exist in the total population such as age, gender, habitat etc and in such a case a powerful design called stratified random sampling can be used. In case of situations where wildlife species remain in groups during all or part of the year, and to draw from such populations or subpopulations, a cluster sampling approach can be used. The formal procedure for cluster sampling consists of three steps: (1) specify the appropriate clusters and make a list of all clusters, (2) draw a simple random sample of clusters, and (3) measure all elements of interest in each cluster selected (Silvy, 2012).

In case of situations when not much information is available before the start of the research and more information can be obtained during the research process, an adaptive sampling method can be used.

Sometimes the sample size is not decided prior to the start of the research and samples are drawn one at a time, and after each sample is taken. Based on the samples taken, the researcher decides whether a conclusion can be reached. Sampling is continued until

the estimate show acceptable precision. The major advantage of this approach is that it usually minimizes sample size, thus saving time and resources. Plots methodology is “widely used to sample habitat characteristics and count animal numbers and sign” (Silvy, 2012). Plots can be circular, square, or rectangular encompassing small geographic areas as an element of the geographically defined population. Another method known as point sampling is used which is more applicable to sample bird density. Similarly, a transect strategy is used using a straight line or series of “straight line segments placed in the area to be sampled”. Transects are useful to obtain systematic samples of spatially distributed populations where sampling plots are established and plots established along transects are actual sample units. In some cases, the sampling strategy called road sampling can be used where sampling from roads is a widely used method for obtaining observations of species sparsely distributed over large areas or for distributing observations of abundant species over a large geographic area.

3.5 Mammal sampling through transect survey

One of the common methods of direct observation, although not an efficient method, is to detect mammal species and most thorough record of specific signs indicating their presence such as tracks, hairs, scats, dung piles, scent marks, or scrapes can be done by employing transect survey. This method is also commonly employed by the Department of Forests and Park Services (DOFPS, 2020). This method is relatively easy and can be done in situations where financial and human resources are limited.

3.6 Survey design and methods

- » The areas to be sampled should be designed with a specific grid as per the requirement of data sets such as rapid snapshot or detailed habitat characteristics.
- » Mammal transect survey should be carried out in the areas of interest of specific species in the mapped grid.
- » Specific protocol can be used as specified in Annexure II.
- » In the specific areas, mammal transect surveys should be carried out periodically such as during the same season and following the same transect trails.
- » Mammals sightings and mammal signs including tracks, scats, dung, scratch marks, etc., encountered on transects should be recorded.
- » GPS location at each sighting and at every 500m distance covered should be recorded in the datasheet even if there is no animal or sign observed.
- » In case of animal sighting, GPS coordinates, the number of animals, sex and age of the animal should be recorded as per the Annexure II.

3.7 Materials required

- » GPS device
- » Compass
- » Clinometer
- » Topographic maps
- » Survey forms
- » Pen/Pencils
- » Plastics bag, in case of sample collection such as scats.
- » Camera

3.8 Monitoring protocols using camera traps

Camera-trapping is a non-invasive tool to study wildlife, although it is one of the expensive approach as to collect data but this approach can be able to gather information on a wide range of species simultaneously and continuously, over large survey areas for a long duration. "Cameras traps can be used as a monitoring tool to document detection/non-detection of a target species or to conduct a species inventory for a given area. The use of camera traps can also advance to major researches such as deducing wildlife abundance and density through Spatially Explicit Capture Recapture (SECR) models well suited for many wild felids with distinct body pattern. Camera trapping also helps in like estimating species occupancy, species interaction and activity pattern, etc." (DOFPS, 2020). For camera trap sampling design refer DOFPS, (2020).

3.9 Camera trap Installation methods

Camera traps can be placed on the trails as travel paths so that it becomes efficient in gathering information and is well suited to surveying terrestrial mammals, especially those known to use roads or trails as travel paths. While installing camera traps, the following points should be kept in mind:

- » Identify the best locations for efficiency capture of information.
- » It is not advisable to place cameras too close to trails but should be placed at 2-4 m from the centre of the trail.
- » Cameras should not be placed beyond 6-8 m as the pictures captured would be blurred especially for smaller species.
- » Place camera at 20-40 cm or knee height by fixing on a tree or any object.
- » Cameras should be placed parallel to the ground to ensure effective capture.
- » Camera should not be placed directly facing each other and also ensure that camera is not placed against the sunlight.

3.10 Small terrestrial mammal assessment

Small mammals such as mice, rats, squirrels, porcupines, etc are seen quite often but they do not have tracts and are at times difficult to identify yet they can be sampled efficiently following the effective protocols and methods. Although some of them such as sampling bats may require specialized equipment but others can be sampled using special kinds of traps.

In most cases, the data generation refers to taking into account the species diversity. While sampling habitat stratification is necessary to compare species diversity and relative abundance of small mammals among different habitat types (Patrick, 2012, DOFPS, 2020).

3.11 Setting of trap line

Within the identified habitat, which may not be stratified based on the homogeneity or heterogeneity of the habitat, sites for sampling can be randomly selected. Either the national standard of sampling grid of 4km X 4km can be followed or if the area to be sampled is relatively small, a random transect line based on the heterogeneity of the habitat can be employed. There are number of types of traps as detailed below:

3.11.1 Dry pitfall traps

Pitfall traps are very widely used in fauna studies for the capture of small vertebrates and sometimes invertebrates as well.

Materials: Any container that can be sunk into the ground such as a 20 litre plastic bucket or PVC pipes can be used as a pitfall trap. However, it should be kept in mind that anything that is used a pit fall trap must have designed for adequate drainage.

Setting up of pitfall traps: Pitfall traps are installed by digging a hole, so it depends on the type of ground surface whether it is a sandy surface or a rocky one. When selecting trap locations, it must be good to keep in mind not to incur direct damage to vegetation, and also it should be made sure that the place where the pitfall trap is placed does not receive direct sunlight from late morning to mid-afternoon. Always ensure there is some sand and leaf-litter at the bottom of a pitfall. This calms animals and offers them some protection from cold, heat and predators.

3.11.2 Sherman Live Traps

Sherman live traps can be used as standard, foldable, portable, and efficient trap choices for most terrestrial small mammals. These traps can be found in several sizes which can be used based on the size of the small mammals. Sherman traps can be placed on the transect lines at an interval of 10 meters, which comprises about 10 traps in a 100 m transect.

Baits should be used to attract the animals and to serve as food while caught in the trap. However, precautions should be taken so that the feed does not cause a faulty trap.

3.11.3 Collapsible Tomahawk trap

These kinds of traps are usually larger than Sherman traps and can be used for species of civets, mongoose, squirrels, etc. since the traps can be of varying sizes, and they also be used to capture small carnivores. Therefore while handling the animals, it is necessary to administer anesthetics through injection.

3.11.4 Monitoring and re-baiting of traps

Traps are baited the first day and as necessary re-baited the following days. Also make sure that the trap lines are run for a period of 3-5 days (Hoffmann *et al.* 2010). Traps should be set before sunset and checked as early as possible the following morning. Trap inspection at shorter intervals can prevent the animals from dying in live traps or being eaten by ants or predators in snap traps. Trapping during days can be done only if the diurnal animals are present.

3.11.5 General handling procedures

Unlike large mammals, small mammals can be handled without anesthesia unless specific samples such as the collection of tissue are required.

The captured animals should be handled carefully and transferred to a handling bag by shaking the animal gently to the side of the trap door. Once the animal is transferred to the handling bag, take the weight of the animal using the Pesola spring scale. Once the animal is on hold firmly, securely and carefully, and note down the species, sex, age, colour, etc. to identify use the following general keys (DOFPS, 2020) as mentioned below:

- » Shrews have long mobile noses, sharp insect-eating teeth and small eyes.
- » Moles live underground, have small or no ears, small or no eyes, and small or no tails, Voles are characterized by a stout body, small and rounded ears, short legs, relatively large eyes, and a tail that is shorter than the head and body.
- » Rodents are all colours and sizes but all have gnawing teeth with a space behind them.

3.11.6 Materials required

- » Sherman live traps,
- » Collapsible Tomahawk trap
- » Cans/bucket for pitfall traps,
- » Ribbon,

- » Cloth sack (handling bag),
- » Permanent marker,
- » Track tubes,
- » Duct tape,
- » Bait (grain, vegetables, meat or locally available fruits),
- » U-shaped wire hold downs,
- » Gloves,
- » Mask,
- » Ear tag,
- » Ear punch,
- » Ruler,
- » Zip lock,
- » GPS,
- » Camera,
- » Pesola scale (different weights)

4.1 Sampling for bird species

A number of techniques are available for bird surveys and methods for rapid assessments enumerating bird species vary depending on the objective of the studies and also type of species focussed. The two different methods of bird survey are recommended for this manual, point counts and line transects. In point counts, it involves walking to and usually marking a particular spot, and then recording all bird sightings for a pre-determined period (often 5 to 10 minutes) before moving on to the next point. In the line transects, observers continuously walk a certain distance and record all the birds observed on either side of the track walk.

The general objective of bird surveys is to cover as many of the natural habitats present within an area as possible, and also focus on specific habitats of certain species if necessary. For the general purpose, a national grid of 1 x 1 km can be used corresponding to the recommended grid size (refer DOFPS, 2020). Methods such as transects, Mackinnon list, general observation, acoustic recording, and mist-netting, can be also used for bird surveys.

For the long-term benefit of monitoring the WBH landscape, it is of greater importance in integrating the distance sampling on point counts or line transects. In the distance sampling method, an estimate is made of the distance from the bird's contact to the center of the point count site or to the line which a transect walk is following. These distance estimates are used to calculate bird densities and, of particular importance, they take account of the fact that some birds are detectable over much greater distances than others, and that a species may be more easily detected in one habitat than another. Thus, even if calculating total population sizes is not the main aim of the project, collecting the distance data will allow you to make direct comparisons between species and between the same species in different habitats.

Distance sampling using Point Count

- » Point counts are often preferred to line transects when surveying less mobile bird species, and in more fine-grained habitats.
- » Sites/stations should be positioned randomly within the sampling units or habitat types. In order to get adequate coverage in each unit, you could adopt a stratified random technique.
- » An approximate spacing of minimum distance between stations in dense forests should be 200 to 250m.

- » Contacts can also be assigned to distance bands in the same way as outlined for line transects.
- » Count period of between five and ten minutes at each station is recommended.
- » Following information to record:
 - Estimated distance of each contact to a designated point and not to the observers, who may not be standing on that exact point.
 - Record the exact time of each contact or assign them to a one or two minute block time period.
 - Information on sex, type of contact, the height of contact in the foliage and group size can be recorded in the same way as with line transects.
 - Birds that fly away from the immediate area are recorded and a distance estimate is made to their point of departure.
 - Birds that fly into the area and land, or fly over the area, can be noted but should be excluded from the data analysis.

Data collection format

The following data collection form could be used to tabulate the survey data.

Station No.	Habitat	Start Time	Time Period	Species	Count	Distance
1						

Distance sampling using Line Transect

- » The site of the transect should be laid out randomly or through a stratified random technique.
- » The total length of each line-transect should be 1 km.
- » Each transect can be partitioned into distance intervals along its length for the convenience of tracking.
- » On each transect, the observers walk at a fairly constant speed, looking at either side of the line, and estimate the perpendicular distance from the line to each bird contact. There are two ways of estimating the distance: 1) distance between the bird and the line, or 2) distance between the observer and the bird, and the angle of the sighting away from the line.
- » Two distance belts is the minimum required for density estimation but it is better to have more and it is usual to vary the widths so the bands closer to the line are

narrower. You could, for example, have 5, 10, 15, 20, 30, 40, 60, 100, 200+ metre limits. The WBH landscape is usually of dense habitats where most of the bird contacts will be close to you, it is better to have narrower bands.

- » Following information to record:
 - Bird species and the sex of the individual birds (if possible);
 - Count of each bird species
 - Distance estimate.
 - The type of contact, e.g. was it a visual sighting or was the birds singing, calling, or flying?
 - The time of day of each contact;
 - The height of the bird e.g. ground, low, mid-strata or canopy.

Data collection format

The following data collection form could be used to tabulate the survey data.

Transect No.	Habitat	Species	Sex	Count	Distance

4.2 Advantages and disadvantages of point counts and transect counts

4.2.1 Advantages of point counts and transect counts

- » Can be standardised and data statistically analysed.
- » Produces species abundance indices with error margins.
- » Widely used, understood and tested in a variety of habitats.
- » Habitat descriptions can be recorded at count stations.
- » Better at describing community structure.
- » Can use distance sampling to produce estimates of abundance.

4.2.2 Disadvantages of point and counts transect counts

- » More time consuming than other approaches.
- » Potential data is lost when walking between point count stations.
- » Less likely to record inconspicuous or rare species.
- » Likely to record fewer species overall than observational surveys.

4.3 MacKinnon lists

This method is a quantitative approach to visual/auditory surveys of birds whereby an observer starts walking a transect route and records the first species heard or seen, and then the second species heard or seen (Patrick et al, 2014)

This process is continued until a list of 20 species has been completed. "If more than one individual of the same species is observed before the list of 20 unique species is complete, the repeat observations are discarded. At the completion of the list, a second list is started from new; this process is continued until sufficient lists are completed" (ibid).

4.3.1 Advantages of the MacKinnon list technique

- » Can be standardised and data statistically analysed.
- » Produces species abundance indices with error margins.
- » Measures the magnitude of species richness.
- » Allows the calculation of species accumulation curves which helps determine when a site is adequately surveyed.
- » One of the most rapid of standardised assessment techniques in that the accumulation of data is largely continuous.
- » Because observations are recorded chronologically, the data from many observers across a site can be pooled.
- » Surveys can be undertaken throughout the day.
- » Potential applications for other taxonomic groups including mammals, insects and other groups.

4.3.2 Disadvantages of the MacKinnon list technique

- » Not very common and relatively few researchers have implemented this approach.
- » Though inter-observer consistency in species abundance indices was high, observers experienced with the avifauna of the area recorded significantly more species than the least experienced.
- » The sequence recording species may have a large influence on the relative abundance of a given species.
- » Not known if it produces consistency of abundance at lower survey efforts, etc.

4.4 Acoustic survey

Sound recordings are a common method of surveys even the bird watchers use to survey and monitor birds and can be employed as part of rapid surveys.

4.4.1 Advantages of acoustic surveys

- » Can be standardised and data statistically analysed.
- » Produces species abundance indices with error margins.
- » Can reduce the variability associated with different observers undertaking point and transect counts.

4.5 Materials required

- » Binoculars & Camera
- » Laser Range Finder
- » Altimeter/GPS Unit/Compass
- » Topographic Map of the
- » Survey Area
- » Field Guide/Bird Songs
- » Data Form/App
- » Pencil/Ball Point Pen

4.6 Ethics

The following must be dully practiced while conducting bird watching or bird surveys (refer DOFPS, 2020).

- » **Maintain Privacy:** Take care not to disturb either the birds or their habitats or offend other people while you are bird watching: respect private property and the privacy of others. Don't disrupt the enjoyment that other people are getting from their own activities.
- » **Observe Silence:** Be quiet, always. Birds have extremely acute hearing. Talk softly and as little as possible.
- » **Steady Motion:** Move carefully and regularly. Do not make sudden movements or point at a bird.
- » **Casual Approach:** Try to approach a bird casually as though you were not interested in it. If a bird feels it is the center of attention it invariably becomes alarmed and moves off.
- » **Avoid Flushing Birds:** Do not disturb birds hidden in the bush/foilage or especially near their nests. Be patient and wait to get a better look. Never throw stones to make a bird fly. Remember the welfare of the bird should always come first.
- » **Avoid Stress:** Avoid disturbing or stressing birds, especially breeding birds, which can be very susceptible to stress. They may abandon a nest with eggs or chicks if

they are unduly disturbed. Similarly, don't harass them with excessive use of bird calls, and don't disturb a nest or handle eggs or chicks that you come across.

- » **No Littering:** Leave nothing but footprints: avoid damaging the natural environment. Leave it just as you found it so that others can enjoy it too. This means taking any garbage away with you when you leave.
- » **Keep your good intentions to yourself:** Your intentions may be good, but it's often best not to interfere with birds that appear to be in distress. Baby birds on the ground can seem abandoned, but maybe just out of the nest and learning how to fly. The parents are probably close by and they will return when you have gone. Don't keep them waiting!
- » **Best Birding Hours:** Start bird watching in the early morning. Birds are most active in the first few hours of the day. They have another smaller peak of activity during the hour or two before dusk.
- » **Avoid Birding:** In general birds don't like windy, wet or very hot weather. Therefore, bird watching will be most successful on warm, sunny, still days.
- » **Avoid Colours:** Avoid wearing bright coloured clothing that will be easily detected by the birds.
- » **Over Play Playbacks:** Avoid excessive use of bird song playbacks. Do not play playbacks at very high volume especially around or breeding bird's territory.

4.7 Safety

- » Always go for bird surveys in the group, with a minimum of two members.
- » Be aware of potentially dangerous wildlife – elephants, bears, tigers, snakes etc.
- » Watch where you are walking. Having been intensely engrossed in bird watching in the air and trees, birdwatchers can easily lose track of immediate surroundings and bump into things or can trip over badly.
- » Prepare for the severe weather conditions, and beware of high altitude sickness. Check the weather forecast beforehand, carry rain gear, and wear appropriate foot wear and long sleeve shirts and pants to cover your arms and legs to avoid bites from snake and insects.
- » Most species of birds are sensitive to disturbance during the breeding season, therefore, extreme caution should be taken while counting the nests or pairs. It is preferable to count the nest from a distance. A bare minimum of time should be spent near a nesting colony. Most counts should be done in the morning or evening, and never during hot mid-day. Avoid going too near to the nests of raptors, especially eagles.
- » Carry adequate consumption provisions (food & water), first aid medicine and energizers (chewing gums, sweets).

5.1 Fish species assessment

5.1.1 Method for fish sampling

Fish sampling depends on the type of rivers. In case of a mountainous environment, fish sampling is relatively difficult. So among many fish sampling methods such as electro-shocker and cast nets can be used. Electro-shocker is efficient in small water bodies while cast nets can be used in rivers.

Timing of the sampling should be well designed to capture migratory species and sampling should be done during the spring season (March, April and May) and the rainy season is not a good time for sampling migratory species. Even non-migratory species may not be captured during rainy season as it is displaced by floods. Similarly, during the winter and full moon phase of the month sampling fish is not recommended.

5.1.2 Electro-shockers

While using electro-shockers for the fish sampling two small scoop nets should be used for the collection of fishes. A scoop mesh net having an eye size of 0.5 x 0.5 cm is recommended.

(CAUTION: electro-shocker using dynamo or car battery could be fatal.

5.1.3 Cast nets

Cast nets with varying mesh sizes are popularly used in almost all river types. However, using cast nets requires training as skilful fishermen tend to catch more fish than an untrained individual. Usually, a cast net with 6 feet, weighing 3-3.5 kg with an eye size of 0.8x0.8 cm is recommended.

5.2 Freshwater macroinvertebrate sampling

While sampling for fish it is vital to also sample macroinvertebrates which determine the quality of the aquatic ecosystem. Since the quality of the aquatic ecosystem affects the diversity of fish species, it should be taken as one of the indicators to assess fish habitat. For sampling macroinvertebrates, spring and autumn seasons are recommended as the best time which is also categorised as pre monsoon and post monsoon seasons in the monsoon affected regions (Shah *et al.* 2011; DOFPS, 2020). However, sampling would not be efficient during or shortly after the floods and also during the drying period of the spring. Following procedures is recommended while sampling macroinvertebrate as follow:

- » Start sampling the microhabitat from the downstream to upstream areas. Starting from the downstream areas would avoid or reduce disturbance to areas to be sampled.
- » It is recommended to place the kick net perpendicular to the flow of water current.
- » Once the net is placed, gently pick the boulders and cobbles over five cm and rub the organism from the stone to the net.
- » To get a maximum sample, it is also recommended to disturb the stream substrate gently with a hand or leg and collect freshwater invertebrates into the net.
- » Place the collected sample in the bucket.
- » Rinse the net thoroughly and add more water to make the water transparent for visibility in case the water is turbid.
- » Identify, sort and count individuals using standard identification keys.

5.3 Physicochemical properties of freshwater

Measurement of physicochemical properties of water must be taken prior to sample collection at the site or from each sampling unit. It is recommended to measure the physicochemical properties at the beginning and at the end of the sampling process.

5.4 Equipment and materials

Following equipment are recommended which may depend on the sampling intensity, frequency and area to be sampled.

- | | |
|---------------------------------|------------------------------------|
| - electro-shocker | - spring balance |
| - scoop nets | - digital calliper for measurement |
| - cast net | - kick net (mesh size 500 µm) |
| - multiparameter testing kits | - kick net size 30 cm X 30 cm) |
| - dissolved oxygen meter | - measuring tape |
| - turbidity meter | - pipette, bucket |
| - boots | - bowls |
| - buckets | - petri dish |
| - formalin | - hand lens |
| - ethanol | - dissection kits |
| - specimen jars | - live jacket |
| - ice-cube trays | - hand gloves |
| - mini aquarium for photography | - sanitizer/soap |
| - GPS devises | - knife |
| - tag guns (voucher specimens) | - label card |
| - tag gun codes | - Reference books |
| - micro-tubes | |

6.1 Sampling of herpetofauna species

In general, herpetofauna are relatively difficult to efficiently sample (Heyer *et al.*, 1994; Tesche and Hodges, 2015). This is because many species of this group are fossorial, sedentary, and cryptic. Also the occurrence and the behaviour patterns of this group species are affected by factors such as temperature, humidity, and precipitation which may result to difficulty in detectability and sampling. The specific objectives of undertaking herpetofauna sampling that this manual would provide are to:

- » understand the diversity and an abundance of herpetofauna in the specific habitat.
- » relate the habitat characteristics to abundance of herpetofauna with habitat characteristics.
- » document the annual changes in species composition and relative abundance patterns across the different habitats.

6.2 Scope and limitations

The sampling techniques detailed in this section should be considered as general techniques only as the different species may need to sample employing specific techniques. Sometimes rather than a single technique, a combination of techniques is recommended. Hence, procedures for sampling for various subspecies dependent on many factors. Techniques used for reptiles may not be relevant for amphibians.

6.3 Sampling procedure

The most prominent methods to sample herpetofauna species are:

- » Pitfall Traps
- » Funnel Trapping
- » Systematic searches
- » Visual Encounter Surveys

The national monitoring protocol recommends Visual Encounter Surveys for Bhutanese landscapes (DOFPS, 2020). This method is relatively cheap and can be employed with ease (Manley *et al.*, 2004; Eekhout, 2010). Furthermore, employing this method can also avoid animal mortality, and reduce site disturbance.

6.4 Sampling effort and techniques

The sampling techniques such as (i) time-constrained searches; (ii) area-constrained searches; (iii) quadrat sampling; and (iv) transect surveys are suitable for both terrestrial and aquatic habitats (Eekhout, 2010).

6.4.1 Time-constrained searches

This technique is to actively search for animals in a given area for a pre-defined time frame and the additional time required to collect additional information such as body measurements or marking individuals would not be considered as part of the search. This search technique is mostly applied during terrestrial surveys. The main limitation of this technique is the requirement of long periods of time that the survey participants need to commit. Also, the time constrained searches are affected by environmental factors such as time of the day, season, and weather. Similarly, the experience of the researchers is also another factor in collecting the intensity and quality of the data.

6.4.2 Area-constrained searches

As the name suggests, the area-constrained searches focus on a certain area and not on an amount of time. This technique would be helpful in collecting data pertaining to the absence or presence of species, in that particular area and information concerning the life history of the species such as time of reproduction, activity patterns, and habitat use. The main limitation of this technique is mainly the environmental conditions and the experience of the workers.

6.4.3 Quadrat sampling

Like the vegetation sampling techniques, the area to be sampled would be randomly distributed and the plots are laid sometimes along transects or distributed randomly where the data concerning the absence or presence of animals is collected. The main limitation of this technique is that the set up itself can be daunting and time consuming.

Within quadrat sampling, point sampling with small squares can be used. 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" (Rodda *et al*, 2005) Sometimes, a modification of this technique is called 'patch sampling', where specific microhabitats are present, can be used. "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" (ibid).

6.4.4 Transect surveys

A linear transect is another sampling design where the whole narrow strip is sampled. This technique is usually useful for surveying herpetofauna across environmental gradients but can also be used within a single habitat. This method can be effective in documenting a good representation of the occurring fauna and overall habitat types. Transects can be set in parallel to the gradient studied or perpendicularly to a gradient and both techniques would be helpful in analysing species abundance across or along the environmental gradient (Eekhout, 2010). Transect surveys need to meet the following assumptions:

- » Specimens are randomly distributed throughout transects.
- » Transect lines are randomly chosen.
- » All the specimens in transect would be observed.
- » Animals will not be counted twice within transect and among transects.

6.4.5 Visual encounter surveys

This technique is one of the safest ways to collect data without disturbing the animals and the habitat. In this technique, observers walk transects and observe individuals and their position relative to the transect path. This survey provides occupancy, density, species richness, location, and frequency for surface-active species.

6.4.6 Pitfall trapping

This is similar to the small mammal sampling where the two different pitfall designs can be used. The first one is using the aluminium drift fences having arrays of pitfall traps and the other is grids of single pitfall traps without fences. The choice to install the specific type of trap depends on the need of the study. Arrays are superior for catching reptiles, relative to the grids of single pitfall traps. The design for pitfall traps are given below:

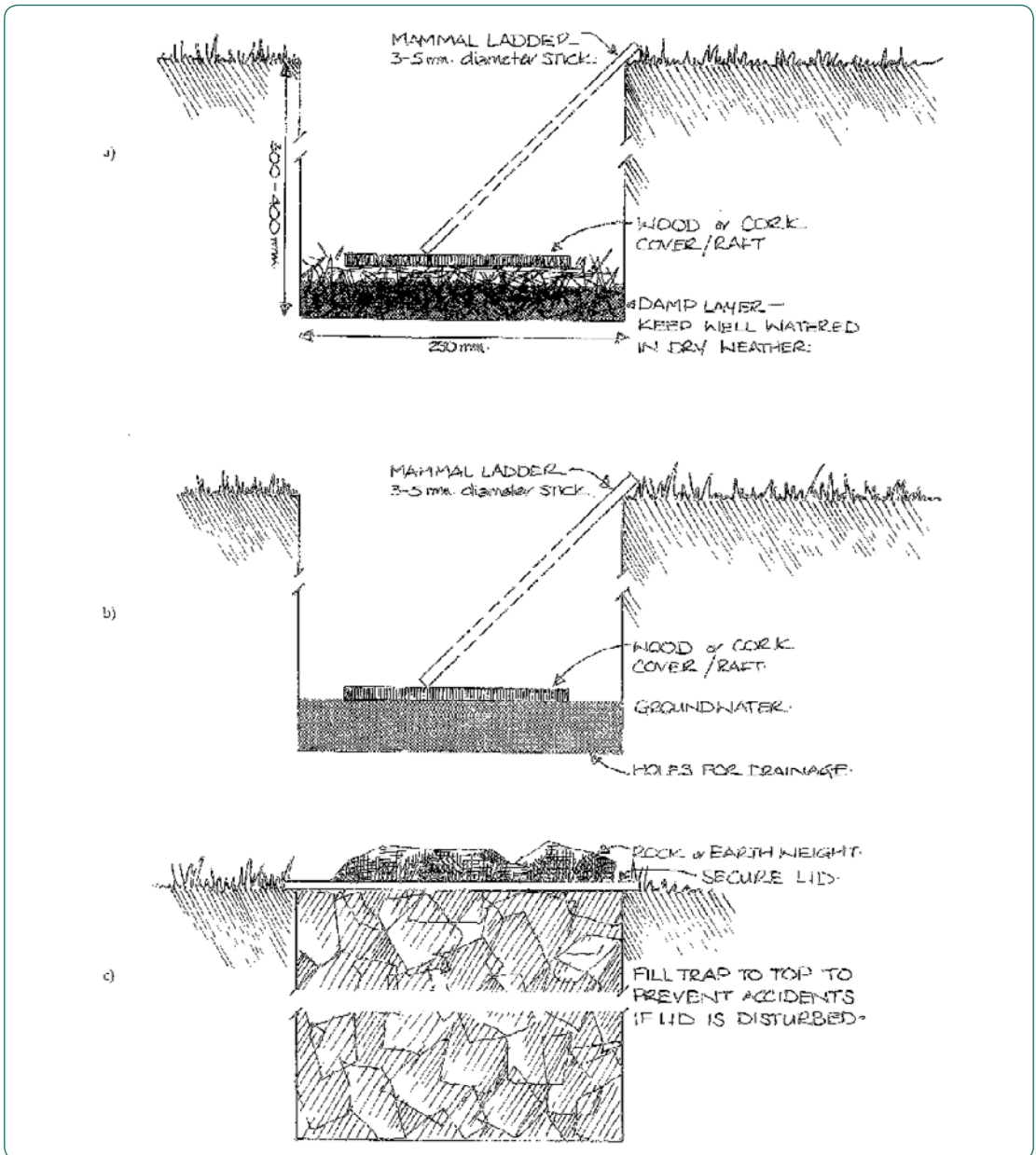


Figure 9: Design for pitfall traps for the safe capture of small amphibians; (a) dry soil conditions in the bottom; (b) Wet soil conditions; (c) closed pitfall trap (Source: Gent and Gibson, 1998).

6.5 Marking reptiles and amphibians

Specific colour of paints that do not affect reptiles or nail polish can be used to mark dots on the scales of snakes and lizards. Each captured animals can be given unique marks. Unlike reptiles, amphibians can be marked by using toe-clipping.

6.6 Materials required

It is necessary to look into the required and necessary equipment. Some of the equipment but not limited to are listed below. The list is also prepared in reference to national standard protocol (DOFPS, 2020).

6.6.1 Materials needed for handling snakes

- » Good quality camera
- » Snake Hook/Tongs
- » Torches
- » Snake boots
- » Snake tubes for handling venomous snakes
- » Protection clothes
- » First aid kits
- » Reference books

6.6.2 Other materials required

- » Water thermometer and pH meter
- » Pair leather gloves
- » Field guides and Anuran call tape for reference
- » Hand lens
- » Pair hip waders
- » Voice recorders for an acoustic study
- » Nail polish, Cuticle scissors, Clipboard
- » Pencils, Sharpener
- » Hand sanitizer
- » Plastic Ziplock Baggies

6.6.3 Materials needed for Pitfall installation and operation

- » Posthole digger
- » 15-m tape, Nylon rope, Plastic flagging
- » Waterproof ink marker
- » Bucket (medium-sized)
- » Wood covers grid or array
- » Waterproof notebook and paper
- » Plastic cup or long handled spoon

7.1 Importance of invertebrate survey and voucher specimen

Surveying insects is an essential practice that helps inform conservation decisions and land management practices. Where possible, species are identified in the field or from photographs, although this is only an option for a small selection of taxa. Many insects are so small, or have very microscopic features between species, that it makes microscope use essential for identification. Unfortunately, this does mean that specimens have to be taken, killed, and preserved. However, once they have been identified and recorded, they become vital data for scientists and conservationists to evidence wildlife and environmental trends. In addition to this, preserved specimens are used as a reference to aid the identification of future samples.

7.2 Selecting sites for the survey

Site selection for surveying will depend on the reason for a survey, permissions and target species. For overall monitoring of insect biodiversity, stratification of the survey sites based on altitude, vegetation, gradient, distances from stream or river, human settlement, cultivation types etc. are critical. Sampling for a range of invertebrates would require sampling in as many different habitats as possible over a considerable period of time and using different methods. Invertebrates have complex life cycles, and aspects such as time of year, time of day, temperature, weather, and sampling method will affect the species collection. While most of the invertebrates are diurnal, some are nocturnal, and amongst diurnal, most are active during bright sunny days.

7.3 Preparation for field survey

7.3.1 Preparing euthanizing jars

Collecting jars are simply any jar that has some sort of euthanizing agent (ethyl acetate) in it and can be of different sizes based on the target taxa. Place a layer of plaster of paris of approximately 1/2 to 1 inch thick at the bottom of the ethyl acetate resistant jar or tube, and pour ethyl acetate until the plaster is saturated. Place folded and cone-shaped papers into the jar to help avoid soaking the specimens. Such jar euthanizing is practiced for most aerial insects. For dragonflies and damselflies, an air tight, flat-bottomed plastic container half-filled with acetone is used as a euthanizing container.

7.3.2 Equipment for field survey

- » Aerial or sweep nets
- » Aspirator
- » Beating tray
- » Sieve
- » DNA vials with 70-90 % ethanol
- » Pen, note book, GPS
- » Other safety gears

7.4 Survey in the field

7.4.1 Active survey

Searching for insects in the environment, and is often used to get particular types of insects, or insects that are found on particular substrates or plants

- » *Netting* – Aerial or sweep netting is one of the easiest ways to survey insects and arthropods, and involves waving a net through vegetation. Invertebrates can then be removed from the net using collection tubes and jars.
- » *Direct Searching* – It includes looking under shelters such as rocks and logs, or searching through vegetation. A pooter can also be useful to aid in capturing invertebrates as you find them. Using this method, you can find a huge variety of invertebrate species, from earthworms to harvestmen and millipedes to beetles.
- » *Sieving* – Comparable to direct searching, sieving can help extract invertebrates from small debris such as leaf litter and rotting wood. This is another method that can pick up a vast range of invertebrates such as larvae of flies or beetles, plus adult organisms such as worms, pseudoscorpions and earwigs.

7.4.2 Passive collection

Involves the use of traps or baits, and is less discriminating in the type of insect collected.

- » *Pitfall Traps* – These traps are set to capture surface-living invertebrates in areas with low vegetation or bare ground. Containers are dug into the ground, with the top becoming level with the ground's surface which invertebrates will fall into. However, we discourage pitfall trapping unless it's essential, as it is an indirect trapping method resulting in lots of by-catches.
- » *Light Traps* – The use of light will attract many night-flying insects. These traps can range in complexity, but essentially they emit white/blueish light, and the insects it attracts can then be temporarily or permanently trapped. This method is perfect for monitoring moths and night-flying beetles and other insects.

7.4.3 Collection of DNA samples

For all the insect groups, the legs are usually preferred because their removal does not affect the general aspect of the specimen. A single leg (or 2) or a leg fragment (2 to 3 mm long) is sufficient for DNA extraction. The parts or whole (small larvae) can be directly stored in DNA vials containing 70-90% ethanol. Label properly.

7.4.4 Euthanization of specimens

Some species are identified in the field or from photographs (based on surveyer's expertise). If unidentified, transfer the gathered specimens from the net into euthanizing jar. Beware of stinging insects. Depending on the size of the specimen, euthanizing time ranges from 5 minutes to several hours. Large butterflies can be euthanized by pinching the thorax between thumb and forefinger, and for further euthanizing, specimen may be placed in the euthanizing jar. Dragonflies and damselflies should be placed in a paper envelope with wings folded and left alive in the envelope for at least six hours to allow them to void their gut contents. Remove specimens from the envelope and immerse them in acetone jar after which most specimens die in 10 to 20 seconds (any labelling should be written in pencil as acetone dissolves most inks). Once the insect is dead, it should be left in the acetone for 8-12 hours. This removes fat, aiding color preservation and making the specimen less attractive to pests.

7.4.5 Labelling with collection information

During euthanization process or upon euthanized, the specimens should be transferred to tubes or containers, and label the information such as collecting site, collector, date, altitude, geo-coordinates etc.

7.5 Laboratory work

7.5.1 Basic requirements for laboratory work with invertebrate specimens

- » Insect pins of varying size
- » Goose-necked table lamp
- » Stereo-microscope
- » Dissecting microscope
- » Stacking microscope
- » Forceps, entomological water soluble glue, pinning block, hair brush
- » Boxes (relaxing, display), white tray, cabinet

7.5.2 Relaxing

If specimens are allowed to dry out or become brittle, they may shatter when being pinned. Dry specimens can be made soft and pliable again by placing them in a relaxing chamber for 1 or 2 days. To make a relaxing chamber, place 1 to 2 inches of clean sand or sawdust in the bottom of a large, airtight jar. The jar must be large enough to allow small dishes to be placed inside, and must have a screw type or other lid to create airtight conditions inside the bottle. Saturate the sand with clean water and add a few drops of carbolic acid (sold at most drug stores) to prevent mold growth. Place the specimens in shallow, open containers on the bottom of the jar, and fit the lid tightly on the relaxing chamber. The amount of relaxing time needed varies with the size of the insect and how dry it is when first introduced. Retrieve and mount specimens as soon as they are pliable enough to pin easily, but remember that they can be ruined if left in a relaxing jar too long. Relaxing the wings of dry butterflies and moths is essential to allow them to be properly spread.

The relaxing chamber should be used for emergencies, not as a general practice. It is always best to pin specimens within a few hours after collection and avoid the need for relaxing chambers.

7.5.3 Mounting

Once the specimens are collected, the next step is to preserve them properly for future study and display. Various insects will require different methods of preservation; soft-bodied insects like larvae or termites are preserved in liquids while larger specimens are mounted on insect pins of varying sizes. Insects that are too small to be mounted on pins are glued to a triangular point.

- » **Preserving Soft-Bodied Insects in Alcohol:** Soft-bodied insects (larvae, scale insects, termites, aphids, silver flies, earwigs etc.) are preserved in plastic or glass vials with ethanol (70%-85% isopropyl or ethanol alcohol is ideal). Non-hairy insects can be preserved in alcohol as well.
- » **Pinning Insects:** Any insect large enough to be pinned without the risk of breaking or distorting it is pinned from the top-right side, straight down through its body. Generally, the area is just slightly right of the midline of the insect's thorax is preferred for pinning basically to reduce the risk of damaging important identifying characteristics. For proper mounting, the use of a pinning block is recommended. Use only pins designed for pinning insects as other household pins may rust and damage your specimens.
- » **Pointing Insects:** Pointing is an option for mounting insects too small to pin through the thorax. The insect is glued on its right side onto a small triangular strip of cardstock, and pinned through the cardstock.

7.5.4 Labelling

Country, location, geo-coordinates, elevation, collection date, collector, a brief description of habitat etc. should be labeled and retained with each specimen. Identification labels can be added separately (family, species, unique specimen number, identifier and year of identification).

7.5.5 Preservation

The well-mounted and labeled specimens should be deposited in a well-facilitated repository center (such as National Biodiversity Centre, Bhutan – NBCB). A simple method to preserve the specimens is to prepare them as dried specimens and store them in a well-concealed box under dry and dark conditions with proper labels. Often collections are readily attacked by a variety of insect pests and destroyed by fungi infestation. Vigilance and precautionary measures must be rigorously enforced. Vigilance includes making at least a quarterly visual examination of each box or drawer and field specimens (alcohol collection) for the sign of pest infestation and the need for the replacement of preservatives. Type specimens for new species should be prioritized for preservation and curated properly.

7.5.6 Identification

Minute morphological structures along with male genitalia are used for species identification. While keys, description, illustrations, and photographs are important for the identification of specimens, comparison with holotypes, paratypes, lectotypes, syntypes etc. are indispensable for correct identification and most importantly for the description of new species.

7.6 Ethics

- » The specimens should be immediately euthanized properly and with respect.
- » No more specimens that are strictly required for a specific purpose should be captured or ethically euthanized.
- » Do as little damage to the habitat as possible.
- » The catch in a trap (e.g. light trap) should be released after being examined, except for any specimens that must be euthanized for voucher purposes or for ecological or other scientific studies.
- » If a trap used for scientific purposes is found to be repeatedly catching rare or local species unnecessarily, it should be re-sited.
- » When coleopterists (or others) work on dead wood or bark, they should leave a substantial proportion untouched in the locality. Where practicable, detached

bark and worked material should be replaced.

- » Overturned stones and logs should be gently replaced in their original positions unless very deeply embedded.
- » Specimen should not be collected from the hive of bees or wasps or poke the hives, since they are aggressive in nature and their sting is lethal in some case.

7.7 Safety

- » Wear a hat and light-colored clothing (so ticks can be easily spotted), including long-sleeved shirts and long pants tucked into boots or socks.
- » Use insect repellent for greater protection.
- » Tuck pants into socks or boots.
- » Workers with a history of severe allergic reactions to insect bites or stings should carry an epinephrine auto injector
- » Seek immediate medical attention if a sting causes severe chest pain, nausea, severe sweating, loss of breath, serious swelling, or slurred speech.
- » Chemicals like ethyl acetate, naphthalene ball, paradichlorobenzene (PDCB) crystals, thymol or phenol are toxic in nature and should be handled with care.

To analyse the wildlife related data, certain concepts such as sampling intensity and statistical power and variability among the sampling units need to be understood clearly. Data obtained with improper sample size exposes animals to risks with incorrect conclusions. There are five interrelated components that influence sample size determinations that eventually influences conclusions (Silvy, 2012).

- » Significance level: “This concept includes type I error and type II error. The former is rejecting the null hypothesis when it is true, and the probability of committing this type of error is controlled by the alpha level (α) of the test (frequently $\alpha = 0.05$) while the latter is failing to reject the null hypothesis when it is false, and the probability of committing this type of error is controlled by the beta level of the test (frequently $\beta = 0.05$).
- » Power: Power is the probability of rejecting the null hypothesis when it is false, and it is controlled by adjusting beta (i.e., power = 1 – β). Increased power results in requisite increases in sample size, due to the relationship between power and beta.
- » Effect size: Effect size (d) is the difference between treatments (e.g., in number of animals seen) relative to the noise in measurements. And it expresses the magnitude of difference between two sample means and therefore is the logical complement to the P-values generated from statistical hypothesis tests. Effect size can be written as $d = \frac{x_1 - x_2}{s}$ which scales the difference in population means 1 and 2 ($x_1 - x_2$) by the standard deviation.
- » The sample variance (s^2) or standard deviation (s): The standard deviation is calculated as positive square root of the sample variance: $s = \sqrt{s^2}$. The standard deviation or the Sample variance would tell us the variation of data among the samples.
- » Sample size: Sample size (n) is the number of samples required to obtain the desired precision in an estimate or the desired power in a hypothesis test. Larger sample sizes generally lead to parameter estimates with smaller variances, giving you a greater ability to detect a significant difference. Sample size is typically the variable being solved for in the planning stages, but it can be an input variable when you are attempting to estimate power. For example, to determine the sample size required for comparing 2 populations with equal variance in a 2-tailed hypothesis: $n = \frac{2(z_{1-\frac{\alpha}{2}} + z_{1-\beta})^2}{\frac{x_1 - x_2}{s}}$, When $\alpha = 0.05$ and $\beta = 0.20$, the corresponding critical values from a standardized normal probability table (z-values or z-scores) become 1.96 (z-score for α , the probability of committing a Type I error: $z_{1-\frac{\alpha}{2}}$) and 0.84 (z-score for β , the probability of committing a Type II error: $z_{1-\beta}$), respectively” (Silvy, 2012).

8.1 Species distribution and detection probability

Wildlife are not randomly distributed, which can create bias in estimates of animal abundance. It is even more complicated when animals exhibit clumped. The survey design such as random sampling or stratified random sampling therefore becomes crucial in alleviating such problem. It is impossible to detect all animals during sampling therefore it is important to use standardized methods when conducting the surveys. The second is the use of covariates in analyses of survey statistics and the third approach is to recognize that detection probability is not constant over space or time.

8.2 Measuring indices

The wildlife detection data would represent mostly the number of individual animals or animal sign information along transects, at quadrats, or points. The index methods include the number of animals seen per kilometer of road, the number of animals present per night at a waterhole, faecal pellets per quadrat, and nest or burrow counts per kilometer of transect. Similarly, the frequency of occurrence index only collects presence or absence data.

8.2.1 Counts on sample plots (fixed area)

In this analysis the area being counted is fixed in terms of length and width prior to the start of the survey.

The mean density from all sample plots is then extrapolated to the entire study area, giving an estimate of average density and/or population abundance for the area of inference.

8.2.2 Strip counts

In this method, the counting unit is a strip or transect, which is merely a long, narrow rectangle of fixed area. Density is calculated as the ratio of the sum of counts to the sum of strip areas (Silvy, 2012):

$D = \frac{\sum xi}{\sum a}$, the density obtained on the sample strips is then multiplied by the size of the study area (area of inference) to obtain population size: $N = DA$.

By combining the 2 formulas, we obtain the simple strip abundance estimator: $N = \frac{A \sum xi}{2Lwns}$

The variance is obtained from the strip counts using

The strip count standard error is then

$$SE_{\bar{x}} = \sqrt{\frac{s_x^2}{n_s}}$$

The variance of the population estimate when sampling with replacement (SWR) is

$$s_N^2 = \frac{(n_t)^2}{n_s} s_x^2,$$

where nt is the total number of samples possible on the area of inference (calculated by A/a). The variance of the population estimate when sampling without replacement (SWOR) is

$$s_N^2 = \frac{(n_t)^2}{n_s} s_x^2 \left(1 - \frac{n_s}{n_t}\right).$$

Example: We wish to estimate the number of grouse on a 2-km² study area. We utilize 5 counting strips, each 100 m in length with a present sighting distance of 10 m (0.5-strip width). We divide the study area into strips and select 5 to survey using a random number table. We count each strip, flushing a total of 15 grouse ($x_i = 4, 3, 3, 2,$ and 3). The total possible number of samples of this size is 1,000 ($nt = A/a$). Therefore, the estimated population abundance would be calculated as follows:

$$N = \frac{(2 \text{ km}^2) (15)}{(2) (0.1 \text{ km}) (0.01 \text{ km}) (5)} = 3,000.$$

The strip count variance ($s^2 = 0.50$) is then used to obtain the strip count standard error ($SE\bar{x} = 0.7071$). The variance of the population estimate when SWR is then

$$s_N^2 = \frac{(1,000)^2}{5} (0.50) = 100,000,$$

and the standard error of the population estimate when SWR is $SEN = \sqrt{100,000} = 316.23$.

We can then calculate the 95% confidence intervals when SWR as $95\%CI = (\pm 2.776)(316.23) = \pm 877.85$. The population estimate, $\pm 95\%CI$ when SWR, is $3,000 \pm 878$ grouse. If we had obtained the counts by SWOR, the Population estimate would remain the same, but the variance of the population estimate would change:

$$s_N^2 = \frac{(1,000)^2}{5} (0.50) \left(1 - \frac{5}{1,000}\right) = 99,500.$$

The standard error of the population estimate would become $SEN = \sqrt{99,500} = 315.44$, and the resulting 95% confidence interval would be $95\%CI = (\pm 2.776)(315.44) = \pm 875.66$. The population estimate, $\pm 95\%CI$ when SWOR, is $3,000 \pm 876$ grouse (Source: Silvy, 2012).

8.2.3 Point counts

This technique is typically used to estimate bird density, it is generally assumed that all birds are detected within the sample radius, and sometimes this assumption could be false. The form of the equation for the simple population estimate, is given as:

$$N = \frac{A \sum x_i}{n \pi r^2}$$
, where N = population abundance; A = area of study area; x_i = number of birds seen within a fixed radius r , of point i , n = total points sampled, π = pi (ratio of the circumference of a circle to its diameter)

r = preset radial distance.

Example: A survey consisting of 10 random points, each with a fixed radius of 50m, is conducted on a 2-km² study area. Surveyors count 50 birds. The estimated population abundance would be calculated as follows:

$$N = \frac{(2 \text{ km}^2) (50)}{(10) (3.1416) (0.050 \text{ km})^2} = 1,273.24.$$

The sample variance (s_x^2), population variance (s_n^2), and population standard error (SE_n) are calculated using the strip count equations for SWOR. We then obtain a point count variance of 0.6667, a population variance of 4,238.13, and population standard error of 65.1. Our calculated 95%CI is then ± 147 birds. Therefore, the population estimate ($\pm 95\%CI$) for the study area is approximately 1,910 ± 107 birds. We would report the population estimate $N \pm SE$ (e.g., 1,273 ± 65 birds), which would allow other investigators to derive confidence intervals of their choice from the data.

8.2.4 Sample units of unequal area

Samples units of unequal area generates different data and it require an average density to be calculated from all units sampled. The average density (D) is then extrapolated to the survey area using $N = DA$. However, the formulas for SWR and SWOR differ for samples of unequal area:

$${}_{SWR} s_N^2 = \frac{(n_t)^2}{n_s(n_s - 1)} [\sum x_i^2 + D^2 \sum a_i^2 - 2D \sum (x_i a_i)]$$

$${}_{SWOR} s_N^2 = \frac{n_t(n_t - n_s)}{n_s(n_s - 1)} [\sum x_i^2 + D^2 \sum a_i^2 - 2D \sum (x_i a_i)],$$

where x_i = count from sample i , a_i = area of sample i , n_s = number of samples taken, n_t = total number of samples in study area, D = average density from the samples.

Example: (Source: Silvy 2012). We wish to estimate the number of grouse on a 2-km² study area. From a total of 784 possible transects, we selected 10 counting strips without replacement. Each strip had a different length, but each was surveyed with a preset

sighting distance of 10 m (0.5-strip width) on each side of the centreline. We counted each strip, flushing a total of 50 grouse, with the counts (x) and area (a) of each strip recorded. There are 784 possible transects on the study area. The estimated population abundance would be calculated as follows:

$$D = \frac{50}{0.0255 \text{ km}^2} 1,960.8$$

and $N = (1,960.8)(2) = 3,922$. The variance of the population estimate (SWOR) would be calculated as

$$\begin{aligned} \text{swor}^2_N &= \frac{784(784 - 10)}{10(10 - 1)} [256 + (1,960.8)^2(0.00006681) - \\ &\quad (2)(1,960.8)(0.1305)] = 7,403.4. \end{aligned}$$

Using the equations for strip counts, we obtain a population standard error (SEN) of 86.0. Our calculated 95%CI is then ± 229 birds. Therefore, the population estimate ($\pm 95\%$ CI) for the study area is approximately $3,922 \pm 229$ birds. Again, we would report the population estimate $N \pm \text{SE}$ (e.g., $3,922 \pm 86$), which would allow other investigators to derive confidence intervals of their choice from the data.

8.2.5 Counts on sample plots (estimating area)

The abundance of animals can be analysed following Hahn method, King method and Hayne method. Each method is highlighted as follow: Hahn method, is commonly used in estimating population density and is similar to the strip method and differs only in the use of distances to estimate the strip width defining the sample area. The Hahn estimate of population abundance is calculated as:

$$N = \frac{A \sum x_i}{2Lv}$$

where N = population abundance, A = area of study area, x_i = number of animals seen on transect l , v = the 0.5-strip width determined by average visibility measurements, L = total length of all transects.

The King method is useful by taking the average radial distance from all observed animals to estimate the 0.5-strip width to be applied in the calculations of density or abundance and is similar to the Hahn method:

$$N = \frac{A \sum x_i}{2L\bar{r}}$$

where N = population abundance, A = area of study area, x_i = number of animals seen on transect l , \bar{r} = the 0.5-strip width determined by average sighting radius, L = total length of all transects.

Hayne Method estimator of density, is

$$D_H = \frac{n}{2L} \left(\frac{1}{n} \sum \frac{1}{r_i} \right)$$

$N = DA$, where N = population abundance, DH = population density, A = area of inference, n = number of animals seen on each transect, r_i = sighting distance to animal i , L = length of transect.

The variance associated with this density estimate is calculated as

$$s_{D_H}^2 = D_H \left[\frac{s_n^2}{n^2} + \frac{\sum (1/r_i - R)^2}{R^2 n(n-1)} \right],$$

where DH = population density, n = number of animals seen s^2_n = variance of n , r_i = sighting distance to animal i

R = mean of the reciprocals of sighting distances r_i .

8.2.6 Counts on sample plots (plotless methods)

Plotless methods can also be used to estimate density and abundance to cover sparse species and also when data collection is constrained by boundary effect when plot technique is used to collect data. Such constrained can be addressed and are relatively easy to apply, so long as the target species remains in place. Under the plotless method two technique such as point to target and target to nearest neighbour method and point quarter method can be applied. Detailed examples can be accessed from Silvy, (2012).

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10.1 Annexure Ia: Vegetation Survey Protocol, ESRAM, Bhutan

Site No. Date: Time: Collector(s):
 Dzongkhag: Locality: Aspect:
 Latitude: Longitude: Altitude:
 Slope: Plot type (circle): Trees (15 x 15m)
 Lopping level (circle): 0, 1, 2, 3, 4 Mining activities (circle): Yes / No
 Grazing (circle): 0, 1, 2, 3, 4 Timber extraction (circle): 0, 1, 2, 3, 4
 Fire evidence (circle): 0, 1, 2 Distance to road in km:
 Distance to nearest settlement in km: Mammals' signs (droppings/scats):
 Soil stability (circle): 0, 1, 2, 3, 4 Potential for plantation (circle): 0, 1, 2, 3, 4
 Forest type (circle): sub-tropical, warm broadleaved, cool broadleaved, temperate, sub-alpine
 Forest canopy cover% (circle): open, partial shade, closed
 Birds (esp. predatory): Present / Absent

Species	Height (m)	DBH (cm)	Remarks*

Notes:

Lopping level: 0=absent, 1=1 to 3 trees lopped, 2=4 to 6 trees lopped, 3=7 to 9 trees lopped, 4=heavy, 10 or more trees lopped

Soil stability: 0=very unstable (erosion/slips visible), 1=unstable, 2=moderate, 3=stable, 4=very stable

Plantation potential: 0=no potential, 1=low potential, 2=moderate, 3=some potential, 4=high potential

Grazing: 0=absent, 1=less, 2=moderate, 3=heavy, 4=very heavy

Timber extraction: 0=absent, 1=less, 2=moderate, 3=heavy, 4=very heavy

Fire evidence: 0=absent, 1=recent, 2 old

*check for invasive species

Any other notes:

10.2 Annexure Ib : Vegetation Assessment Protocol, ESRAM, Bhutan

Site No.

(Shrubs – 4 x 4m)

Species	Height (m)	Number	Remarks

Notes: Plot size can be increased up to 5 x 5 m if resources permit

10.3 Annexure Ic: Vegetation Assessment Protocol, ESRAM, Bhutan

Site No.

(Herbs – 1 x 1m)

Species	Cover % OR number	Remarks

Notes: Plot size can be increased up to 2 x 2 m if resources permit

10.4 Annexure Id : Variables and parameters for plant diversity survey

Area of Inquiry	Variables	Parameters	Remarks
Vegetation	Trees (DBH \geq 1.3 m)	Height	For vegetation analysis
		Diameter	
		Species	
		Count	
	Shrubs (DBH \leq 1.3 m)	Height	

Area of Inquiry	Variables	Parameters	Remarks
		Species	
		Count	
	Herbs	Cover percent	
		Species	
Environmental	Physical	Slope/gradient	
		Altitude	
		Geo-coordinates	
		Aspect	
	Air	Air temperature	
Human disturbance	Resource use	Lopping	Visual estimate in Likert scale of 5
		Timber extraction	Visual estimate in Likert scale of 5
		Mining/quarry if any	Present or absent
		Fire evidence	Recent or old
		Grazing	Present or absent
	Infrastructure	Estimated distance to road	
		Estimated distance to nearest settlement	

10.5 Annexure IIa. Scat collection field note

Survey area	Date
Survey team	Site ID
Surveyor	
Landscape	
Landscape	Altitude
	Vegetation
Sight description	

Scat collection no.	Microhabitat type	Name of species	Notes

10.6 Annexure IIb. Opportunistic animal sighting field note

Survey area			Date		
Survey team			Site ID		
Surveyor					
Name of species	Time observed	Observation type	Habitat type	Locality description	Remarks

10.7 Annexure IIc. Data form for Small Mammal sampling

Name of enumerator: _____, _____

GPS Coordinates

Latitude: _____ Longitude: _____

Elevation: _____

Transect ID: _____ Trap ID: _____

Start Date: _____ End Date: _____

GPS Location/elevation for start & end points trap;

1) 1st Trap: _____ 2) End Trap: _____

Habitat: _____

Trap Location of the capture:

Latitude: _____ Longitude: _____

Elevation: _____

Morphometric Measurement

Body weight: _____ gms; Head body length: _____ cm;

Tail length; _____ cm;

Hind foot length: _____ cm; Ear length: _____ cm

Males:

Testes: _____ Descended: _____ Non descended: _____

Females:

Nipples: _____ Prominent _____ Not Prominent _____

Pelage:

Juvenile: _____ Sub adult _____ Adult: _____

Date of collection: _____ Collection No: _____

Remarks and other notes: _____

10.8 Annexure III. Data form for Bird sampling (field notes)

Name of enumerator: _____, _____

Transect ID: _____

Start Date: _____ End Date: _____

Sl no	Name of species	Male	Female	Juvenile	Number	GPS coordinates	Remarks

Date of collection: _____

Remarks and other notes: _____

10.9 Annexure IVa. Areas of inquiry for aquatic survey and associated variables and parameters

Area of Inquiry	Variables	Parameters	Remarks
Aquatic biodiversity	Fish diversity	Species	Diversity Indices & abundance
		Species count	
		Length	
		Weight	
	Amphibians	Species and its count	Opportunistic survey (if found)

Area of Inquiry	Variables	Parameters	Remarks
	Crustacean (crabs)	Species and its count	Opportunistic survey (if found)
Vegetation (terrestrial)	Trees (DBH ≥ 1.3 m)	Height	For vegetation analysis
		Diameter	
		Species	
	Shrubs (DBH ≤ 1.3 m)	Height	
		Species	
		Count	
	Herbs	Cover percent	
		Species	

10.10 Annexure IVb: Physical & Chemical Parameters for Water Quality Assessment

Sl No.	Parameters
1.	pH
2.	Temperature
3.	Total Dissolved Salinity
4.	Salinity
5.	Electrical Conductivity
6.	Dissolve Oxygen
7.	Total hardness
8.	Nitrate
9.	Chloride
10.	Phosphate
11.	Ammonia
12.	Turbidity
13.	Total Suspended Solids

10.11 Annexure IVc: Ichthyological Collection Protocol, ESRAM, Bhutan

Site No. Date: Time: Collector(s):

Dzongkhag: Locality: Water body name:

Latitude: Longitude: Altitude:

Water body type (circle): seasonal stream, river, lake, spring water, dam, ditch, swamp, cave water

Water bed/substrate (circle): boulder, cobble, sand, mud, vegetable matter, other (name):

Water reach type (circle): riffle, pool, cascade, run, other (name)

Water characteristics (circle): clear, turbid, muddy, sandy. Water temp.:

pH: Conductivity: TDS: Salinity:

Stream depth: Stream width: Capture depth: Capture method:

Weather (circle): sunny, rainy, cloudy, partly cloudy, windy Water velocity:

Forest type (circle): sub-tropical, warm broadleaved, cool broadleaved, temperate, sub-alpine

Cover over water body (circle): open, partial shade, closed, other (name):

Fixation in: Preservation in:

Species	Length (cm)	Weight (g)	No. of juvenile	Remarks

Notes if any:

10.12 Annexure V. Protocol to record herpetofauna species

Location:					
Locality description:					
Habitat Description:					
Method of effort:					
Date:					
Time:					
Temperature:					
Wind velocity:					
Precipitation:					
Observer:					
Shade (yes/No):					
Search method	Name of species	Species code	No of individuals	Microhabitat	Remarks



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