

## Chapter 2

# Methodology for Biodiversity (Flora and Fauna) Study

### 2.1 Plant Biodiversity and Phytosociological Study

For enumeration and quantification of plant biodiversity, in-depth studies are to be conducted in the region. Considering the potential impact of development on biodiversity, this need to be assessed from a biodiversity viewpoint to indicate the extent to which the disturbance will have impact on biodiversity. Several field studies were undertaken in order to gather authentic information on enumeration, quantification, and distribution of plant biodiversity in the study region. Standard field and laboratory methods of biodiversity studies were followed for data collection and data analysis. In plant biodiversity study, standard field and laboratory methods were followed for data collection and data analysis. However, such a detailed information regarding plant diversity of the study region is not available from the study area, and hence, this study can be considered a baseline study on plant biodiversity of the study area. The photoplates (2.1, 2.2, 2.3, and 2.4) illustrate the procedures for conducting phytosociological study, specimen collection, field observations, and herbarium preparation.

#### 2.1.1 Duration of Survey

The ecological documentation of biodiversity and the survey were started in the month of April 2010 and completed in December 2013. During the field visits, various experiments were conducted. This was followed by surveys, exploration, collection, and preparation of specimens toward building an inventory of floral diversity of the area. Phytosociological studies were conducted to assess the composition, diversity, distribution, and their status in the nature. During the study, the phenological aspects of trees and shrubs were taken into consideration and the annual cycles of these groups recorded based on field observations. This was

**Photoplate 2.1** Conducting phytosociology study in the study region



Positioning quadrat for phytosociological study



Recording the GPS points for vegetation analysis



Collection of various plant species for herbarium



Recording taxonomic features of the species

Measuring Tree GBH

**Photoplate 2.2** Specimen collection

cross-checked with the traditional knowledge of the people of the study region. A comprehensive study of all the plant species along with their abundance, density, and frequency in the area including diversity indices has been given in this book. The documentation of traditional knowledge relating to the biodiversity forms a very important component of the study. Apart from this, emphasis has been given on the plant species belonging to rare, threatened, and endangered categories included in the IUCN Red Data Book. A comprehensive work on plant biodiversity of the study area is not available as a ready reference barring this study.



**Photoplate 2.3** Field observations

### ***2.1.2 Field Equipments***

Following tools/equipments were used for conducting phytosociological study.

- Alcohol and mercuric chloride
- Ballpoint pen
- Binoculars
- Camera
- Collecting picks
- Field bags
- Field notebooks





Specimens Trimming



Poisoning



Drying



Tied with Field Press

#### Photoplate 2.4 Herbarium preparation

- Field shoes
- Gloves
- Global positioning system (GPS)
- Identity cards
- Measuring tapes and scales

- Old newspaper
- Plant cutters
- Plant pressers
- Plant tags
- Pocket lens
- Polythene bags for specimen carrying from field to laboratory
- Ropes
- Pruning hooks
- Vasculum

### ***2.1.3 Methods Used***

The following groups of plants were surveyed for floral diversity.

- Angiosperms
- Gymnosperms
- Pteridophytes
- Bryophytes

There are different methodologies proposed by ecologists for sampling of angiosperms. The most important and widely used method for a general assessment is belt transect method. The study area was divided according to habitat types followed the random sampling method in the selected area. For plant biodiversity study in the ecosystems, the transect method was followed, and accordingly, transects or straight lines were marked starting from the base of the study area to the end of the vegetation zone in each selected site. The length of a transect was 500 m to 1 km in each of the selected habitat. In each selected site, 30 quadrates were laid down with the size of each quadrate being  $10 \times 10$  m for tree strata,  $5 \times 5$  m for shrubs, and  $1 \times 1$  m for herbs. This is the standard scientific method followed by various workers in respect of phytosociological studies (Cottam and Curtis 1956; Ralhan et al. 1982; Saxena and Singh 1982; Nayak et al. 2000; Lu et al. 2004; Nautiyal 2008). While sampling, circumference at breast height (CBH) of tree species was measured at 1.37 m from ground level, along with the name of the species, phenology (flowering, fruiting, and flushes), and uses. However, in this respect, the circumferences of shrubs were measured at 5 cm above the ground level. The methodology used for conducting the experiments and completing phytosociological studies is given in Fig. 2.1 (Nautiyal and Kaechele 2008).

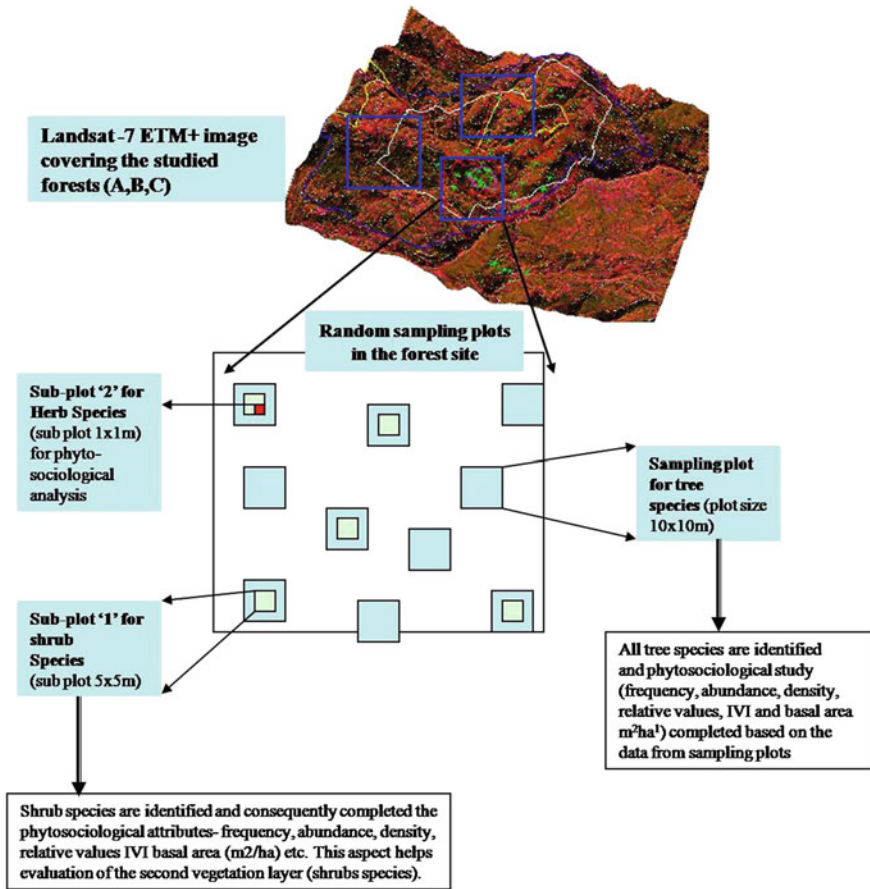


Fig. 2.1 Experimental design for conducting phytodiversity and phytosociological studies

### 2.1.4 Collection Methods

For studying floral diversity, specimen collection is one of the important aspects. During the specimen collection, field notebooks were carried along for noting down plant-related descriptions. All details related to each plant species were recorded in the field notebooks including the date of collection, local name of species, family, locality of the collection, altitude, habit and habitat, scientific name, vegetation, local use, and collector's name. Such characteristics are recorded for developing proper herbaria. Along with the above characteristics, other special characteristics of each specimen such as latex present or absent and its color, color of the flower, size of the flower and smell, stem shape and size, hairs present or absent, height of the plant, thrones present and size and species association, and root and rhizome character were also recorded.

#### **2.1.4.1 Collection of Trees, Shrubs, and Herbs**

The collection of plant specimen is very important for preparing a good herbarium. The size of a herbarium sheet is  $28 \times 42$  cm. The plant collection is always according to the herbarium sheet size, but sometimes, long specimens such as tall grass, creepers, and other large plant parts can bend V, M, N, and V shape. In case of angiosperms (flowering plants) the flowers are the key characters for identifying a specimen's scientific name. During field visits, specimens of trees and shrubs were collected with flowering and fruiting twigs; small herbs were collected with the whole plant and sometimes with roots also; creepers were collected with flowering and fruiting; and grasses were collected with underground parts. Plant parts such as bark, root, leaves, and fruits were also collected and preserved in a laboratory.

#### **2.1.4.2 Collection of Succulents**

In the study area, around ten types of succulent plant species were recorded. Generally, the collection of these specimens and making a herbarium is difficult by following general methods. These plants contain more tissues, and their thickness takes a very long time to dry, and hence, they require special methods for drying. After the collection of these plants, their thick parts were cut or made into straight lines with the help of a knife and then boiled in hot water for killing tissues before keeping the specimen on newspapers for up drying with newspapers being continuously changed till they dried up completely.

#### **2.1.4.3 Collection of Minute Plants**

The minute plant species required special care for the collection and preservation and the methods for preparing herbaria is different from the higher plants. In view of plants being small, the herbarium of minute plants cannot be prepared through usual methods. After collection, some of the plants were dried and kept in packets which mounted on a herbarium sheet (for example, *Lemna polyrhiza*).

#### **2.1.4.4 Collection of Large Plants like Palms**

In the study area, the details of palm trees such as coconut, borassus, and bamboo species were recorded. Generally, these are very large, and the shape of leaves is irregular, and hence, it is difficult to prepare a good herbarium with full characteristics. During the field survey, good photographs of their main parts such as scale trunk, inflorescence, and leaves were taken for the records and identification.



### 2.1.4.5 Collection of Aquatic Plants

From the aquatic ecosystems, plants were collected. Some specimens were long, and a few of them were small that float on water. The tall specimens were bent into M, N, and V shape to prepare a herbarium. For removing the moisture content from aquatic plants, muslin cloth was used.

### 2.1.5 Herbarium Preparation

The specimens were collected according to habitat as explained above. After the collection of specimens, press and drying are important to make a herbarium. Plant pressers are of different types: wooden, iron, and book pressers. For pressing and drying, small iron rod pressers and old newspapers were used. The collected specimens were trimmed nicely before poisoning process. The specimens were poisoned with alcohol and mercuric chloride and placed in between the newspapers before being tied with field pressers. Dipping of specimen in mercuric chloride is called dry method. The newspaper needs to be changed every alternative day till the specimen dried up completely. Dried specimens were mounted on a herbarium sheets (28 × 42 cm), while large specimens were mounted on different herbarium sheets shaped like M, N, V, and W identified by professional taxonomists (Allen et al. 1996; Smith 1971; Anderson 1999; Maden 2004; Wondafrash 2008).

### 2.1.6 Data Analysis

For calculating the species composition, abundance, and diversity indices at the transect level, the following common variables were used: basal area, relative dominance, and relative frequency following Phillips (1959), while the sum of the relative dominance and relative frequency gave the importance value indices (IVI) for various species (Curtis 1959). Species richness, concentration of dominance, evenness, and similarity index were also analyzed. For developing the land-use and land-cover change for the study area, GPS data were recorded.

$$\text{Frequency} = \frac{\text{Number of sampling units (quadrates) in which a species occurs}}{\text{Total number of sampled units studied}} \times 100$$

$$\text{Density} = \frac{\text{Total number of individual in all sampling units}}{\text{Total number of sampled units studied}} \times 100$$

$$\text{Abundance} = \frac{\text{Total number of individuals in all sampling units}}{\text{Total number of sampling units of occurrence}}$$

The basal area was calculated using the following formula:

Basal area of a single tree =  $\pi \times r^2$

$r$  = radius,  $\pi$  = 3.14

Basal cover (m<sup>2</sup>/ha) for shrub and tree species obtained by adding value of all species together and presented as follows:

$$BC = \frac{\sum_{i=1}^{sh} BASH}{PA}, \quad \frac{\sum_{k=1}^m BAT}{PA}$$

where BC = basal cover or basal area, Sh = shrubs, and  $m$  = tree and BASH and BAT are basal area for shrub, tree species respectively, and PA = plot area or quadrat. The total basal cover calculated by the multiplying mean basal cover and density of the species.

$$\text{Relative density (RD)} = \frac{\text{Number of individual of a species}}{\text{Total number of individual of all species}} \times 100$$

$$\text{Relative frequency (RF)} = \frac{\text{Number of occurrences of a species}}{\text{Total number of occurrences of all species}} \times 100$$

$$\text{Relative dominance (RDo)} = \frac{\text{Total basal cover of individual species}}{\text{Total basal cover of all species}} \times 100$$

$$\text{Importance Value Index} = \text{RD} + \text{RF} + \text{RDo}.$$

### 2.1.7 Diversity Indices

Diversity is a combination of two factors; the number of species present, species richness, and the distribution of individuals among the species are referred to as evenness or equitability. Whittaker distinguishes three types of diversity.

1. *alpha* diversity—diversity within a particular area or ecosystem,
2. *beta*-diversity—the change in diversity between ecosystems, and
3. *gamma* diversity—the overall diversity of a landscape comprising of several ecosystems.

The two most widely used species diversity indices are Shannon and Simpson indices. They are adopted by ecologists to describe the average degree of uncertainty in predicting the species of an individual picked at random from a given community. As the number of species increases, the uncertainty of occurrence also increases along with distribution of individuals, more evenly among the species already present.

The Shannon–Wiener Index or species diversity (Whitt, when properly manipulated, always results in a diversity value ( $H'$ ) ranging between 0, indicating a low community complexity and 4 and above indicating high community complexity.

**Species diversity ( $H'$ )** was computed following the Shannon and Weiner (1963) information index as follows

$$H = \sum \frac{n_i}{N} \log_n \frac{n_i}{N}$$

where  $n_i$  is the total density value for species,  $i$ , and  $N$  is the sum of the density values of all the species in that site.

**Beta-diversity ( $\beta$ )** among all the studied forests was calculated following the method given by Whittaker (1975).

$$\beta = \sum \frac{Sc}{S}$$

**Richness:** The number of species per sample is a measure of richness. The more species present in a sample, the “richer” the sample.

**Evenness:** Evenness is a measure of the relative abundance of different species making up the richness of an area. A community dominated by one or two species is considered less diverse than the one in which several different species a similar abundance. Species richness and evenness increase, so does diversity. Simpson diversity index is a measure of diversity which takes into accounts both richness and evenness.

The term “Simpson’s Diversity Index” can actually refer to any one of 3 closely related indices.

**Simpson’s Index ( $D$ )** measures the probability of two individuals randomly selected from a sample belonging to the same species (or some category other than species).

$$\text{Simpson index } D = \frac{\sum n(n-1)}{\sum N(N-1)}$$

where  $N$  = total number of species and  $n$  = number of species in a given community.

**Simpson’s Index of Diversity  $1 - D$ :** The value of this index also ranges between 0 and 1, but now, the greater the value, the greater the sample diversity. This makes more sense. In this case, the index represents the probability of two individuals randomly selected from a sample belonging to different species.

**Simpson Reciprocal Index  $1/D$ :** The value of this index starts with 1 as the lowest possible figure. This figure represents a community containing only one species. **Simpson reciprocal index increases with an increase in diversity.** The maximum value is the number of species (or other category being used) present in the sample (Simpson’s Diversity Index online).

## 2.2 Phytoplankton Study Materials and Methods

Phytoplankton are single-celled free floating algae and easiest food source for most of the aquatic life form like zooplankton and fishes thus are the basic food producers in any aquatic ecosystem (Suseela 2009). Random sampling method has been applied for the collection of sample from the selected site. Samples were collected in two different seasons from January 2011 to December 2011. The sample were collected using plankton mesh net which consisting of a cylindrical tube with stoppers at each end and closing device, and water sample was collected up to 6 l at each station and passed through the mesh net. Collection of whole water samples from the site, all size classes of plankton can be collected. Different size and categories of plankton were separated by subsequently filtering these whole water samples through netting of the appropriate mesh size. The final volume of water sample was collected 100 ml from the mesh for further study.

Algal samples were also collected small streams and canals surrounding the lake; the algal sample growth was abundant and visible on the surface of the rocks (Pandey and Kashyap 1995).

### 2.2.1 Phytoplankton Preservation

The following methods were used for the preservation of phytoplankton sample.

**Lugol's solution:** After collection, plankton sample preservation has done using the Lugol's solution. Add 0.3 ml Lugol's solution to 100 ml sample and stored in the dark place. For long time preservation, Lugol's solution add 0.7 ml per 100 ml of sample and buffered formaldehyde add 2.5 % final concentration after 1 h (Grace Analytical Lab 1994). (\*\*Lugol's solution: Dissolve 20 g potassium iodide (KI) and 10 g iodine crystals in 200 ml distilled water containing 20 ml glacial acetic acid)

**Formalin:** After collection of the sample, 40 ml buffered formalin (20 g sodium borate,  $\text{Na}_2\text{B}_2\text{O}_4$ , +1 l 37 % formaldehyde) add to 1 l sample immediately (Manickam et al. 2012).

### 2.2.2 Brief Procedure for Phytoplankton Study

- Phytoplankton survey has been carried in two seasons summer (April to May) and rainy season (July to September).
- Four lakes were chosen to study the phytoplankton.
- The lakes are around 200 m length and 65 m breadth.
- The collections were done using phytoplankton net.
- Random sampling method has been applied in the plankton collection procedure. Samples were collected in 20 localities in each lake.
- After collection, the phytoplankton samples were preserved in 4 % of formalin (aqueous solution of formaldehyde).

- After sample collection, it has stored in dark place and later sent to the laboratory for identification.
- Samples are identified with images and documented according to protocol (BARC 2009). The samples were identified in Phycology lab at the Madras University, Gundy campus. Glycerin was used for mounting the material. The centric organism was photographed using a LABOMED microscope with attached SANYO ccd camera (Arulmurugan et al. 2011)

## 2.3 Animal Biodiversity

### 2.3.1 Fauna Survey and Study of Insects

Studying the insects is a very big task, and insect taxonomy requires continuous work in selected sampling plots. The numbers of insects are more than 90 % of various life-forms on planet earth; therefore, they are too diverse groups of the kingdom Animalia. And their structure also varies from one species to another species. Collection is the best practice methods for studying the insect taxonomic positions. Not only collection, but insect preservation is also very important process for future references and study. Standard methodologies (Triplehorn and Johnson 2005; Ragumoorthi et al. 2003) were followed for taxonomic study of the insects. The data collection method for fauna is given in Fig. 2.2.

#### 2.3.1.1 Insects: When and Where to Collect

Insects appear in each and every ecosystem (terrestrial or aquatic ecosystems). Some insects are diurnal and some insects are nocturnal. Some insects are active in summer or some are active during winter, but most of the insect will go to hibernate during winter season. Some insect species prefer particular plant for their food purpose. It shows that vegetation also very important for insect collection. Insect may available season wise and habitat wise, but for getting various types of insects, the experiments are to be conducted throughout the year in each and every habitat. Photoplates show the netting method for collection of insects from the study region (Photoplate 2.5), collection and preservation of invertebrates (Photoplate 2.6), below ground diversity survey methods (Photoplate 2.7).

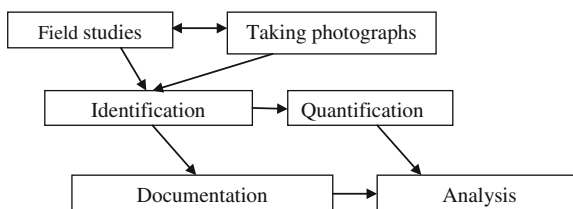


Fig. 2.2 Procedure for data collection in faunal studies





**Photoplate 2.5** Netting method for collection of insects from the study region

### 2.3.1.2 Collecting Equipment

- Insect net
- Killing jars
- Forceps
- Vials
- Envelopes
- Preservatives
- Aspirator
- Beating umbrella/sheet
- Traps
- Knife
- Headlamp
- Box
- 10× lens

### 2.3.1.3 Collection Methods

Various methods have been used for insect collection. For collecting insects, the methods were (1) hand collection, (2) net collection, (3) traps, (4) aspirator, and (5) beating.

1. **Hand collection:** Large insect's (grasshoppers and beetles) collection can be done by hand. This collection method is unsuitable for poisonous and dangerous (which have stings) insects. In this method, insects were collected by hand and transferred into killing jars for further procedure.



Wet preservation



Collection of insects by using net



Insects preservation in Insect cabinet box



Samples preservation in envelopes



Samples collection near water



Segregation of samples for identification

**Photoplate 2.6** Collection and preservation of invertebrates

2. **Net collection:** Two types of insect nets were used for collection of insects. They are (1) aerial net and (2) sweep net. All flying insects have been collected by using nets, while they are active (mid-morning/late afternoon).

*Aerial net:* This net will be used for active fliers such as butterflies, moths, dragonflies, and wasps. This net is very light weight.

*Sweep net:* This net is suitable for collecting grasshoppers and leafhoppers and other insects. This net is heavier than aerial net.



Collection of litter



Berlese funnels



Extraction of samples from funnels



Identification of samples

**Photoplate 2.7** Below ground diversity survey methods**Photoplate 2.8** Fishes survey

- Traps:** Various types of traps were used for collection of insects. This is very effective and easy method for collecting insects (mainly small insects). Any device, often containing something to which the insects are attracted is a trap. A trap needs to be arranged in a manner so that once the insects get into it, cannot be escaped (Triplehorn and Johnson 2005). Following traps were used for insects collection.

*Bait trap*—This method is very effective method for flies sampling. The egg yolk, fried coconut, and honey were used as bait.





**Photoplate 2.9** Reptiles survey



Phytoplankton net



Sample collection



Sample Preservation



Identification

**Photoplate 2.10** Methods of plankton (phyto and zoo) collection

*Light trap*—This light trap is effectively useful for sampling nocturnal insects. The high-power florescent or mercury lamp arranged behind the white cloth for trapping the insects. For that light, nocturnal insects (moths

and some another flies and beetles) get attract and they will come to nearby light. Under the light, insects can be collected.

*Note: This light trap is effectively works in late evening, and everyday timing should be maintained constantly.*

*Sticky trap*—White flies

*Pitfall trap*—This is a kind of passive collection, unlike the active collection where the collector catches each animal from the sampling sites. By using this method terrestrial insects and spiders can be collected. However, this method is also useful for sampling herpetofauna (reptiles and amphibians) in addition to collection of invertebrates. For preparing pitfall trap to collect insects and spiders, the cylindrical plastic bottles (11 cm depth and 10 cm diameter) were used (Churchill and Arthur 1999). In this, 69 % water, 30 % ethyl acetate, and 1 % detergent were used as a preservative. Specimens were removed from traps after seven days for further laboratory processing identification (Hore and Uniyal 2008). This pitfall traps could be useful to assess species presence and relative abundance (Bury and Corn 1987) in the study sites.

4. **Berlese funnel:** The berlese funnels are used for collection of soil insect (soil-dwelling insects). This is very effective method for collecting insects from soil and leaf litter. In this method, soil and leaf litter collected from various parts and then transferred into berlese funnel. Funnel contains killing jar or alcohol container below of it. And electric light bulb is placed above the funnel is used to heat the upper part of the soil or litter which makes insects and other animals to move downward and fall into killing jar or alcohol container. After collection of insects, standard procedure was followed for preservation of the insects, spiders and other animals.
5. **Aspirator:** The aspirator was used for capturing small insects.
6. **Beating:** Beating umbrella was used as a sheet beneath the vegetation/bushes where insects/spiders have the dwellings. Upon beating the vegetation/bushes from top the insects and spiders were collected in the beating sheets/umbrella placed underneath. Thereafter, the insect are picked up by hand and transferred into killing jar.

*Note: An umbrella can be used in beating, but it should be made up of muslin or light white color cloth.*

#### 2.3.1.4 Killing of Insects

After collection of insects, killing is one of the most important procedures for preservation of insects. It should be done immediately after capturing of insects. While killing insect, proper handling should be necessary (insect should not get damage and should not break their body parts). Various materials can be used for killing insects such as cyanide, ethyl acetate, and chloroform as a toxic agent in killing jars.



*Killing Jars:* This killing jar is the equipment where insect can be killed. For killing of insects, traditional killing jar was prepared by using various toxic agents. For preparing killing jar, cyanide and chloroform used as a toxic agent in various size bottles (for various insect sizes). The lepidopteron and other insects were kept in separate killing jars. The reason is that if kept in same containers or killing jars, their wings and other body parts may get damaged thus identification of species would be difficult.

### 2.3.1.5 Preservation

The preservation of specimens is very important for records in the laboratory. For preservation of specimens two methods namely dry preservation and wet preservation were followed.

- (a) **Dry preservation:** For dry preservation, specimens are preserved by using pins in insect cabinet box. In dry preservation, all hard-bodied insects were preserved by using paper envelopes and pinning of insects. The following materials were used for preservation of insects.
- Paper envelopes: Paper envelopes were used for preserving the large winged insects such as butterflies, dragonflies, and moths.
  - Spreading board: It is used for spreading the wings of dead insects.
  - Pins: For pinning the nickel-plated (rust resistant) pins were used, which are specially prepared for preserving the insects.

*Pinning:* Pinning is a most common and suitable method for preserving hard-bodied insects. Pinning should be done in a proper way (to identify diagnostic characters clearly). Based on the size of the insect, pins are selected (small size pins for smaller insects and large size pins for larger insects). Place of pinning varies from insects to insects. The pinning region in various groups of insects is depicted in Table 2.1. Once pinning procedure is over, the insects have to be shifted to insect cabinet boxes for long-term preservation.

**Table 2.1** Showing pinning position for various insects group

S. no.	Insect group	Pinning region
1	Grasshoppers, crickets, praying mantids, and cockroaches	Pronotum
2	Bugs	Scutellum
3	Stick insects	Metanotum
4	Beetles and weevils	Right elytron
5	Earwigs	Right tegmen
6	Dragonfly, damselfly, green lacewings, moths, butterflies, bees, wasps ants, and true flies	Thorax

Source Ragumoorthi et al. (2003)

- (b) ***Wet preservation or liquid preservation:*** Soft-bodied insects are nymphs, larvae, caddisflies, and mayflies. Mainly, ethyl alcohol (70–80 %) was used for preservation of soft-bodied specimens. Various solutions such as Hood’s solution, Kahle’s solution, and alcoholic Bouin’s solution can be used for preservation. For this study Hood’s solution was used for preservation of insects.

Hood’s solution: 95 ml 70–80 % ethyl alcohol + 5 ml Glycerin.

### ***2.3.2 Spider Collection Methods***

Hand collection, pitfall trap, beating, and berlese funnels as mentioned above have been used for sampling and collection of spiders in various habitats. After collection, the spiders were placed in Hood’s solution for identification and preservation.

### ***2.3.3 Study of Vertebrates***

For sampling and monitoring the vertebrates from the study area, the standard methodologies were followed which are given in the “Handbook of Biodiversity methods Survey, Evolution and Monitoring” (Hill et al. 2005) and “Practical Methods in Ecology” (Henderson 2003). The vertebrate species were not preserved, however, along with visual observations, and vocal sounds good photographs were taken for identifying vertebrate species. The standard field guides were referred for proper identification of the species. (Daniel 2002; Daniels 2002; Prater 2005; Manakadan et al. 2012). Survey methods for fishes and reptiles are depicted in photoplates 2.8 and 2.9.

#### **2.3.3.1 Survey and Monitoring of Fishes**

Fishes were collected from the water body using locally available fishing gears from different sampling stations. Fishes collected during premonsoon, monsoon, and post-monsoon seasons. The direct count method (visual survey) and netting were used for surveying the fish species.

#### **2.3.3.2 Survey and Monitoring of Amphibians**

Survey and sampling was done in both aquatic and terrestrial systems. Sampling required day and night search during all three seasons in specified habitats (under the logs and stones, digging through litter and soil, searching short bushes and tree hollows, and under fallen barks and water-catchments). For surveying and monitoring

amphibians, torch count method (aquatic frogs) and pitfall trap methods (terrestrial frogs) were followed. By using vocal sound and photographs, the amphibian species were identified. Continuous surveys are required to obtain reasonably good estimates of amphibian species diversity and abundance in study sites.

### 2.3.3.3 Survey and Monitoring of Reptiles

Several survey techniques such as standard walk transect, visual encounter survey and pitfall trap methods were used to sampling reptiles in each and every habitat of the study area. While doing this survey, photographs were taken for identification of species. Species identification was done by using standard field guides in consultation with experts.

*Visual Encounter Survey method (VES)*: This method is useful for studying the species richness and abundance in a survey path (Crump and Scott 1994). VES are standard method for inventory of terrestrial herpatofauna (Campbell and Christman 1982; Corn and Bury 1990).

### 2.3.3.4 Survey and Monitoring of Birds

Birds are sampled by using line transect method, point count method, and opportunistic bird sightings. By using bird vocal sounds and photographs, the species were identified in consultation with ornithologists.

*Line transects*: In this method, a straight line of 1 km is drawn, and all birds seen or heard till a range of 25 m on either side of the transect were recorded. The transect was worked for one hour.

*Point counts*: In this method, the observer will stand in a randomly chosen point and birds seen or heard in 50 m radius are recorded for 5 min. This observation is repeated in another point at least 300 m from the first point.

*Opportunistic bird sightings*: While traveling in study area, many bird species will be detected in survey time. Such species are recorded by their appearance or by their call.

### 2.3.3.5 Survey and Monitoring of Mammals

Intensive survey has been done by transect method (walking and in vehicle) for all major habitats for surveying of mammals by direct and indirect evidence. Direct observation technique has been used for surveying large and medium sized mammals. But this technique is perfectly suitable for surveying of diurnal mammals. But, for nocturnal mammals, camera traps were used and other evidences (pellets, hair, foot prints, and vocal sounds) also collected for surveying mammals (Martin 2009). Bait traps and visual encounters have been used for small mammals (rodents and squirrels). However, good photographs were also taken for species identification.

### 2.3.3.6 Identification

The field guides (as cited above) and standard keys were used for species (invertebrates and vertebrates) identification. The experts' laboratories (The Department of Entomology at the University of Agricultural Sciences, Bangalore, National Bureau of Agricultural Insect Resources (NBAIR) Bangalore, regional office of the Zoological Survey of India (ZSI), Pune were visited for the purpose of proper identification of insects. However, frequent visits were made to Anand Agricultural University (AAU) located in Anand, Gujarat for the identification of spiders. The survey methods for various groups of animals are given in Table 2.2.

**Table 2.2** Various survey methods used for various groups of animals

Group of animals	Methods used for survey	
Insects	Hand collection	
	Net collection	Aerial net, sweep net
	– Aerial net	
	– Sweep net	
	Traps	Bait trap, light trap, sticky trap, pitfall trap
	– Bait trap	
	– Light trap, sticky trap	
	– Pitfall trap	
	Berlese funnel	
	Beating	
Aspirator		
Spiders	Pitfall trap	
	Beating	
	Berlese funnel	
Fishes	Direct count method (visual survey)	By using locally available fishing gears
	Netting	
Amphibians	Visual encounter survey	
	Torch count method	
	Pitfall trap	
Reptiles	Visual encounter survey	
	Pitfall trap	
Birds	Line transect method	
	Point count method	
	Opportunistic sightings	
Mammals	Line transect method	All mammals
	Visual encounter survey	Large mammals
	Pellet count method	Large mammals
	Bait traps	Small mammalian groups

## 2.4 Zooplankton Survey and Monitoring

Zooplankton are micro organisms present in water bodies and play an important role in aquatic ecosystems in energy transfer from primary level to higher level (Tiwari 2011). The role of zooplankton is significant in assessing the water quality and they act as biological indicators of water pollution (Shivashankar and Venkataramana 2013; Gayathri et al. 2014). For collecting zooplankton (20–200  $\mu\text{m}$ ) such as protozoa, rotifers, and immature micro-crustacean, the methodology used for phytoplankton collection was followed. The zooplankton usually are sufficiently abundant to yield adequate samples in 5–10 l bottles as described in several research papers. However, during time of sample collection, the experts recommend composite samples over depth and time and bottle samplers are suitable especially for discrete depth samples. If depth-integrated samples are desired, the nets are used. The larger and more robust micro-zooplankters (e.g., loricate forms and crustacea) may be concentrated by passing the water through a 64- $\mu\text{m}$  mesh net. Various methods regarding zooplankton study are described by Pennak (1978), Battish (1992), APHA (1998) and Altaff (2003). The detailed methodology is given in the protocol developed for flora fauna study (BARC 2009). Methods for plankton (phyto and zoo) collection depicted in photoplate 2.10.

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