Faunal Survey
A Methodology Manual

ZOOLOGICAL SURVEY OF INDIA

Ministry of Environment, Forest and Climate Change
Government of India

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Edited by

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Citation

Banerjee, D., Raghunathan, C., Subramanian, K.A., Pathania, P.C. and Rizvi, A.N. Eds. (2026). *Faunal Survey- A Methodology Manual* : 1 – 146. (Published by the Director, Zool. Surv. India, Kolkata).

Published : January, 2026
ISBN : 978-81-995835-3-5

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Published at the Publication Division by the Director, Zoological Survey of India, M-Block, New Alipore, Kolkata-700 053.

Designed & Printed by Cygnus Advertising (India) Pvt. Ltd. Kolkata – 700 091

FOREWORD

India is the home for diversified floral and faunal communities spread across different kinds of ecosystems and it shares nearly 7 to 8 percent of the world's known species whereas geographically it is only 2.4 percent of the Earth's land. The 10 biogeographic zones, 4 biodiversity hotspots, wide ranges of ecosystems from mountain ranges to deep-sea, alpine forest to coral reef ecosystems of India represents a total of 1,05,244 faunal species.

The Zoological Survey of India is a leading taxonomic organization in the nation, consistently engaged in the exploration and discovery of India's faunal diversity since its establishment on 1st July 1916, and systematically documenting these findings for the purposes of conservation, management, and sustainable utilization of bioresources. With a legacy spanning over 110 years, the Zoological Survey of India has conducted extensive faunal surveys, concentrating on taxonomic studies and biodiversity assessments across terrestrial, freshwater and marine ecosystems. The scientific knowledge produced by the ZSI has aided the government in formalizing conservation policies and executing them to ensure the successful conservation and sustainable utilization of our invaluable biological resources. The ZSI has played a crucial role in the formulation of the Indian Wildlife (Protection) Act of 1972 and its most recent amendment in 2022, while also providing vital contributions to international agreements such as the CBD, IUCN, CMS, and CITES.

The present publication, '***Faunal Survey: A Methodology Manual***', signifies a landmark contribution to faunal exploration by the scientists of the Zoological Survey of India with the cohesion of internationally recognized standard methodologies. With the ease of technological development and uses in methodology across the world, the methodologies employed have often varied among taxa, areas, and organizations, leading to challenges in data standardization, comparability, and integration. The book thoroughly outlines methodological strategies for sampling, observation, specimen collection, identification, documentation and the long-term monitoring of faunal taxa under 23 chapters. It combines traditional taxonomic methods with contemporary tools, including molecular techniques, remote sensing, and Geographic Information System (GIS) applications. Researchers, conservation agencies, and policymakers have long acknowledged the necessity for a comprehensive and standardized methodological framework that encompasses all principal taxonomic groups, from Protozoa to Mammalia.

This publication is significant as the first comprehensive and integrative methodological compendium for faunal surveys in India, covering both invertebrate and vertebrate taxa while connecting traditional taxonomy with modern technological advancements. I strongly believe that it will be an invaluable resource for taxonomists, ecologists, conservation biologists, environmental managers, academics, and students to pursue their research interest in faunal exploration and documentation.

Dr. Dhriti Banerjee
Director
Zoological Survey of India

Contents

I Developing a Faunal Inventory

Introduction:

The scientific discipline of taxonomy has given the universal naming and classification system of biodiversity. It is through taxonomic research, that our understanding of biodiversity and classifications of living organisms have progressed (Thomson et al 2018). The basic understanding about the components of biodiversity is necessary for effective decision-making in conservation and sustainable use (https://www.cbd.int/gti/importance.shtml). The Zoological Survey of India (ZSI) is a premier taxonomic organization which undertakes faunal inventorisation, explorations and taxonomic research leading to the advancement of knowledge with regard to the rich and varied fauna of the country. The inventories lead to documentation of fauna under different categories like State Fauna Series, Fauna of Conservation Areas, and Fauna of specific ecosystems or habitats. These documents in addition to providing elementary baseline data, also yield operational frameworks for conservation of biodiversity, biocontrol, forest management etc., also serving as an essential reference aid for fundamental as well as applied faunal research.

Faunal sampling for taxonomic research:

While integrating taxonomic research with biodiversity conservation, for inventorying and monitoring specific taxa or habitats, it is important to use standardised methodology and accepted protocols (Häuser et al, 2010b). Since species distributions are heterogeneous in space and time, adequate spatio-temporal replicates are essential to obtain a representative biodiversity inventory, that too through coordinated efforts by a multidisciplinary team (Leponce et al, 2010). Both qualitative and structured sampling are needed for the inventory to be complete (Nichols & Langdon, 2007), they being complementary to each other. This is much relevant in the case of the highly diverse invertebrate microscopic taxa, because, a structured sampling approach alone, like the stratified sampling, while allowing quantification of biodiversity and statistical comparisons, may many a times miss out at least a few taxa being reliably sampled. Though the faunal inventories are exhaustive and are unable to sample the entire components, it is essential that the inventories should adequately have samples representing the habitat or the taxa investigated. Hence it is recommended to employ a variety of procedures and collection gadgets, so that the sampling refelcts the species richness. For example, while aiming for an insect survey at a given locality, different techniques employing diverse gadgets like light traps, malaise traps, sweep nets, pitfall traps and yellow pan traps, operating on different principles are to be utilized. Faunal sampling requires knowledge of their biology, habitat preference and activity patterns (Grootaert *et al.*,). Developing and maintaining a faunal inventory is an expensive and laborious task. Many steps are involved in this, starting from procuring legal permissions from the authorities to collect specimens, conducting field surveys and collection of samples, proper curation, preservation and adequate labelling of the samples collected, and finally accurate identification

and cataloging of the collected samples. DNA barcoding assists in framing sound taxonomic conclusions and is undoubtedly a powerful tool to unearth the hidden biodiversity, especially when sibling species are considered. Processing the samples in the field itself, as soon as the specimens are collected, that too as per the recommended preservation techniques and procedures are a requisite for molecular works.

Surveying specialized habitats:

Tree related microhabitats serve as important substrates and structures for biodiversity in both commercial and protected forests, and are now being increasingly acknowledged in management, conservation and research (Larrieu et al, 2017). Such specialized microhabitats like tree holes are known to hold a diverse array of vertebrate and invertebrate fauna. Water-filled tree holes often register good diversity of aquatic insect species, several mosquito species are known to breed in tree holes. It has been noted that availability of small water collections in tree holes are crucial for the survival and reproductive success of the tree hole frog *Frankixalus jerdonii* (Biju, etal 2016). Little attention has been paid in India in inventorying these specialized habitats. Integrating tree hole surveys into regular stream biodiversity inventories, involve systematic sampling, by laying 10m X 10m contiguous subplots, in huge areas and investigating a fixed number of selected tree species at random (Blakely and Didham, 2008). Several studies in the recent acknowledge forest canopies as one of the most species rich and highly threatened terrestrial habitats. Forest canopies in fact are considered as hotspots of biodiversity (Nakamura et al, 2016). This is because the complex three-dimensional structure of the canopy affords opportunities for niche diversification and vertical stratification (August 1983, Ozanne, et al. 2003). Erwin (1982) observed that canopies can hold about two thirds of the arthropods in a dry tropical forest. Though forest canopy science has become a lucrative and active discipline in the recent, their limited accessibility renders very slow progress in this field. Canopy exploration requires multilateral and collaborative research efforts and is highly technologically intensive. Facilitating access to canopies involve mountain climbing methods with the help of technical climbing hardwares and ropes, fogging using non persistent insecticides, construction equipments like cranes and also canopy walkways (Lowman et al, 1993). Canopy cranes allow access to the upper canopy layer (Nakamura et al, 2016). Canopy fauna in India remain largely unexplored, however, a few projects were executed in the canopies of south Western Ghats.

GIS Studies:

The geographical distributions of organisms yield important hints in interpreting their natural history. Such distributional data will also lead to meaningful inferences on earth in the past, and even on the place of origin of species (Kirby, 1877). Hence location of an organism is now one of the most essential attributes to be recorded in biodiversity science. Geographic information systems (GIS), uses georeferenced information based on the occurrence or location of an organism. Mapping quantities and densities help in categorising the dominant taxa, area wise. Combining the visual aspects of a map and analytical power of a database, several questions, can be answered and interpretations made on distribution patterns, revealing hitherto unknown relationships and trends. In the current scenario of biodiversity decline, resource overuse, species extinction and climate change, GIS functions as a handy tool in formulating solutions, for confronting issues.

Conclusions:

Faunal inventories are irreplaceable biological assets, on the basis of which organisms are discovered and described. An ideal faunal inventory, employs uniform and standard field collection techniques and protocols, taxa wise, and will also consider seasonal variants, thus representing the diversity as well as abundance of the taxa. Well preserved, adequately labeled, georeferenced faunal inventories, with validated scientific names function as invaluable scientific knowledge banks for biodiversity research.

II Standard Survey Protocols for Terrestrial and Freshwater Ecosystems

Purpose of the Survey:

Faunistic surveys are conducted to document the biodiversity of a geographic area. The spatial scale of this geographic area varies from microhabitat (few square meters) to biomes (thousands of square kilometres). The survey methods employed to comprehensively document faunal diversity at different spatial scales are as varied as the taxa. This is because of the diversity of the habitat they occupy, life cycle and complex behaviours repertoire of the taxa. Scientific surveys aims to acquire maximum data on species diversity through efficient and economic means using geospatial and statistical tools. In addition, the methods employed are to be uniform following a standard protocol for comparison across spatial scales. The survey results accumulated over time and space are important data for formulating biodiversity conservation policies and management interventional measures at local, regional and national level.

Selection of Survey Localities:

Survey of India (SOI) has been producing very detailed maps of India at various spatial scales. These maps are available in print and digital formats. The SOI topo sheets providing a very detailed information on topography, habitats, road networks etc. at a scale of 1: 50,000 (1cm=500m) should be the basis for the survey. In the SOI topo sheets, the entire country is divided into grids with unique identification number. To locate a topo sheet for a particular area, index map of SOI may be used. One latitude-longitude degree grid in SOI 1: 50,000 topo sheet is further divided into 16 equal quarter degree grids. One quarter degree grid is equivalent to 27.5 Km X 27.5 Km = 756.271 Sq Km. This will form the basic survey unit. Since this basic unit is very large, a stratified approach across habitat, altitude and bioclimatic gradient is to be followed for conducting sampling area. This schema of spatially nested approach is graphically represented.

Types of Surveys:

Faunistic surveys can be broadly divided into four types:

1. **General Faunistic Survey:** The survey aims to comprehensively document the faunal diversity by surveying all habitats in the survey area. A suite of methodologies to be employed to document all taxa ranging from Protozoa to Mammals in diverse ecosystems. Such surveys are time consuming and require a team of expertise in various groups. Examples for such studies are State Fauna, Fauna of Protected Area etc.

2. **Taxonomic surveys:** These surveys are specifically aimed at particular taxa. The survey is conducted on a particular habitat most suitable for the taxa using standardized methods and sampling equipments. Examples of such surveys are fish surveys, soil arthropods, amphibians etc.
3. **Ecosystem surveys:** Surveys targeted at specific ecosystems such as wetlands, estuaries, coral reefs etc. Within these ecosystems, different sampling methods are to be adopted to document diversity of different faunal groups.
4. **Status surveys:** These surveys are targeted towards a specific species usually of conservation importance. However, status surveys could also be targeted for species of economic/health/agriculture/ invasive importance or specific species communities eg. Coral reefs, wetland birds, rodents, raptors, pollinators etc.

Conducting survey following standard protocol is very important in collecting data which can be compared across ecosystems and biomes. In long term, data which is collected following standard protocol is very valuable in understanding long term trends in biodiversity and developing conservation plans. Hence, following standard protocol is to be followed for data collection in terrestrial and freshwater ecosystems. Standard protocol for marine survey is separately dealt with.

I. Pre Survey Preparations:

Preparation of baseline Maps:

As mentioned earlier SOI 1: 50,000 will form the basis for preparation of baseline maps. Base line maps should be prepared and studied by the survey team before the commencement of the survey. In addition to SOI toposheets, online satellite based data bases such as Google Earth and BHUVAN are also very useful. Once the survey grid as per SOI toposheet is fixed (0.25 X 0.25 Degrees), base maps indicating altitude, water bodies, vegetation, rain fall, temperature, road network and major landscape features are to be created. To prepare such maps, desktop GIS applications such as DIVA-GIS and Q-GIS can be used. Basic Shape files for preparing such maps are freely available online. Once such a map in GIS software is prepared, it can be used for plotting GPS data and species distribution.

Ecological Stratification of Survey localities:

The spatial distribution of species within a geographic area is generally determined by altitude, rainfall, temperature, water bodies, vegetation and human disturbance level. A map showing these bioclimatic and physical variables can be easily prepared using desktop GIS applications. Once such maps are prepared, using Google Earth or BHUVAN a study of the proposed survey area should be conducted and important landscape features, their names and names of villages, settlements etc are to be noted down. Standard classification of biomes, ecosystems and vegetation types are to be followed in the ecological stratification of the survey localities. The difference between stratified and other sampling methods are illustrated.

Selection of sampling localities:

Once the survey area is stratified based on ecological and bioclimatic features, sampling localities are to be spread across representing altitudinal zone (low, medium and high), rainfall gradient (low to high), vegetation (disturbed to pristine), wetland types etc. A discussion with local villagers, fishermen and forest officials will be useful in refining the sampling localities based on accessibility and logistics.

Step 1: Preparation of survey maps:

1. Obtaining Survey of India 1:50,000 and if available 1:25,000 scale toposheets (Print or Digital) for the survey area.
2. Delineating natural and manmade ecosystems in the survey area.
3. Identifying altitudinal and rainfall gradients in the study area.
4. Ecological stratification of survey area into to ecosystems (natural and manmade), altitudinal and rainfall gradients.
5. Preparation of final survey maps incorporating above details in GIS platform/BHUVAN/Google Earth which can be digitally carried in a laptop/TAB/ mobile phone while conducting the survey.

Step 2: Baseline data collection:

1. Literature collection on previous faunal studies from the survey area.
2. Details of forest management plan (if available).
3. Detailed forest map with land marks.
4. Details of presence/absence of major fauna.
5. Interaction with local knowledgeable individuals (fisherman, hunters, forest personals and other researchers).

Step 3: Reconnaissance Survey:

1. Conducted to understand the survey landscape.
2. Identifying and marking sampling locations in all previously identified ecosystems, altitudinal and rainfall gradients using GPS.
3. Identifying and marking important land marks (lakes, ponds, streams, rivers, bridges, road junctions, caves etc), villages, human settlement etc.
4. Fine tuning and updating survey maps.
5. Collecting baseline data on fauna, bird roosting colonies, nesting colonies, bat roosts etc.
6. Updating survey maps in GIS platform/BHUVAN/Google Earth indicating sampling locations.

II. Conducting Survey (Terrestrial and Freshwater):

Survey Season and Time: The temporal distribution of species is highly depended upon season. Species diversity increase or decrease across seasons and several species are found only during certain season for a short window. This is especially true of many insect species. Hence it is important and prescribed to cover all seasons for comprehensive documentation of the fauna. However, it is not possible to cover all seasons in several ecosystems due to extreme weather events and dangerous field conditions (eg. High altitude Himalaya, Monsoon, flooded rivers, extreme summer in desert etc.).

In addition to seasonal changes in species diversity, species show marked circadian rhythm. Hence, species are active during day (diurnal), night (nocturnal) or twilight (crepuscular) hours. Conducting surveys to capture this circadian variation will help in maximizing data acquisition.

Survey duration: It is prescribed to survey one quarter degree grid in each survey session. This is equivalent to 27.5 Km X 27.5 Km = 756.271 Sq Km. and to be surveyed for 20 days in a season. Ideally, four such surveys are to be conducted in that particular grid spread across seasons. However, considering the human and financial resources required, logistically it may not be possible to complete the same in a year and efforts should be made to cover the seasons in the project period.

Survey team: Ideally Faunistic survey team should comprise of different taxa experts, scientific, field assistants. However, in practise it is not possible to include all experts in a single survey. Hence two or three experts may join together to conduct the survey.

Recording the data: The survey data comprise of voucher specimens, photographic or video graphic evidence and observational records. Voucher specimens are to be collected and preserved as per the standard protocols. Details records of collected specimens to be maintained in the field note book and converted into electronic format. Photographic and video graphic records should be sorted taxa and locality wise and archived for long term use. Observational records are to be maintained as per the standard format.

Collection Ethics: Voucher specimens are the primary data of the survey and they should be collected and preserved at outmost care. Collection of voucher specimens of species in the Wildlife (Protection) Act, 1972, species listed as Critically Endangered, endangered and threatened by IUCN should be avoided. A photographic record of such species will serve the purpose. Over collection of common and wide spread species should also be avoided. Collection methods which are destructive to the habitat of the species should not be practiced. It is important to remember that the surveys are aimed at maximizing documentation of diversity and not number of individuals.

Steps in conducting survey

1. **Defining the objective of the survey as:**

   1.1. General faunstic survey for documenting diversity (Go to 2).

   1.2. Status survey of a particular species or community (Go to 3).

2. For documenting faunal diversity organizing the survey in following components is required since the methodology, season and timing vary among different faunal elements. It is also important to note that survey need to be conducted during day and night to record diurnal, crepuscular and nocturnal fauna.

   2.1. Terrestrial invertebrates

   2.2. Terrestrial insects

   2.3. Terrestrial vertebrates

   2.4. Aquatic invertebrates and insects

   2.5. Aquatic vertebrates

3. Status surveys are conducted to ascertain the conservation status of a species or community. Detailed quantitative data is required to scientifically assess the status and methodologies for the same vary with the organism under study. Well established standard quantitative protocol is to be followed for such surveys and multiple protocols (eg: line transects, camera trapping, nest and roost count etc.) may be required. Since the results from status survey will have direct implications on future conservation and management plans, it is recommended to have detailed consultation on methodology and data analysis before commencing the status surveys.

4. **Survey Strategy:** For conducting general Faunistic survey, stratified random sampling or two stage sampling is to be followed. In addition to this, opportunistic sampling is also to be carried out as several species are not encountered during routine sampling procedures. If specimens are collected through opportunistic sampling, the details of the voucher specimens with geographic coordinates is to be maintained in in the records.

5. **Sampling Units:** In terrestrial ecosystems, within predetermined ecologically homogenous survey area belt transects and nested quadrates of different sizes to be laid for sampling different vertebrate and invertebrate taxa. Recommended sampling units for different taxa are provided in tables below.

SL. NO.	TRANSECTS	TIME	TAXA
01	1 Transect on foot= 500mX20m (10m on each side) belt transect for 1 hour.	9.00-14hrs. Timing will vary with ambient temperature and season	Butterflies (forest trails, stream bed, rivers, grasslands), adult odonates (forest trails, stream bed, rivers, grasslands).
02	1 Transect on foot= 1000mX100m (10m on each side) belt transect for 1 hour.	Sun rise to 9.00 AM and Sunset to 10 PM	Birds, Mammals (forest trails, stream bed, rivers).
03	Vehicle transects to cover large uniform habitat such as grasslands and deserts.	Different time periods of day	For large mammals and birds.

SL. NO.	QUADRATE SIZE	TAXA
1.	1mX1m	Soil protozoan's, nematodes, flat worms, earth worms, soil mites, collembolans and other micro arthropods
2.	5mX5m	Land snails, millipedes, centipedes,
3.	10mX10m	Ants, spiders, scorpions, terrestrial arthropods
4.	20mX20m	Amphibians, Reptiles, terrestrial arthropods
5.	50mX50m	Amphibians, reptiles, rodents, shrews

Sampling in inland wetlands such as streams, rivers, lakes, ponds and marshes require different approach and recommended sampling units are summarized below.

SL. NO.	WETLAND HABITAT TYPE	METHODS	TAXA
1.	Hill Streams	10mX10m quadrates. The total area is to be covered is100m2. Depending on the dimensions of the stream the quadrate size may change. For example for small first order streams it could me 1mX100m.	Aquatic invertebrates, fishes, frogs, reptiles
2.	Large streams	10m X 10m quadrates to be laid every 50m. Ideally on alternative side of the stream bank covering different stream habitats such as cascades, riffles, pools etc. Riparian area to be sampled as per standard terrestrial sampling protocol (transects and nested quadrates).	Aquatic invertebrates, fishes, frogs, reptiles

SL. NO.	WETLAND HABITAT TYPE	METHODS	TAXA
3.	Large Rivers	10m X 10m quadrates to be laid every 100m. Ideally on alternative side of the river bank covering different river habitats such as cascades, riffles, pools etc. Riparian area to be sampled as per standard terrestrial sampling protocol (transects and nested quadrates).	Aquatic invertebrates, fishes, frogs, reptiles, mammals
4.	Ponds	10mX10m quadrates. The total area is to be covered is100m2. Riparian area to be sampled as per standard terrestrial sampling protocol (transects and nested quadrates).	Zoo Planktons, Aquatic invertebrates, fishes, frogs, reptiles
5.	Lakes and Reservoirs	10m X 50m quadrates Riparian area to be sampled as per standard terrestrial sampling protocol (transects and nested quadrates).	Zoo Planktons, Aquatic invertebrates, fishes, frogs, reptiles
6.	Marshes	10m X 50m quadrates. Riparian area to be sampled as per standard terrestrial sampling protocol (transects and nested quadrates).	Zoo Planktons, Aquatic invertebrates, fishes, frogs, reptiles

6. **Data collection**: Standard data collection format is to be followed for field survey data (Ref. Appendix). Data on voucher specimens and observations should be recorded and kept separately. Field data is to be computerized for future data analysis and interpretation. Methodology for standard collection and preservation techniques are separately provided.

7. **Sampling Effort**: The sampling effort required to completely document all the fauna in a given area is enormous and resource intensive. Hence sampling effort should be maximized to survey all distinct ecosystems and cover all the taxa. The sampling units (transects and quadrates) should be proportionately distributed across ecosystems to cover spatial and temporal variations in species distribution. Statistical tools such as species area curve, rarefaction and estimators of biodiversity can be used to quantitatively represent the sampling effort.

8. **Photo/Video graphic Records**: Good photo and video graphic records of fauna, especially for birds, mammals and other fauna included in Wildlife Act (Protection)-1972, landscape, habitat is to be made during the survey.

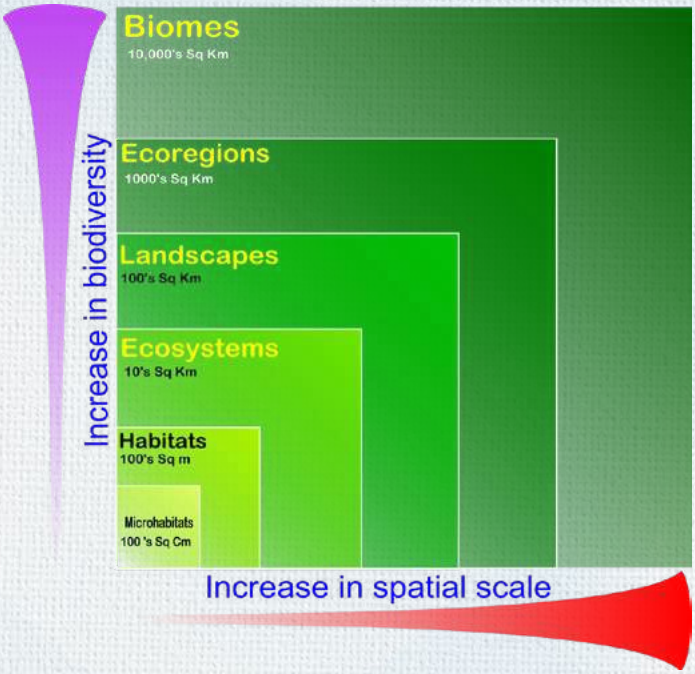
III. Data Analysis and interpretation:

In Faunistic surveys following types of georefernced data are likely to be generated:

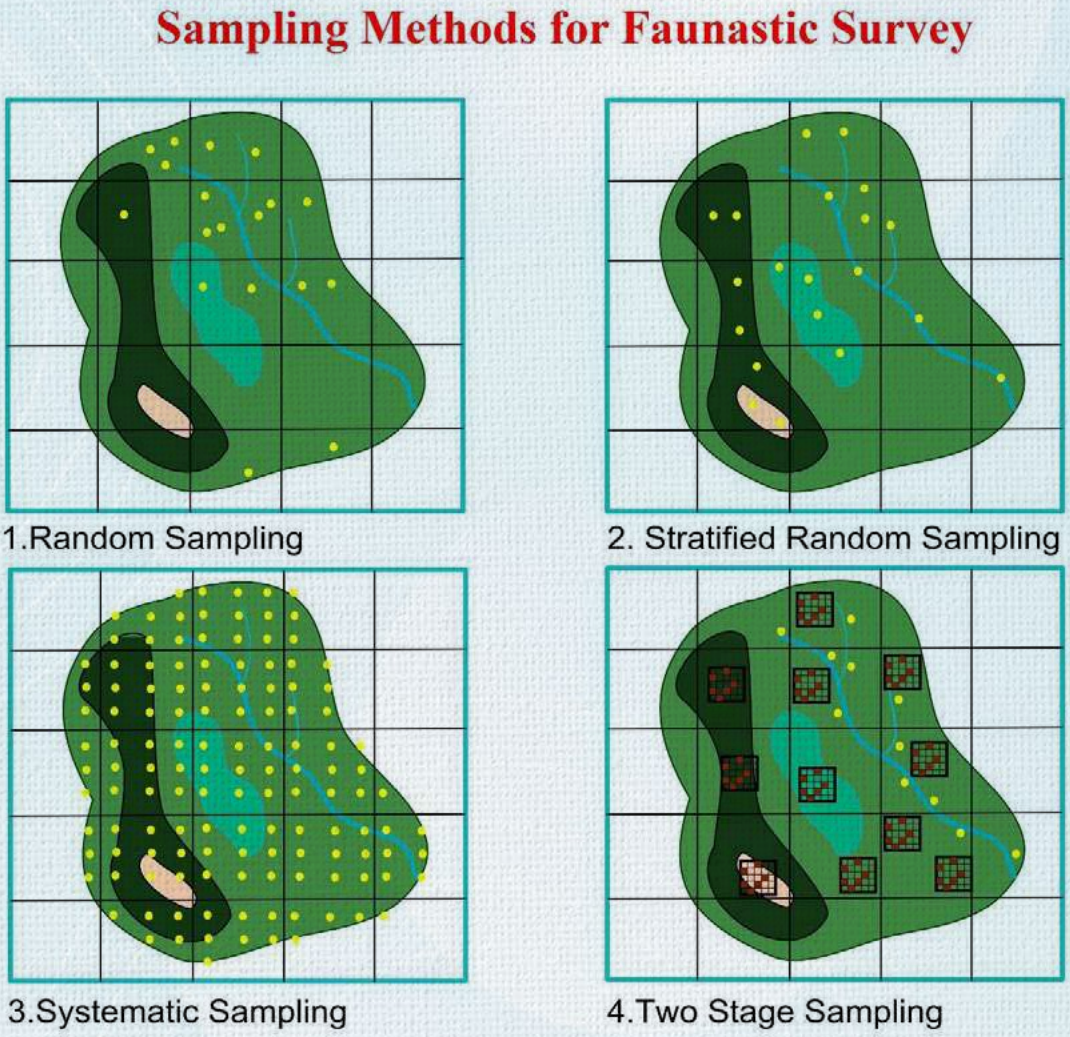
1. Species checklists across habitats/ecosystems/landscapes.
2. Species presence/absence data across habitats/ecosystems/landscapes.
3. Species relative abundance data across habitats/ecosystems/landscapes.
4. New taxa to science.
5. New reports to the country.
6. Data on endemic, endangered and threatened species across habitats/ecosystems/landscapes.
7. Data on exploited species.
8. Data on invasive aliens across habitats/ecosystems/landscapes.

These data once organized into spread sheets such as MS Excel can be converted to tables, charts and graphs. Presence/absence data and relative abundance data can be used for calculating various alpha and beta diversity indices. Open source software such as PAST can be used for calculating biodiversity indices and preparing graphs.

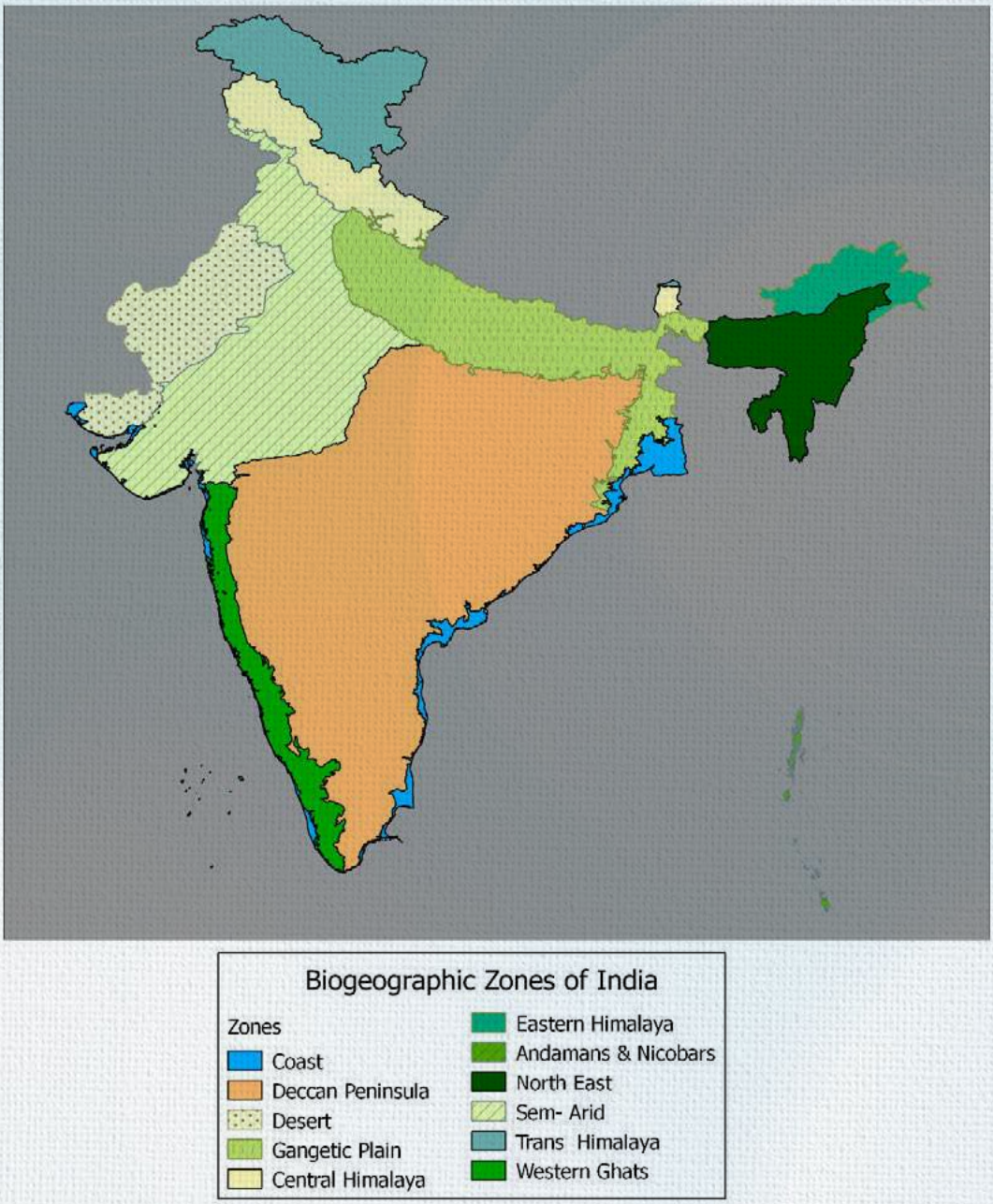
The georeferenced data can be converted into Shape files which are basic file format for GIS analysis. Once data is brought into GIS platform detailed analysis can be carried out using spatial statistics tools and visualized as maps. Distribution and abundance data on endemic, endangered, threatened, exploited and invasive alien species across habitats, ecosystems and landscapes is of immense conservation value



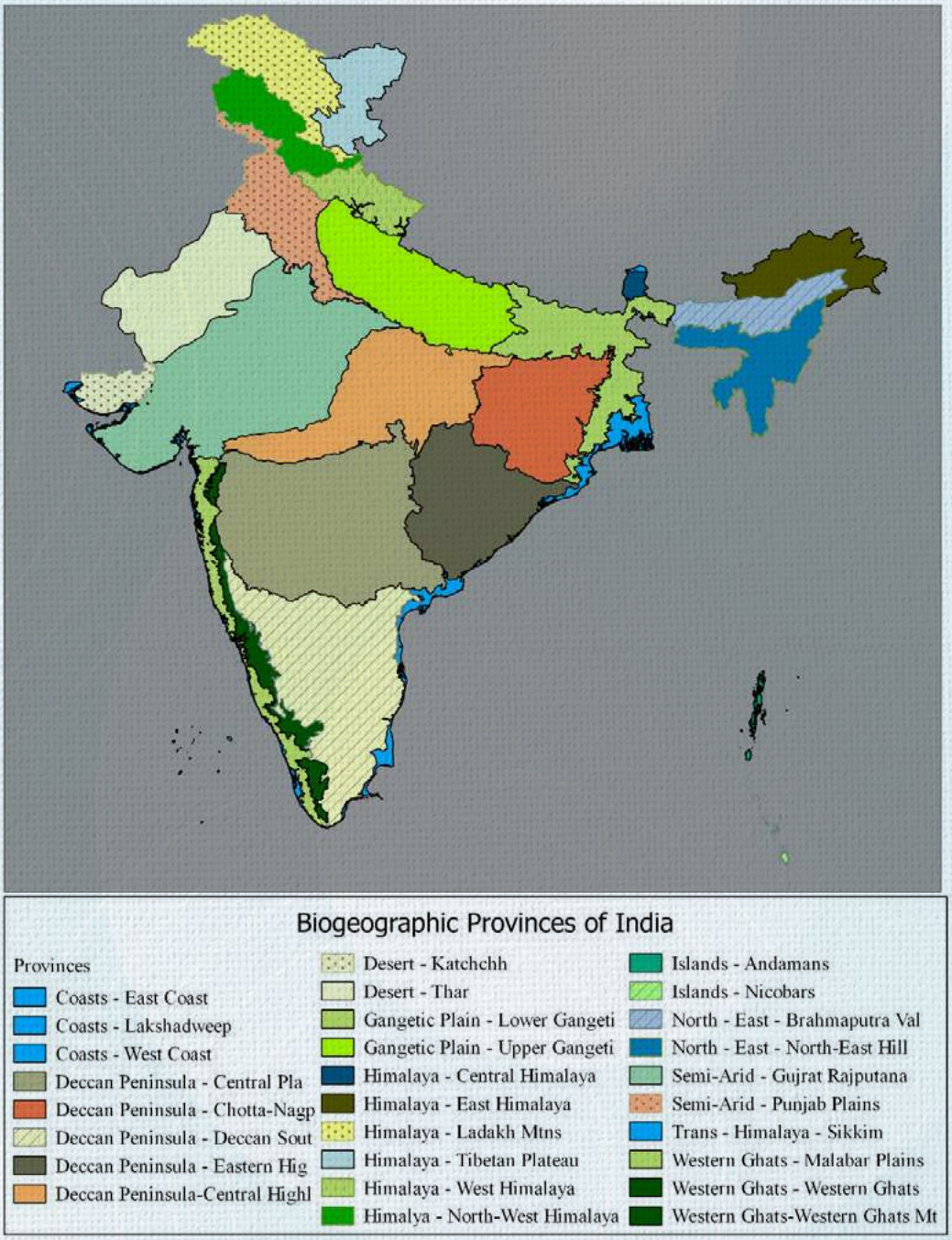
Graphic showing nestedness of spatial units and change in biodiversity across spatial scales. Faunistic surveys are conducted at landscape level, within which ecosystems, habitats and microhabitats are nested. Several landscape level surveys will add on to generate data on ecoregions.



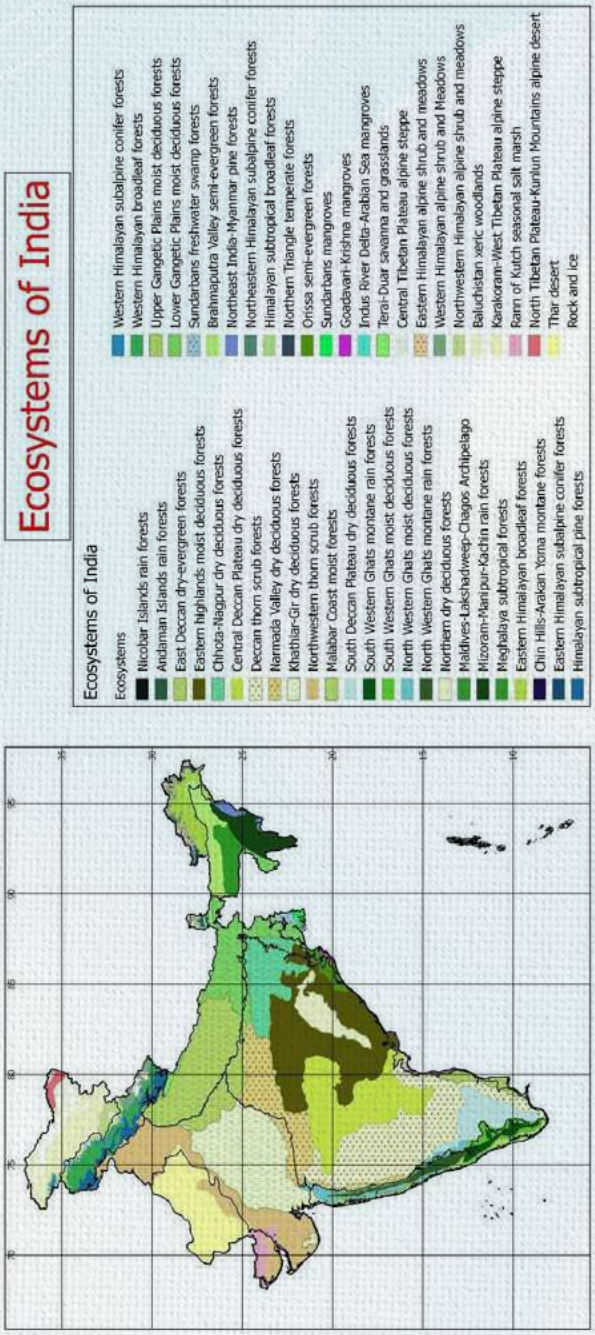
Important sampling strategies of Faunistic surveys. Stratified Random Sampling and Two Stage Sampling is recommended to maximize documentation of faunal diversity.



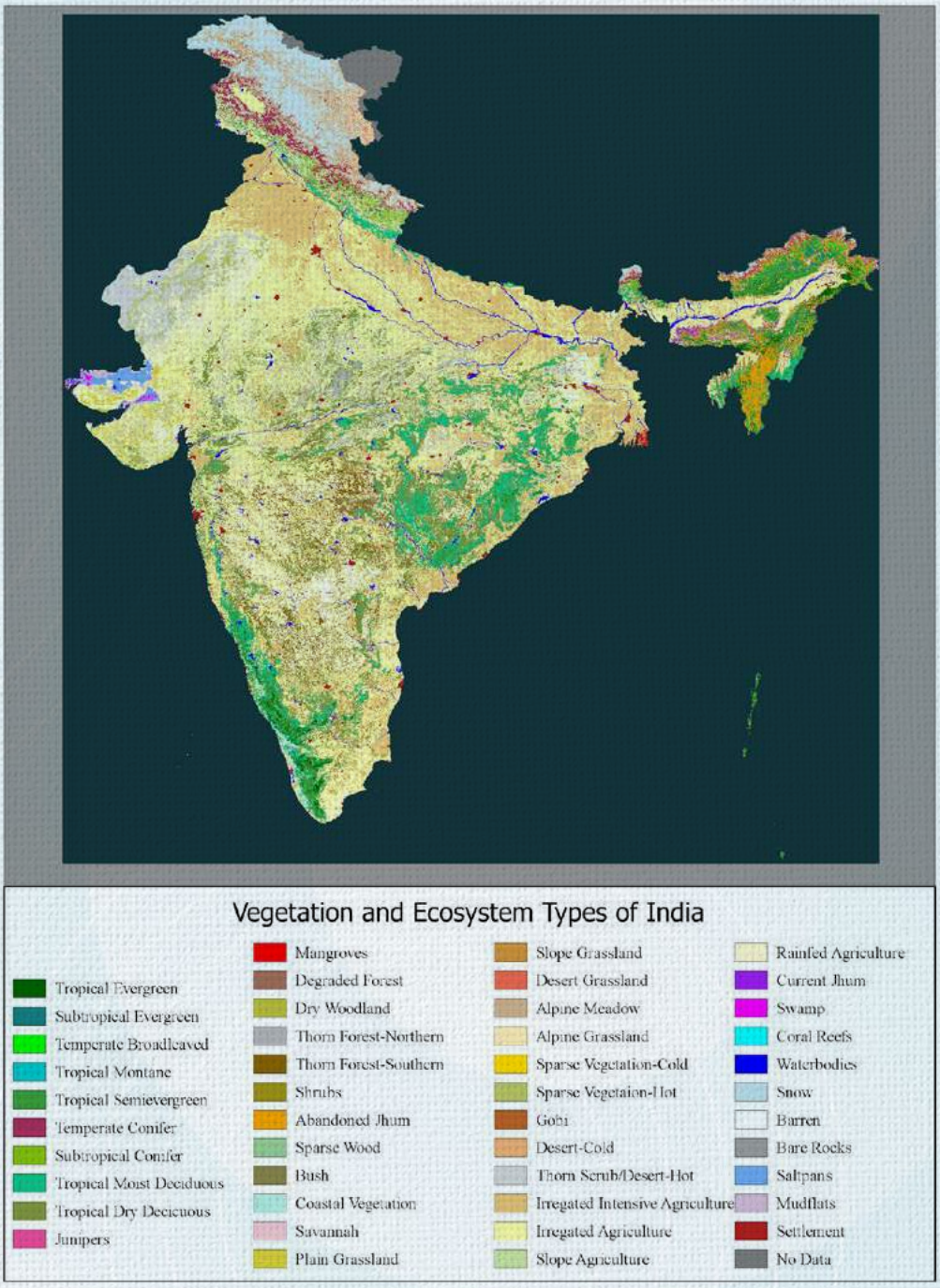
Ten biogeoraphic zones of India. Biodiversity varies across the biogeographic zones.



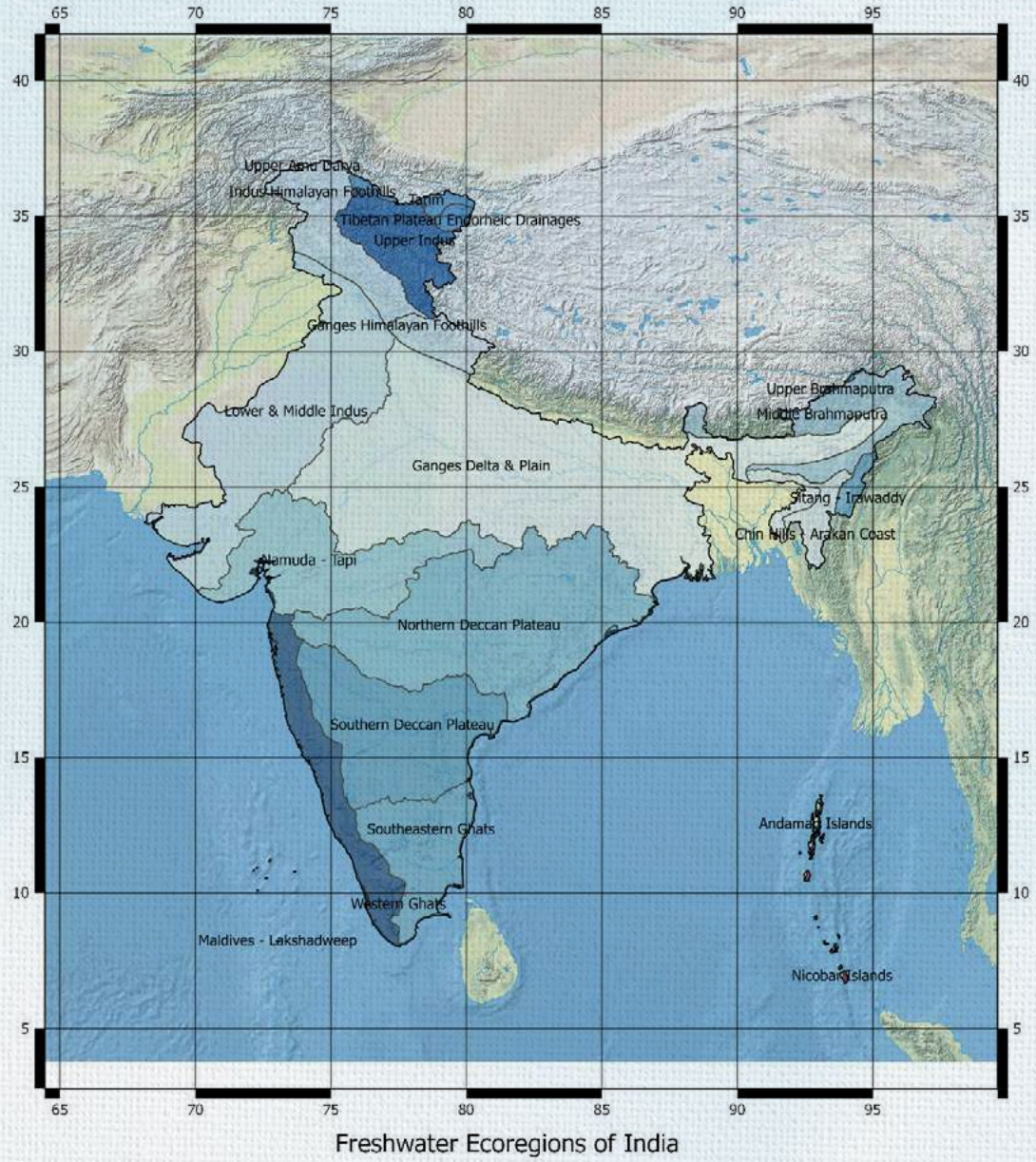
Ten biogeographic zones are further divided into twenty eight biogeographic provinces based on bioclimatic variables to which fauna and flora respond.



Ecosystems of India as per international classification scheme.

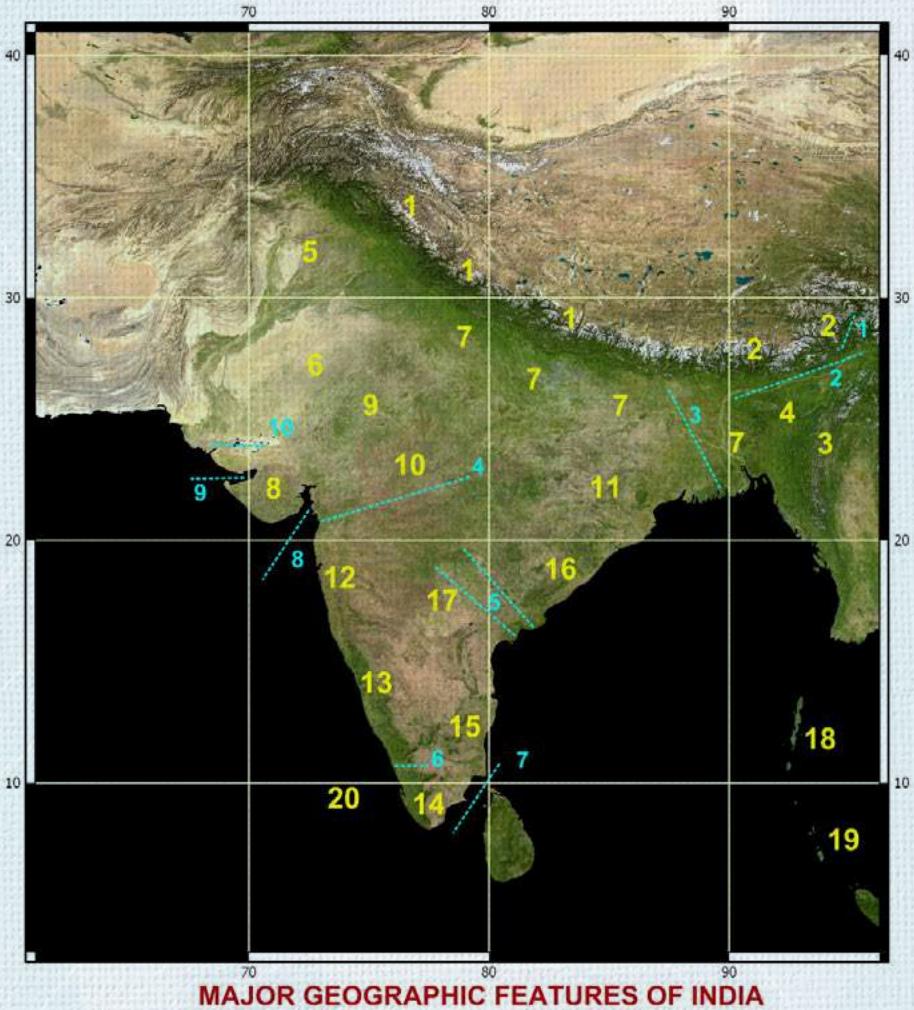


Major vegetation and ecosystems of India.

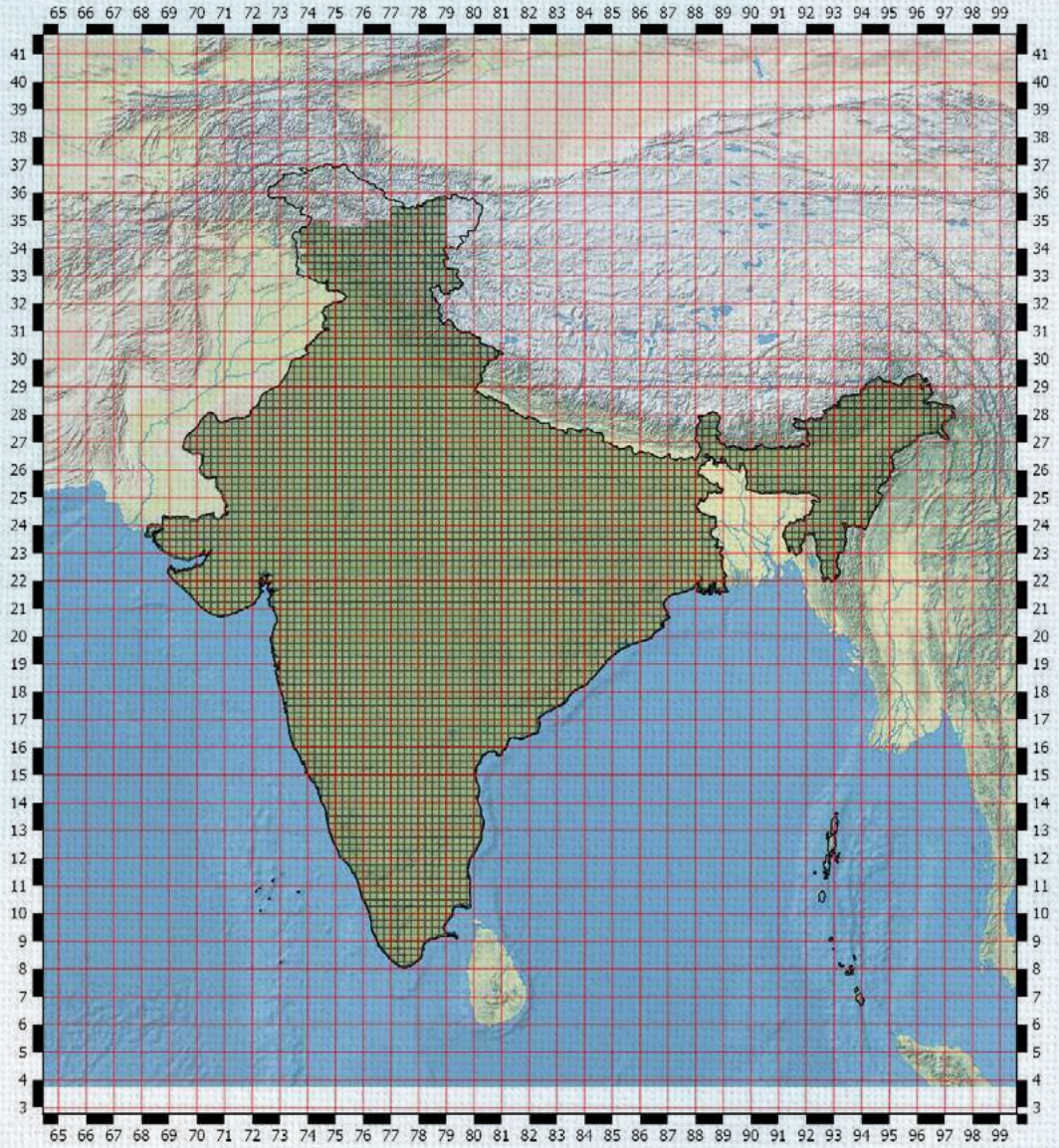


Freshwater Ecoregions of India

Freshwater ecosystems of India are divided into freshwater ecoregions as per international classification.

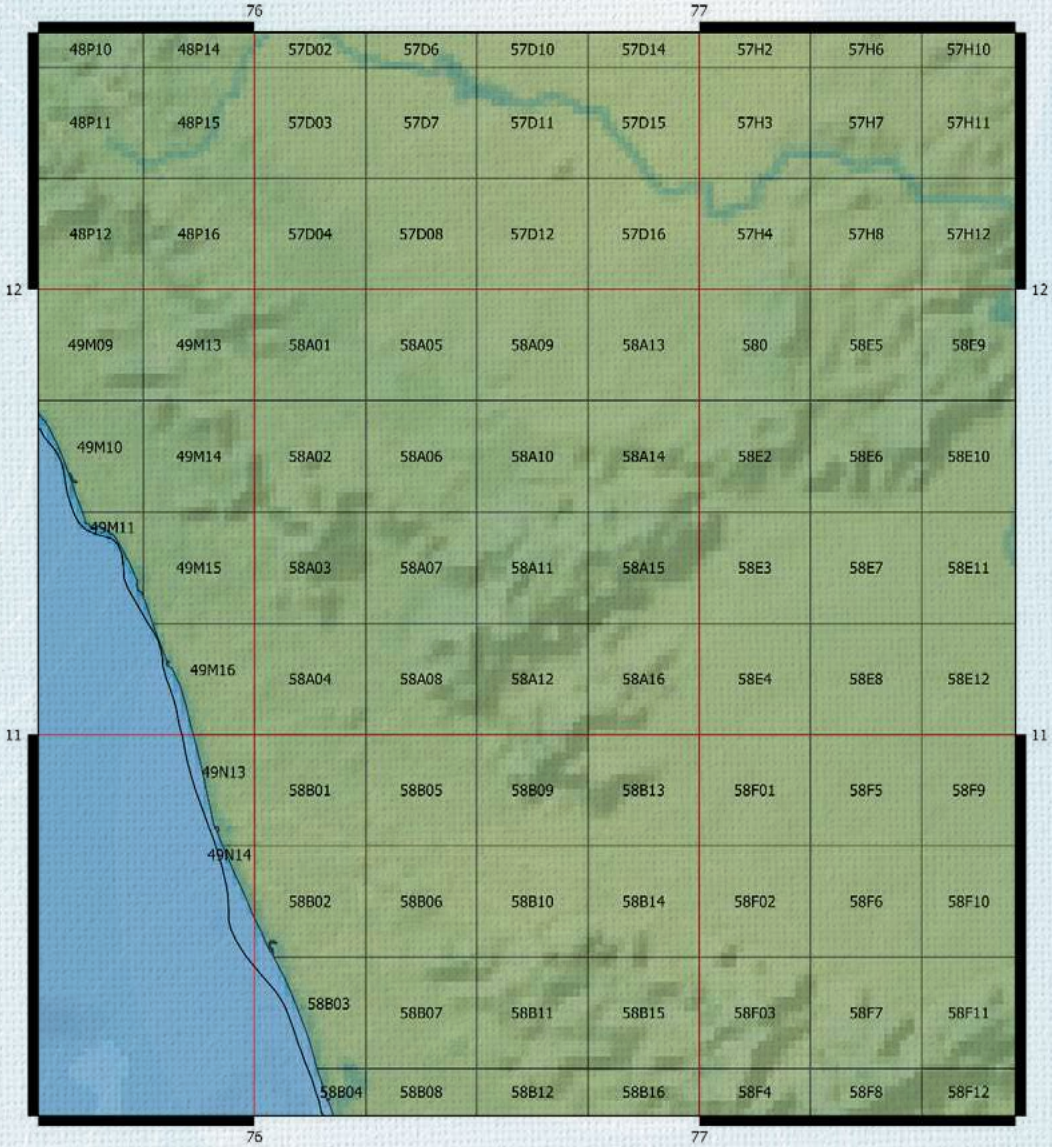


Geographic features such as mountains and rivers play a major role in determining species distribution.



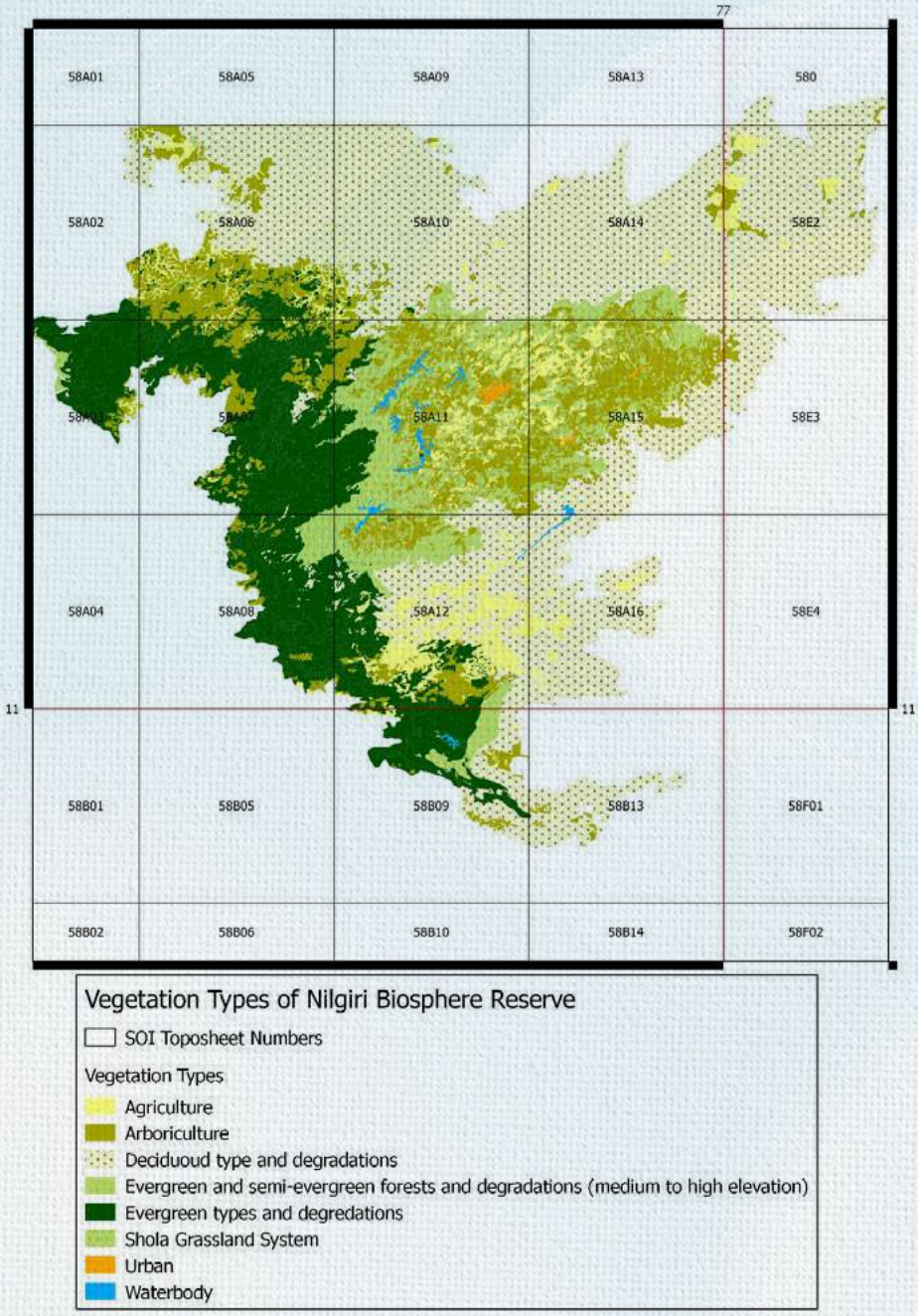
Coverage of 1:50,000 Survey of India Topo Sheets

Survey of India has divided the terrestrial region of India into 15 X 15 minute grids at a scale of 1: 50,000 (1cm=500m). One degree grid is covered by sixteen topo sheets which are uniquely numbered and provide all details required for a survey.

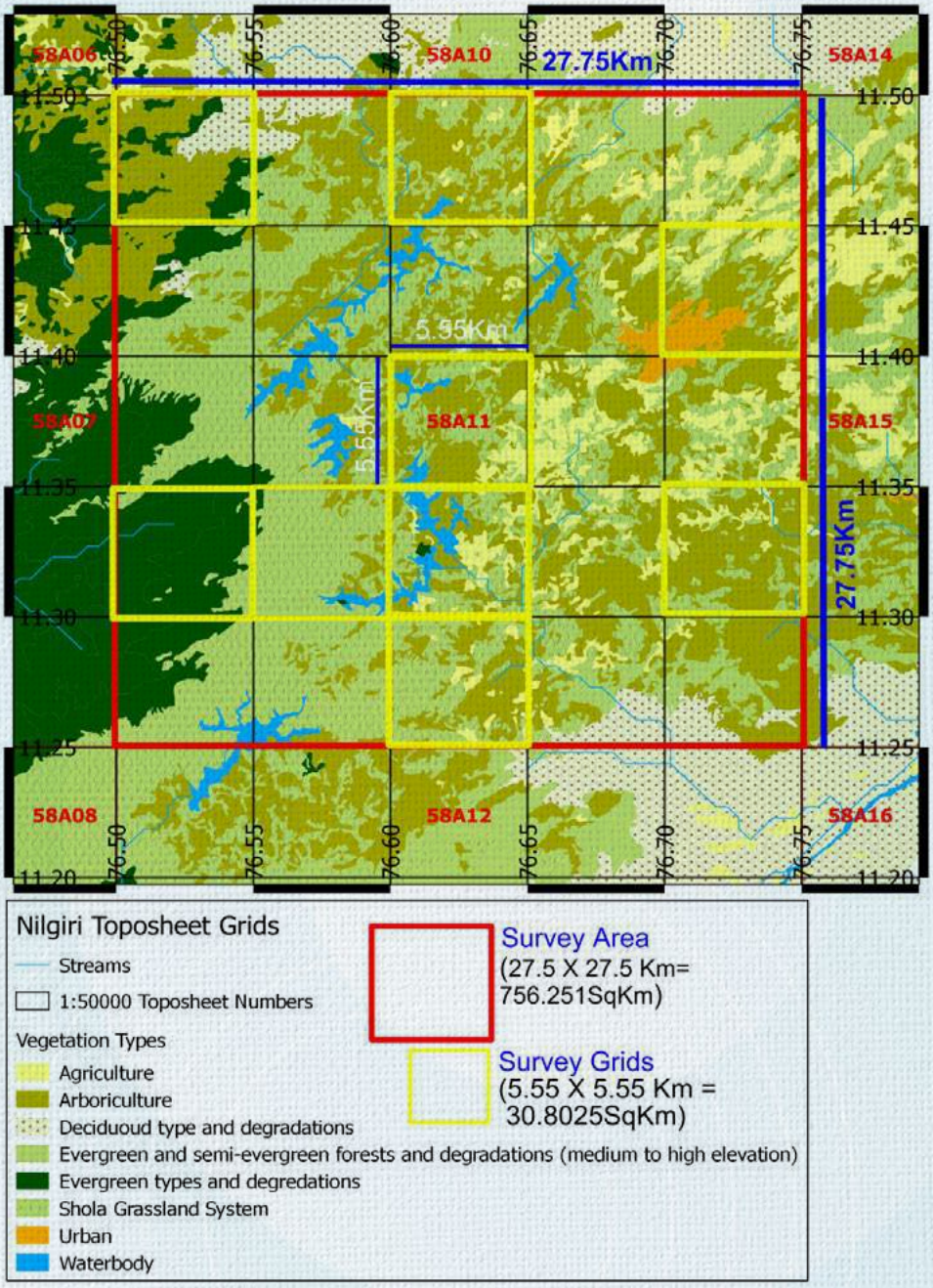


Coverage of 1:50,000 Survey of India Topo Sheets
South Western India

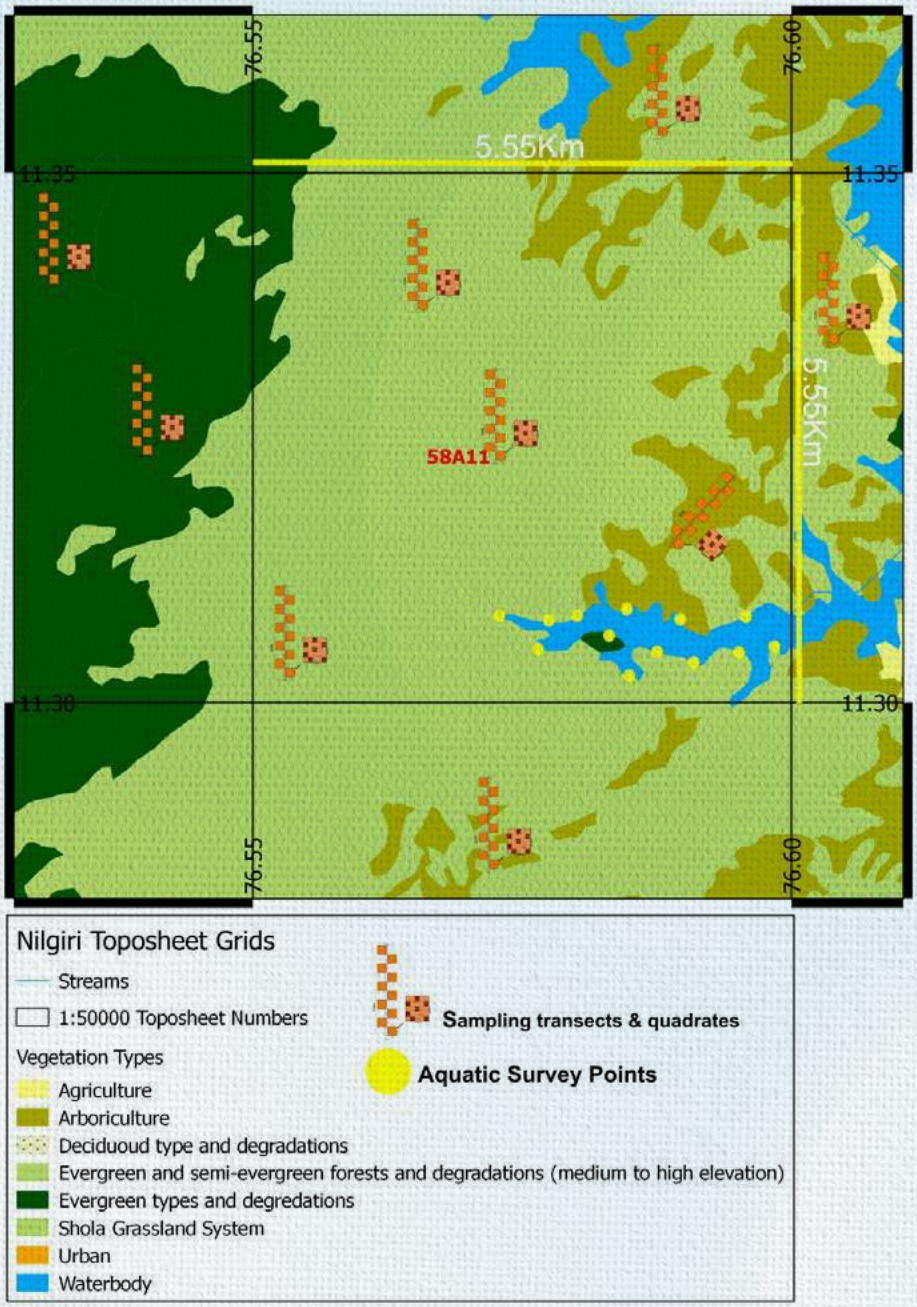
Coverage of Survey of India topo sheets showing unique numbers.



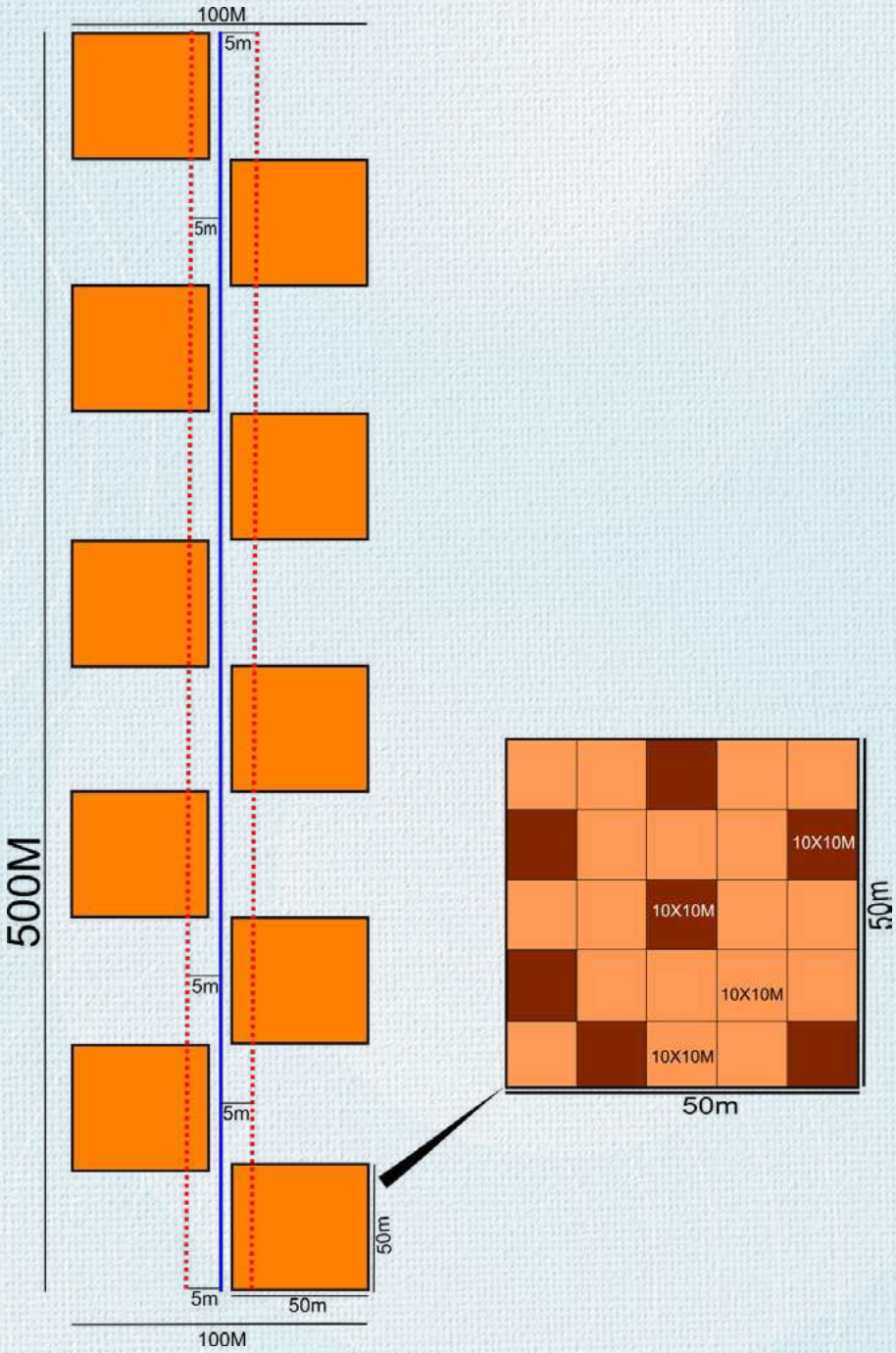
Overlaying of vegetation map of Nilgiri Biosphere Reserve and survey of India topo sheet grid with reference numbers.



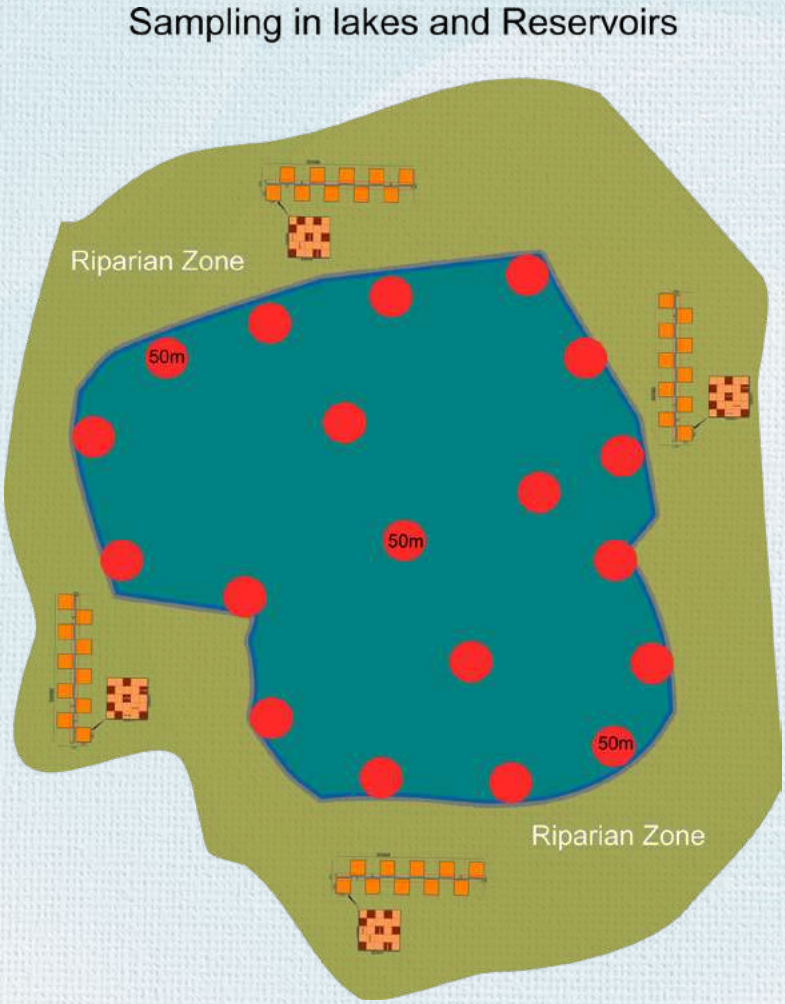
Survey scheme showing survey area and survey grids. The survey area is stratified to cover different vegetation types (ecosystems).



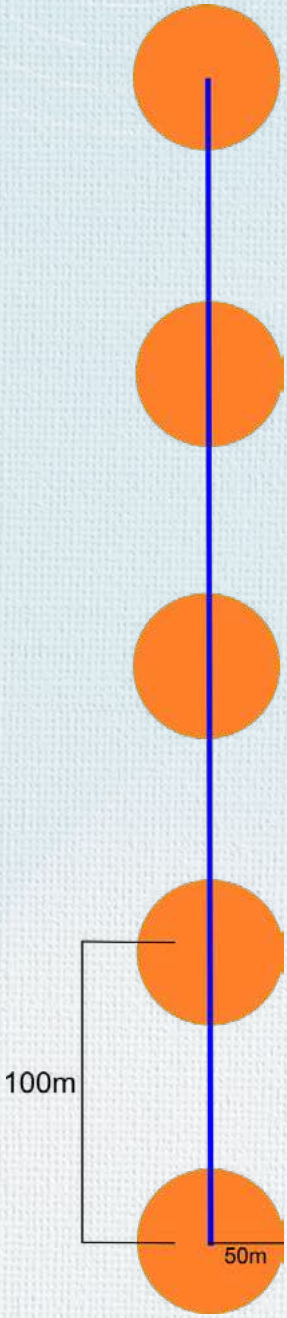
Within a survey grid, terrestrial and aquatic ecosystems are sampled as per the protocol. Note that the sampling points are evenly spread to cover different ecosystems.



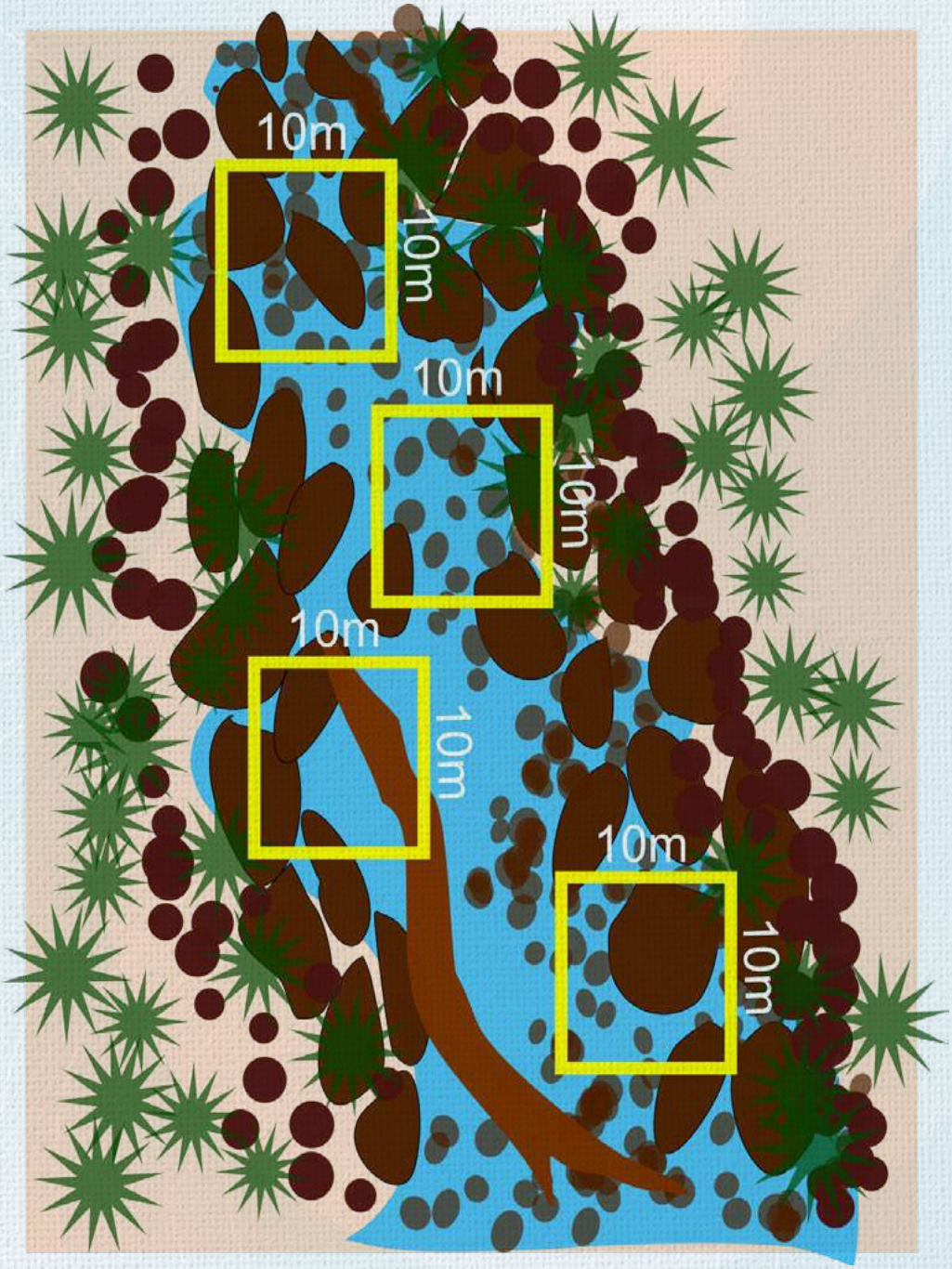
Nested quadrate and belt transect sampling scheme for terrestrial vertebrates and invertebrates.



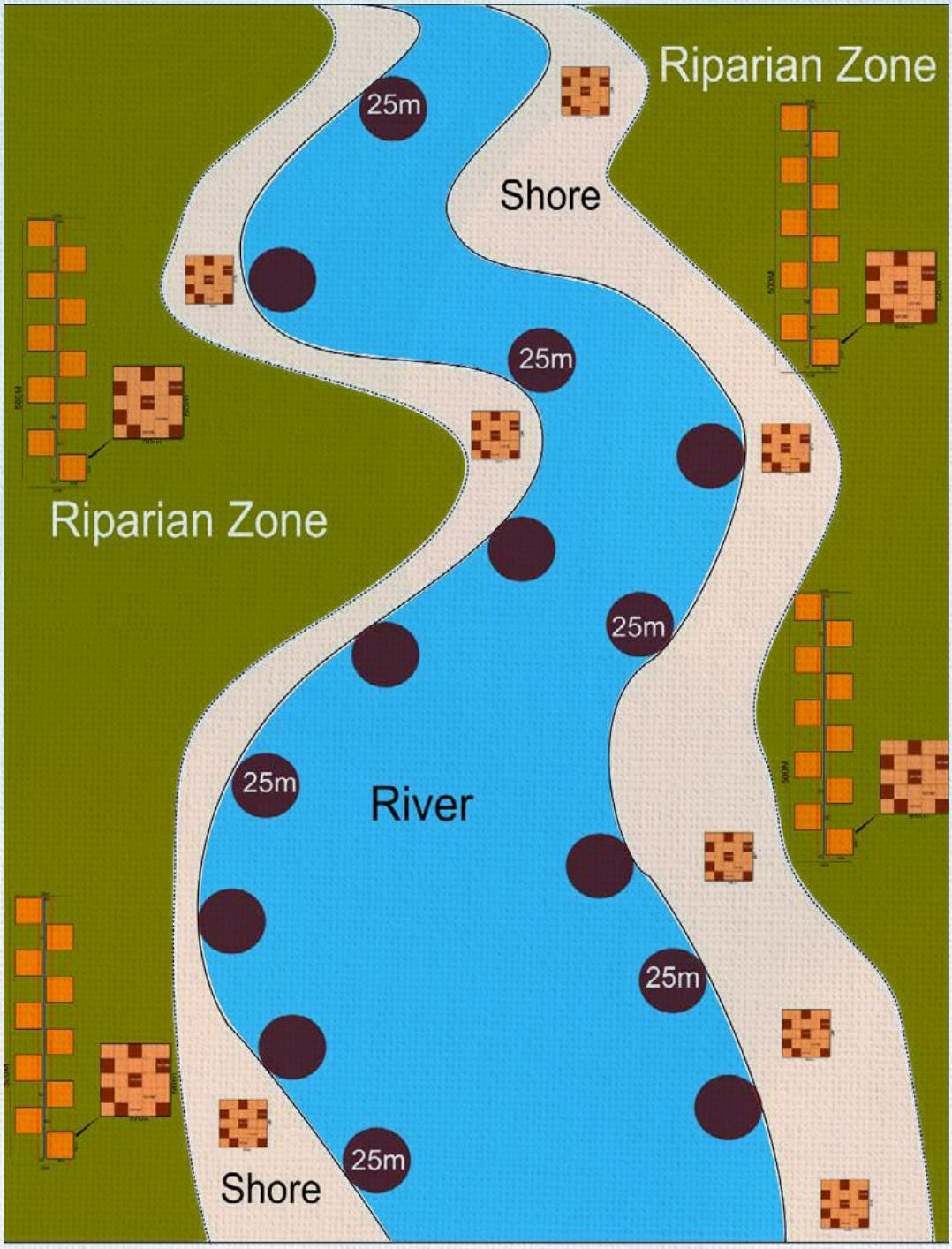
Terrestrial and freshwater sampling scheme for large lakes and reservoirs.



Scheme for point count method for birds.



Sampling Scheme for Hill Streams



Terrestrial and freshwater sampling scheme for rivers

III Collection and Preservation Techniques for different Faunal groups

1. PROTOZOA

INTRODUCTION

Ideally protozoa are the first eukaryotic animals, mostly small and microscopic, generally unicellular, and sometimes simple, but some of them are regarded as the most complex cells for carrying on all the biological and biochemical processes for a complex life-style within the single cell. According to the revised classification developed in 1980 by the Society of Protozoologists, the subkingdom Protozoa is divided into seven phyla, viz., Sarcomastigophora, Labyrinthomorpha, Apicomplexa, Microspora, Myxozoa and Ciliophora (Levine *et al.*, 1980). These unicellular organisms with one or more nuclei usually range between 5µm to 250 µm and may exceptionally be as large as 5000 µm (e.g., some foraminiferans and trophozoite stage of *Myxobolus*). These animals with very simple to most complex life cycles occupy multifarious habitats and lead a diversified mode of life, such as, free-living, parasitic, symbiotic, *etc.* Hence, collection and preservation of protozoa require special efforts and modern techniques depending on the group to be collected. Ecologically, Protozoa are distributed in all moist habitats and hosts including invertebrates and vertebrates and plants, and can be categorised into 4 major groups. So, it would be wise to explore protozoans either habitat-wise or group-wise. The habitat-wise or ecological category-wise survey is conducted for general collection of protozoa whereas taxa or group-wise investigation is adopted for specialised collection. The ecological categories of Protozoa along with habitats are presented (**Table 1**).

Table 1. Ecological categories of Protozoa and their habitats.

ECOLOGICAL CATEGORIES	MAJOR GROUPS	HABITATS
A. Freeliving Protozoa		
(i) Freshwater forms	Flagellates, rhizopods, ciliates	Ponds, lakes, rivers, etc.
(ii) Brackishwater forms	Flagellates, rhizopods, ciliates	Backwaters, estuaries, etc.
(iii) Saltwater forms	Flagellates, rhizopods, ciliates	Coastal/ Oceanic waters
(iv) Soil-inhabiting forms	Flagellates, rhizopods, ciliates	Wet/ Semi-dry soils

ECOLOGICAL CATEGORIES	MAJOR GROUPS	HABITATS
(v) Moss-inhabiting forms	Flagellates, rhizopods, ciliates	Rock/Tree moss, etc.
(vi) Epiphytal/ Periphytal	Flagellates, rhizopods, ciliates forms	Hydrophytes/ Macrophytes, etc
B. Symbiotic Protozoa		
(i) Intestinal forms	Flagellates Gut of wood eating termites	
(ii) Rumen-dwelling forms	Ciliates	Rumen of ruminants
C. Commensalistic Protozoa		
(i) Ectocommensals	Mostly ciliates	Body surface
(ii) Endocommensals	Flagellates and ciliates	Gut/ Rectum
D. Parasitic Protozoa		
(i) Lumenicolous forms	Flagellates, ciliates, gregarines, Coccidians, etc.	Alimentary canal
(ii). Histozoic forms	Haemoflagellates, myxozoans, microsporans, etc.	Body organs/ tissues including blood
(iii). Coelozoic forms	Myxozoans	Gall bladder/ Urinary bladder

COLLECTION METHODS

Collection of protozoa is made from various aquatic and terrestrial habitats and also from different organs or micro-habitats of the host concerned. For freeliving forms, samples are taken from fresh-, brackish- and saltwater in wide mouthed glass jars along with some algae, water weeds and/ or bottom ooze and kept in a sun-lit place at room temperature for subsequent detection of protozoa under a compound microscope. Samples of parasitic protozoa including symbionts and commensals are obtained by dissecting organs/ systems of the hosts. The detection of parasites involves microscopical examination of the gut content, faeces, blood smears, organ imprints, tissue sections, etc. Dissecting instruments, culture tubes, cavity blocks, etc., are required to be sterilized at the time of dissection and physiological saline is essential for microscopical examination in live condition. There are several techniques even for collecting / detecting protozoa from a single habitat type or host organ. However, some common and easy to adopt laboratory techniques (**Table 2**) are summarized hereunder.

Table 2. Common collection techniques for major ecological categories of Protozoa.

ECOLOGICAL FORMS	COMMON LABORATORY TECHNIQUES
A. Freeliving forms	
(i) Freshwater Protozoa	1. Collecting water samples with weeds, flocculent matters and bottom ooze 2. Using plankton net/ sieve particularly for plankton.
(ii)Marine Protozoa	1. Mostly by using nets. 2. Sometimes by sieving bottom samples for foraminiferans and testaceans.
(iii) Soil Protozoa	1. Using soil corers, dredge, etc. 2. Sieving soil samples.
(iv) Moss-inhabiting Protozoa	1. Culturing moss samples at room temperature
B. Parasitic/Symbiotic/ Commensal forms	
(i) Lumenicolous Protozoa	1. Dissecting digestive tract and examining gut content/ faeces in physiological saline. 2. Concentrating cysts in 33.1% aqueous zinc sulphate sulphate solution specially in case of scanty infrctions.

ECOLOGICAL FORMS	COMMON LABORATORY TECHNIQUES
(ii) Coelozoic Protozoa	1. Dissecting gall bladder, urinary bladder. 2. Examining coelozoic fluid under microscope on cavity slides and/ or making smears.
(iii) Histozoic Protozoa	1. Dissecting suspected organs and making imprint smears/ blood smears. 2. Making microtome sections after fixing the suspected organs in Carnoy's fixative/ Bouin's fixative and embedding in paraffin.

LIVE PREPARATION TECHNIQUES

For taxonomic study, live temporary preparations of some freeliving and lumenicolous protozoans are required to be made to observe them under microscope in live condition because certain diagnostic features can not be seen after preservation on slides. For example, live prepration helps to distinguish colonial peritrich ciliates, which contract individually (e.g., *Carcheslum*) or all individuals in the colony as a whole (e.g., Zoothamnium). These temporary preparations are usually observed under a suitable phase contrast microscope with image capture computer attachment system.

Unstained preparation

Freeliving forms with a drop of of their natural/culture medium or lumenicolous flagellates and ciliates in the gut content/faeces with a small amount of physiological saline (0.5–0.8% Normal saline/Ringer's solution/Locke's solution) is placed on a slide, covered with a cover slip and, if necessary, sealed with liquid paraffin for microscopical examination. For slowing down the movement of freeliving protozoa a ring of Methocel solution on the slide may be added at the time of preparation of the slides. Both ciliary and flagellar movements as well as contraction of colonial/epizoic peritrichs can be seen in such preparation along with protrusion of pseudopodia.

Stained preparation

Stained temporary preparation can be made using intra-vitam stains, such as, neutral red and methyl blue (0.01%). A drop of stain is allowed to dry on slide and then a drop of sample is placed on the stained area of the slide and covered with a cover slip. Aqueous Eosin solution (1%) is often used to differentiate unstained protozoan cysts (and helminth ova) against the coloured background of Eosin solution, while Lugol's iodine solution (4% Potassium iodide and 2% Iodine in distilled water) is used to stain the nuclei of cysts and their internal structures.

PRESERVATION TECHNIQUES

The preservation of protozoan specimens are usually made on slides as permanently mount preparation and sometimes preserved as cultures. These two types of techniques are briefly discussed here referring interested workers to consult Mandal and Nandi (1980) and Mandal *et al.*(1991).

Permanent preparation

The preparation of permanent slides involves fixation, staining, differentiation, dehydration and mounting in a neutral medium as follows :

(i). Freeliving and lumenicolous Protozoa

Killing and fixation	:	Killing and fixation can be done using Schaudinn's fixative, Bouin's fixative, etc., in semi-dried condition.
Staining and differentiation	:	Staining is done in Heidenhain's iron-haematoxylin and differentiation in 1% iron alum solution.
Dehydration and mounting	:	Dehydration is made through ascending series of alcohol (30 – 100%), clearing in xylene and mounting in DPX.

Labeling: Labeling of slides should be made with all collection data on printed labels.

Note: 1. Stains are used in accordance with the fixatives and with respect to organelle to be studied. For example, Feulgen stain is used for nucleus and Potargal stain for ciliary lines.

2. Best results are obtained with materials stained soon after fixation.

3. Gradual changes in alcohol grades are required in all staining and dehydration procedures to avoid distortion.

4. Smears should not be allowed to dry at any stage of processing of the slides.

(ii). Histozoic and Coelozoic Protozoa

Suspected organ-imprint smears and blood films are made on clean grease free slides, drying them in air and fixed in absolute acetone-free methyl alcohol for 5 minutes. The fixed and dried smears are subsequently stained with Geima's stain for 45 minutes and then washed in neutral distilled water, dried and preserved with or without mounting the slides in DPX.

Preservation as culture

Freshwater protozoa are usually cultured in hay/wheat infusion, while Lwoff and Lwoff medium is used for flagellates. Rice-agar medium is suitable for rhizopods and hay infusion for ciliates. Soil-extract agar or chopped hay infusion is used for culturing soil protozoa. For cultivation of parasitic protozoa, in-vitro culture in a simple medium of peptone, glucose, sodium chloride and water is employed for monogenetic flagellates. However, host erythrocyte suspension is useful for malarial parasites; NNN medium for digenetic trypanosomes and TTY (Tryptose, Trypticase, Yeast extract) medium for termite flagellates, etc.

2. PORIFERA (Sponges)

INTRODUCTION

Sponges are the simplest of all animals and are sessile and asymmetrical adults; with pores on the body surface for water circulation, contain specialized choanocytes or collar cells, but no tissue. A single large osculum or a few oscula open on the top for water circulation from the body cavity. Spicules, tiny hard structure made of calcium carbonate or silica, and/or spongin are present to form the skeleton of the body. Sponges are filter feeders that remove planktons (food) from the water that is brought in through pores lined with collar cells. Sponges reproduce asexually by external buds that break off and form new sponges or stay attached to form a sponge colony or by gemmules which are specialized, internal buds formed during cold or dry weather, that can survive harsh conditions. Sponges vary in their size from 2 cm to 2 meters and are distributed in freshwater as well as marine environments. Sexual oviparous or viviparous and development is by parenchymula or amphiblastula larva.

COLLECTION METHODS

Sponges may be removed from the substrate with a knife or chisel, preferably using protecting gloves and protective clothing. Collection of sponges intended for identification should be accompanied by *in situ* photographs and adequate documentation (locally, habitat, surface features, colour notes etc.). In many species both colouration and morphology may change dramatically following collection and preservation. Identifications, even by specialists, are often greatly facilitated if there are adequate colour photographs of live materials.

FIXATION AND PRESERVATION

Sponges should be frozen immediately upon collection, which to a certain extent fixes the colour, or live material may be placed directly in 80-90% ethanol solution. Sponges should not be fixed or preserved in formaline.

3. CNIDARIA

INTRODUCTION

Cnidarians are a diverse group of organisms, which are placed in the phylum Cnidaria, solely due to the presence of specialised defensive cells called nematocysts in their bodies. The word 'cnidos' in Greek means stinging nettle. Nematocysts eject stings tipped with poison with barbed threads at their end and cause irritation to the animals, which come in contact with it. Some are lethal too, due to the potency of the poison in their body.

Cnidarians are grouped into four major groups. *Anthozoa* is the most primitive of the four groups and includes true corals, which build giant reefs in tropical waters as well as sea anemones, sea fans and sea pens. *Cubozoa* consists of deadly box jellies with complex eyes. *Hydrozoa* is the most diverse group with hydroids, siphanophores, fire corals and many medusae. *Scyphozoa* are the big jellyfishes.

Cnidarians pose difficulties to systematics due to their diverse form and simple morphology. They are considered to be organisms with simplest tissue grade of organisation. Simple morphology creates difficulty in comparing taxa. Their cells are organised into true tissues. Cnidarians are described as animals with hollow cylindrical body with tentacles on top. Their bag like body is composed of two cell layers, outer ectoderm or epidermis, inner endoderm or gastrodermis which in some may be divided into septa (Anthozoa) or into elaborated branching canals (Scyphozoa) and an intermediate layer of jelly like substance called mesoglea which is made of scattered cells and collagen fibers. A ring of tentacles often surrounds the mouth.

Cnidarians are predominantly marine occurring from the tropics to the poles from the shallow depths to the deepest bottoms. Some are burrowing too. They occur in small numbers in rivers and fresh water lakes. Even though many thousands of species are discovered to date, taxonomists consider that as a meager percentage.

COLLECTION METHODS

Majority of the Cnidarian species are sessile (attached to the substratum) followed by planktonic medusoid forms and very few docile swimming forms. Hence, in addition to general equipment, specialised equipment is also needed for their collection.

Equipment

In the field, general equipment include collecting instruments like Forceps, Gloves and Scoops are needed in addition to containers like bottles, tubes, bags etc., along with dishes, troughs for general observation and magnifying lenses and photographic equipment for close observation and recording the details of a specimen. In the laboratory, chemicals for relaxing, fixing and preserving the specimens along with needles, scissors, microscopes for further preservation and analysis and labeling material for labeling the specimens are a must. Further, special equipment is needed depending on the type of substrata, and depth (intertidal or subtidal).

Collection

Method of collection basically varies depending on the depth of the sampling site and substratum. Intertidal micro fauna (which are few millimeters in size) can be well collected during spring tide. Large non-sessile animals can be picked up either with the help of forceps or by hand. Sessile animals are to be picked with the rock/seaweed to which they are attached using a hammer, chisel/scissors. Rocks or weeds are moved to expose the motile fauna. Burrowing animals can be collected by squirting a weak solution of formalin or if necessary by digging the burrow.

Planktonic forms can be collected by using specific nets. Scyphozoans and cubozoans can feebly swim against the water currents and are collected with the help of plankton nets and are mostly found as by catch in trawling nets. Sieves are used to separate the specimens from unwanted material in the sandy and muddy environments.

Majority of the marine cnidarians are benthic and collected from the subtidal regions by divers from the coral reef areas. Collections are made using a hammer, chisel and cutting pliers to separate from the substratum to which they are attached divers generally use 'Slurp gun' to suck out the burrowing forms.

Dredges, Grabs and Trawls are also used in deep open seas to collect the samples remotely.

PRESERVATION

The main objective of preservation is to keep the dead animal in its original shape and form (as in the living condition) for a longer duration to document ecological, biogeographic, physiological or other scientific status related study over years.

Many fauna, especially invertebrates are highly contractile, hence relaxation through anesthetics before death and fixation is needed. Fixation stabilizes proteins in tissue and keeps the form of the animal intact even after death. Suitable preservatives maintain the fixed specimen for a longer period. Hence, the process of preservation is a sequence of relaxation or narcotisation, fixation and preservation.

The duration of narcotisation or fixation is a matter of preference and is mostly dependent on the size of the specimen. For better preservation of specific groups preliminary experimental observations are to be made.

A. Relaxation or Narcotisation

Magnesium Chloride is the most recommended narcotising agent for Cnidarians. The animals are exposed to approximately 7% Magnesium Chloride which is isotonic to seawater for about 5 hours for narcotisation.

B. Fixation

In general, after narcotisation the specimens are exposed to different fixing agents for killing and fixing the specimen. Main objective of fixation is to make the cell contents into insoluble substances to prevent degradation of the tissue. This is best achieved on the relaxed material. Formalin and Ethyl Alcohol are the mostly widely used fixing and preserving agent for most of the biological specimens. Formalin is sold as 37-40% Formaldehyde in aqueous state. To make a solution of 10% formalin, which is mostly used for fixation and preservation of animals, 9 parts of water is added to one part of 40% Formaldehyde (aqueous). The draw back with the Formalin/Formaldehyde is that they readily oxidize into formic acid with a pH range

of 2.5 to 5, which dissolve the calcified tissue of the specimens in due course. Since seawater has got a pH of 8.2 it is sometimes used as a buffer. Calcium Carbonate and Borax (Sodium Borate) are also used for short term buffering. For long term buffering of Formalin/Formaldehyde solution a phosphate buffer is used. In addition to this, Formalin/Formaldehyde is hazardous and must be avoided contact with skin and eyes or inhalation.

4–10% Phosphate buffered formalin is the most preferred fixing agent for Cnidarians. A suitable 10% phosphate buffer (Sorensen's Buffer) is prepared by adding four grams of monobasic sodium phosphate monohydrate (NaH2PO4.H2O) and six grams of dibasic sodium phosphate anhydrate (Na2HPO4) to 100 ml Formaldehyde (40%) and 900 ml Seawater or distilled water.

C. Preservation

Cnidarians are found both in the polyp form and medusae form. Ethyl Alcohol is the best-known preservative for archival storage of the specimen tissues. This helps in the analysis of DNA even after many years. The draw back with the Alcohol is that it shrinks the medusae. Hence, the medusoid Cnidarians are preferred to be preserved in buffered formalin for retaining the shape and form.

PRESERVATION OF SPECIFIC TAXA

No two groups of animals can be preserved using the same technique due to the diversity in their chemical composition and form. Cnidarians are incredibly diverse in form. The present day identification of Cnidarians is mostly done with the help of the morphology of the animal body or skeleton. Hence, the preparation of the different groups in Cnidaria for identification involves different methods. The following is a brief account of those methods.

Narcotisation

7% magnesium chloride is the relaxing agent for all classes of cnidarians.

Fixation and preservation

Scyphozoa is represented by big jellyfishes and **Cubozoa** by sea wasps and box jellies. Since the medusoid forms are shrunk by Alcohol, animals belonging to these groups are preserved in 5% phosphate buffered formalin after fixing them in 10% phosphate buffered formalin. Sometimes 5% solution of chromic acid is used to make the jelly tough and more pliable.

Hydrozoa is represented Marine hydroids, Freshwater hydras, Small jelly fish, special Corals (ex. Fire coral) and Siphanophores (ex. The Portuguese man of war) consisting of both medusoid and polyp forms.

The medusoid forms of Hydrozoa are fixed and preserved in 5% phosphate buffered formalin. The Hydroid polyps are first fixed in 4% phosphate buffered formalin and preserved in 70% Ethyl Alcohol after passing them in 30%, 50%, and 70% Ethyl Alcohol. The skeletons of fire corals are preserved in dry state. Siphanophores are placed in Zenker's fluid for sometime to harden the tissue before fixing them in 5% phosphate buffered formalin and finally preserved in 5% phosphate buffered formalin. Zenker's fluid is prepared by adding 5 grams of Mercuric chloride, 5 ml Glacial Acetic Acid, 2 grams of Potassium dichromate and 1 gram of Sodium Sulphate in 100ml of distilled water.

Anthozoa is one of the most difficult groups in the animal kingdom for identification and is exclusively with marine polyps and without a medusoid stage. It is represented by soft bodied animals (Sea Anemones (colonial & non-colonial), Coral like anemones (Corallimorpharians) and animals with some skeleton (Hard Corals, Octocorallians (Soft corals, Horny corals (sea fans & sea whips), Blue corals, Sea pens and sea pancies)). When the soft bodied animals and the tissues of the animals with some skeleton are preserved in 70% Ethyl alcohol after fixation in 4-10% phosphate buffered formalin and subsequent washing in 30%, 50% and 70% Ethyl alcohol. The hard skeleton containing Scleractinians (stony corals) and the soft skeleton containing Octocorals (soft corals, stoloniferans and gorgonians) are treated separately for preserving their skeletal parts for identification. Anthozoans can be very well preserved in Alcohol since there is no medusoid stage and cannot be preserved in Formalin since it dissolves their skeleton.

Hard corals (Scleractinians)

Hard corals belong to the subclass Hexacorallina have a skeleton made up of calcium carbonate. After the collection the samples are immersed in freshwater for a day or two to allow the fleshy part to putrefy. After the putrefaction the skeleton is washed clean under a jet of water. The skeletons totally free of fleshy material are sun dried until the moisture is totally evaporated from the sample. Partial drying of the samples, result in the growth of the fungus leading to the deterioration of the skeleton. Transparent and thick fleshy part found in some corals, like mussids have to be removed with the help of forceps. The dried samples are properly labeled and stored in plastic containers or plastic covers to keep them away from dust.

Octocorals (soft corals, stoloniferans and gorgonians)

The skeletal structure of this group of animals is primarily composed of spicules called sclerites. The identification is based on the shape, size and arrangement of these spicules. Since the characteristics of the spicules are different in different tissues of the colonies, spicule samples are extracted from different parts of the body. For the extraction of the spicules a thin portion of the living tissue is taken in a cavity slide and is treated with few drops of 10% Sodium hypochlorite solution for 3-8 minutes until the tissue is dissolved and spicules are found intact. The spicules are washed with distilled water and observed under the microscope. For the preparation of permanent slides, spicules are treated with xylol and mounted using Canada balsam or DPX mountant under a cover slip.

Storage

Unbreakable bottles or plastic covers must be used to carry the collected samples in the field. Wet specimens must be stored in glass containers for better visibility and to avoid reaction with the preservative. The containers must be airtight to avoid evaporation or leakage of the preservative. Dried samples must be either kept in containers (preferably transparent plastic containers) or closed plastic bags and must be monitored regularly to keep watch on the unwanted growth of fungus on the samples due to accumulation of moisture which leads to the degeneration of the sample.

The Cnidarians are a fascinating group of animals with very interesting associations among themselves and with other group of animals in the animal kingdom. Very little is known about them and their taxonomy and is still a less explored field. They offer a scope and challenge to those who wanted to learn more about them. Many species belonging to this group are becoming increasingly popular because of their economic importance especially in their role in providing fisheries and bioactive substances to the society. Unfortunately, many species have disappeared before being named and many more are in the queue to endangerment. An increase in the number of taxonomists working on this group will not only help in understanding this group but also help those who work on these groups since the taxonomists on this group themselves are an endangered lot.

4. PLATYHELMINTHES

INTRODUCTION

Helminth parasites mainly represent the invertebrate phyla Platyhelminthes (including Monogena, Trematoda and Cestoda), Nematoda and Acanthocephala. They harbour the various habitats (external surfaces such as gills or skin and internal organs) in a wide range of vertebrates (from fishes up to mammals). The guidelines that follow are intended as a help to beginners in the field of Taxonomy of parasites in collecting, storing and staining the worm parasites as a prerequisite to identification based on morphology.

COLLECTION AND FIXATION

To the extent possible the parasites must be collected at the earliest after the death of the host while they are still alive and active so that deterioration or post mortem changes do not occur. Parasites collected may be allowed to relax and flattend prior to fixation.

Polyzoic (*i.e.*, segmented) cestodes should be collected intact or in such a way that scolex and immaure, mature and gravid parts of the strobila are kept. Nematodes and acanthocephalans being diocoeous, both male and female worms need to be collected. If the parasite is gripping fast to the host's tissue (e.g. gut wall, gills etc.) by means of its anchoring structures, it should be freed by teasing away the surrounding tissue or fixed in tissue by cutting out the wall of tissue to which it is attached.

RELAXATION

Live specimens should be relaxed, as they would make better preparation than contracted ones. One of the several methods can be followed:

Place the specimen in tap water or normal saline at 4°C in a refrigerator or at room temperature and keep until relaxed.

Swirl platyhelminth and acanthocephalan worms in warm water or saline from time to time to induce muscle fatigue and prevent strong muscle contraction.

Narcotize platyhelminth parasites in 5–10% ethanol at room temperature.

Stretch the nematodes by plunging them in hot (70°C) fixative.

FIXATION AND PRESERVATION

The choice of fixative depends on how the specimens are to be processed.

For whole mounts, AFA (alcohol-formalin-acetic acid), 70% ethyl alcohol or 4–5% formalin is frequently used.

For general hisological preparations, AFA, buffered 10% formalin, Bouin's fluid and 70% alcohol are suitable fixatives.

For scanning electron microscopy, 10% neutral buffered formalin or 2–3% glutaraldehyde (in 0.2 M phosphate or cacodylate buffer) are used.

For specific histochemical study, the material may be fixed as per the test required (Pearse, 1968, 1972).

FLATTENING

Helminths other than nematodes (which have a cylindrical body contour) are usually flattened and fixed under gentle pressure from a cover slip or slide depending on size and thickness.

Place the specimen on a slide in a drop of water or saline and straighten it gently with a brush. Cover with a cover slip and secure with thread. Fix by pipeting fixative at one side of the cover slip while drawing off fluid at the opposite side. Drop the slide into fixative.

Large trematodes can be placed between slides secured with thread and dropped into fixative.

Cestode scoleces should not be fixed under pressure to avoid distortion.

When the worm is opaque, transfer it to a large volume of fixative for appropriate length of time and then transfer it to a suitable storage medium (usually 70% alcohol).

FIXATIVE AND PRESERVATIVES

AFA	
95% ethyl alcohol	50 parts
Commercial formalin	6 parts
Glacial acetic acid	4 parts
Distilled water	40 parts

Fix for 48 hours specimens can be stored in AFA or transferred to 70% glycerol alcohol.

GLYCEROL ALCOHOL	
Ethyl alcohol (70%)	90 parts
Glycerol	10 parts

(Glycerol prevents the specimen from drying out completely if the liquid evaporates or leaks out of the container vial.)

70% ALCOHOL	
Ethyl alcohol	70 parts
Distilled water	30 parts

BOUIN'S FLUID	
Picric acid (saturated aqueous solution)	75 ml
Commercial formalin	25 ml
Glacial acetic acid	5 ml

4% FORMALIN

4 parts of commercial formalin (which is a saturated aqueous solution of formaldehyde and is treated as 100% formalin) made up to 100 parts with water or buffer. 70% glycerol alcohol is suitable for long-term preservation and storage of the material. Use glass vials with screw tops sealed with Parafilm or melted wax to reduce evaporation.

GOWER'S CARMALUM

To 100 ml. of 45% glacial acetic acid add 10 g of Carmine. Dissolve by heating and allow to boil. Cool and filter. The residue left on the filter paper is acidified carmine; spread it out flat to dry. Store it in a tightly closed container for future use. The staining solution made up of the following:

Acidified Carmine	1 g
Alum	10 g
Distilled water	200 ml

Dissolve the ingredients by the aid of heat. Cool and filter the mixture and add a crystal of thymol to prevent mould growth.

BORAX CARMINE	
Carmine	3 g
Borax	4 g
Distilled water	100 ml

Boil until carmine is dissolved. Cool the mixture and add 70% ethyl alcohol.

In worms fixed in formalin or Bouin's fluid, the stain may fail to penetrate properly, possibly because of coagulated proteins on the surface. Prior to staining, this material should be washed in running water, or soaked in 70% acid alcohol for several hours followed by washing in distilled water. For thick specimens this procedure should be followed in routine. Cestodes with a long strobila should be cut into segments of convenient length (4-5 cm) before staining.

HYDRATION

Bring specimens preserved in 70% alcohol to distilled water through a graded series of alcohols (70%-50%-30%-distilled water), keeping in each solution for a few minutes to several hours depending on the thickness of the material.

STAINING

The length of time taken to stain varies according to the size of the specimen from about 10 minutes for small delicate specimens to several hours for large, thick worms. Large, thick worms may be left in stain overnight. Wash in distilled water/70% alcohol (according to the stain used) to remove excess surface stain.

DIFFERENTIATION

Differentiate in acid solution (1% conc. HCl in distilled water for stain in aqueous solution; 1% HCl in 70% alcohol if an alcoholic stain was used) till stain is lightend from the body surface and underlying parenchyma while leaving the organs deeply stained. If organs cannot be seen under a stereoscopic microscope because the specimen is too thick, differentiation should be continued till the body surface becomes pale pink. To stop the process, wash specimens in non-acid solution (water or 70% alcohol).

DEHYDRATION

After washing the differentiated specimens, dehydrate them in ascending series of graded alcohols from 30% to absolute alcohol; in case differentiation was done in 70% acid alcohol then dehydration would begin at the 70% alcohol stage.

If the worms are not flat and show a tendency to curl up, it will be necessary, before dehydration, to straighten them by placing between two slides or between a slide and cover slip secured with thread.

CLEARING

After completing dehydration the specimen is cleared to render it translucent. The clearing agent is miscible with both alcohol and mounting medium.

Clove oil and cedar wood oil, though expensive, are non-toxic and good clearing agents and can be re-used many times.

Xylene and benzene evaporate quickly and make the specimens hard.

Methyl benzoate is a good clearing agent but, like benzene, gives fumes.

Creosote is also very good, as it can be used after dehydration of specimens in since it has a dehydrating effect, but is corrosive.

If specimens are not properly dehydrated, they will not clear well and will look opaque. Return them to absolute alcohol, leave for more time and repeat the clearing process.

MOUNTING

Mount specimens in Canada Balsam, a natural resin, which is the most preferred and most stable mountant in use currently. It has refractive index very close to that of fixed and cleared cell constituents. However it dries very slowly. Synthetic mountants like DPX (Distrene dibutylphthalate Xylene) can also be used; DPX dries quickly and the mount is ready for keeping in a day's time.

Mounted slides should be labeled with all relevent information, as in the collection vial.

PREPARATION OF NEMATODES

Because of having a thick cuticle covering the body, nematodes are generally not stained but are cleared before mounting.

CLEARING

There is a choice of clearing procedures to follow:

Glycerine alcohol (10-50% glycerine in 70% alcohol) - Transfer the specimen to the solution. Keep the specimen vial without lid in a descicator and allow the alcohol to evaporate. It is a slow method of clearing but causes minimum damage to the worms.

Lactic acid - Preserved specimen can be directly mounted in lactic acid from the collection vial. This solution is quick to use and restore the shape of the worm.

Lactophenol - Preserved specimen can be directly mounted as for lactic acid. Alternatively, place the worms in lactophenol lightly coloured with cotton blue (0.1% solution) until sufficiently coloured and mount in lactophenol (Lactic acid + Phenol + distilled water + glycerine in the ratio of 1:1:1:2).

Beechwood Creosote - Mount specimens directly in Creosote (good for old specimens or those that will not clear by other methods). However, since it is a corrosive and hazardous chemical, it should be used carefully under a fuming hood.

PERMANENT PREPARATIONS

Clear the worms in glycerine alcohol, mount in pure glycerine or melted glycerine jelly, and seal with glyceel.

GLYCERINE JELLY	
Glycerol	50.0 ml
Gelatin	8.0 g
Distilled water	50.0 ml

Allow gelatin to soak for 1-2 h in water, add glycerol and preservative (0.1 g phenol). Warm for 10-15 min and stir untill mixture is homogeneous. Store in cool place. Melt the jelly in a water bath before use.

Mount in lactophenol/lactic acid and seal with glyceel. Clear the specimens, wash in glycerine, mount in Berlese fluid and seal with glycerol.

BERLESE FLUID	
Dextrose syrup	5.0 ml
Water	10.0 ml
Glacial acetic acid	3.0 ml
Gum Arabic (acacia)	8.0 g

Mix water with acid and syrup. Add gum to dissolve in this. This requires a week or more. Stir at intervals. When solution is made, add chloral hydrate.

Double mounting; put the cleared specimen in the center of a cover slip and mount in melted glycerine jelly using a cover slip one size smaller than the base cover slip, so that the margines of the latter remain uncovered. Wipe the mountant off the margins and allow the jelly to solidify. Take a clean slide, put a drop of mountant over it and lower the mounted cover slips - the smaller cover slip downwards (i.e., touching the mounting medium). Seal the margins with glyceel.

NON-PERMANENT PREPARATIONS

Non-permanent mounted specimens can be returned to the collection vial by carefully removing the cover slip and transferring the specimen to acid alcohol. Leave the specimen for washing in the acid alcohol until it has become opaque. Wash in two changes of 70% alcohol, before returning the material to the collection vial.

5. NEMATODA (Plant and Soil Nematodes)

INTRODUCTION

Nematodes are lower invertebrates, highly diverse, inhabiting wide range of habitats and constitute nearly 90% of all metazoans in number and have 26646 recorded species (Hugot *et al*., 2001). Nematodes have been successful in adapting to every ecosystem, from soils, to freshwater to marine ecosystem and they have been reported from polar regions to the tropics and are found from highest to lowest elevations, even in oceanic trenches and also within the earth's lithosphere (Borgonie *et. al*., 2011). Recently, Shatilovich *et.al.* (2018) have reported the viable soil nematodes from the samples of Pleistocene permafrost deposits of the Kolyma River Lowland corresponding to the age of deposits, which is 30000-40000 years.

Soil inhabiting nematodes predominate over all other soil animals, both in number and species. The nematodes that occur in soil belong to more than 10 orders, and are calso lassified on the basis of their feeding habits which emphasizes the diverse habitats exploited and their roles in soil ecosystems (Yeates *et al.* 1993).

1. Plant: parasites or herbivores: feed on vascular plants causing damage to plant roots resulting in root systems which are not able to take required nutrients and water and threaten the agricultural crops throughout the world, particularly in the tropics and subtropics.
2. Predators: feed on invertebrates such as protozoa, nematodes, rotifers and enchytraeids and have the potential to be utilized as biological control agent
3. Omnivorous: feed on a wide range of foods.
4. Fungivorous: Have a weak stylet which is used to puncture fungal hyphae Feed on saprophytic, pathogenic and beneficial mycorrhizal fungi. Play important role in the decomposition process.
5. Bacterivorous Have unarmed open stoma for ingestion of bacteria. Feed on symbiotic, pathogenic & saprophytic bacteria .Play an important role in the decomposition of dead organic matter.

Taxonomically, the orders Tylenchida and Triplonchida constitute plant feeders, fungal feeders and predaceous forms. Aphelenchida comprises of fungal feeders and predators. Order Dorylaimida includes species that are plant feeders, fungal feeders, predators and omnivores. Bacterial feeders are found in orders Rhabditida, Diplogasterida, Monhysterida, Alaimida and Araeolaimida, whereas predators occur in various orders like Mononchida, Diplogasterida, Aphelenchida and Dorylaimida. All these types of nematodes have been found to co-occur in soil. The average annual yield loss worldwide by plant parasitic nematodes is around 10% or even more (Ahmad, 1996). On the other extreme, bacterial and fungal feeders are very important and beneficial in the decomposition of organic matter and recycling of nutrients in soil. These nematodes possess several attributes that make them useful ecological indicators (Freckman, 1988; Bongers, 1990; Neher, 2001).

The basics of studying soil nematode involves the following procedures

1. Soil Sampling
2. Processing of soil and root samples

3. Extraction of nematodes
4. Killing, fixation and preservation of nematodes
5. Permanent slide preparation
6. Identification

1. Soil sampling:

The design of soil sampling will depend on the purpose of the study. Nematodes are not evenly distributed in a field and therefore soil sampling procedures need to take this into account to obtain a truly representative sample. The reliability of nematode counts depends on the sampling procedure used. In case of taxonomic studies, the required accuracy is relatively low and a simple sampling plan is sufficient. Whereas, in case of ecological studies, like the species composition of a nematode population, sampling needs to be done more accurately.

Soil nematodes are very small in size (0.3 to 10mm), population abundance is very high (generally million/m^2) and diverse (> 30 taxa /m^2) in all soils (Yeates, 1979). The soil inhabiting nematodes are found in every cubic foot of soil, and although they are most abundant in the upper strata of the rhizosphere, they may also be found up to the depth of over 20 feet (Jenkins and Taylor, 1967). Most of the soil contains 90% nematodes at top six inches of soil surface (Crofton, 1966) as the soil moisture and oxygen are favorable factors for dense population (Nicholas, 1984).

Sampling equipment: Sampling auger can be used for soil sampling to a depth of 20 cm or more. A soil corer or auger is also useful for taking many sub-samples (cores). Narrow-bladed shovels or spades, garden trowels, are also useful especially when sampling agricultural fields or areas with rocky soil. (Kimenju *et.al.*, 2004)

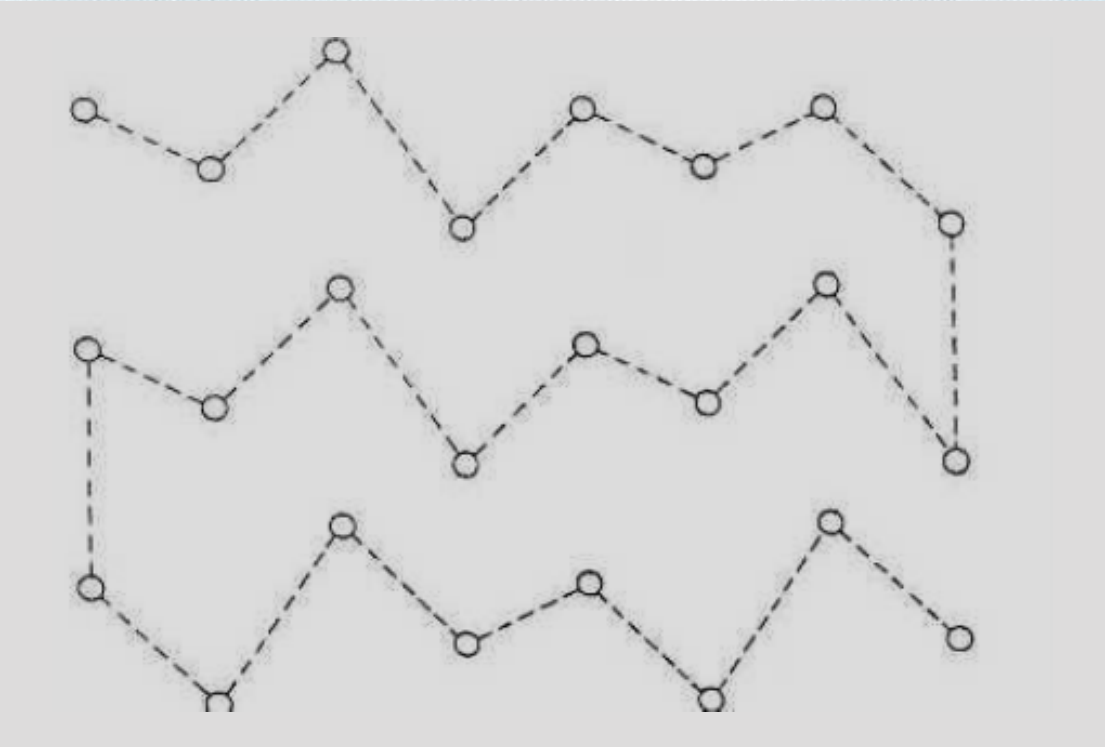
Sample size: The nematode sampling goal should be to obtain information about the presence of nematode species occurring in one or more ecosystems, landscapes, or geographic regions. The breadth and depth of the rae will determine the number of samples to be taken, where the relative numbers of sites from which samples are collected sampling denotes breadth, and the extent of taxonomic characterization per sampling site determines sampling the depth.

Quadrant sampling: A 10 x 10 metre area is marked out for quadrant sampling and a sample, consisting of 50 cores is taken from this area. An auger of 10 cm length and a diameter of 1.7cm, yields a sample of approximately 1 litre (> 1 kg soil). Five samples consisting of 10 cores each are taken within a quadrant.

Shovels and spades for soil sampling

A soil tube or auger for soil sampling

Sampling pattern: Basically, sampling can be carried out at random or systematically. In general, sampling is usually carried out in a systematic way because it is more favourable and achieved in less time. In case of systematic sampling, soil samples are collected along a line or at points on a grid, at a fixed distance from each other. Prior to sampling. A field plot may be divided into strips or blocks before sampling both for random as well as systematic sampling.



Sampling in a field in zigzag manner

Sub-sampling: Relatively large sample size is often needed to achieve sufficient accuracy. however, these can be achieved completely by obtaining sub-samples. The subsamples should be homogenised by mixing. If homogenisation is carried out thoroughly, analysis of one sub-sample is sufficient

Root sampling: For the investagation of the endo and semi-endo-parastites, the roots and shoots should also be brought to the laboratory.

Sampling data: All relevant collection data like name of the host plant (associated plant or vegetation), habitat, symptoms of the plant, if any, details of locality, geographical co-ordinates, date of collection, name of the collector etc. should be noted.

Storage of soil samples: To avoid dehydration, samples are collected and stored in plastic bags. Label may be stapled outside the bags or it can be marked on the bag with waterproof marked. Avoid exposure of

samples to high temperatures. The samples must be processed as soon as possible, to avoid changes in nematode numbers and species composition. The samples should be stored at 4°C for better recovery.

Pre-soaking of soil samples: Usually samples of manure, litter and other uncommon substances can be washed without any problem using one of the extraction methods for plant material or soil. However, these samples need to be soaked prior to extraction, to facilitate the extraction process and to increase the extraction yield. (van Bezooijen, 2006).

Drying: Extraction requires dried samples in the case for some of the cyst nematode study. For best results sub-samples should be taken before drying, rather than drying the whole sample. Drying can be done at room temperature by spreading out the (sub) sample out and allowing it to dry naturally, or it can be achieved by drying in a drying chamber/ drying cabinet.

2. Processesing of soil nematodes:

The collected soil samples are generally processed by Cobb's sieving and decantation technique (Cobb, 1918) followed by modified Baermann funnel technique (Christie &Perry, 1951) as discussed below:

- Soil /sediment placed in a bucket, thoroughly mix with water
- Debris & pebbles, if any should be removed
- Fill the bucket with water & stir the suspension thoroughly
- Leave for about a minute & pass it to another bucket through a coarse sieve (2mm pore size) which retains debris, roots & leaves
- Stir the suspension in the second bucket & leave it for a minute & then pour through 300 mesh sieve (pore size 53 um)
- The catch on the sieve containing nematodes along with fine soil particles collected in a beaker.

Coarse Sieve (2mm) and 300 mesh sieve (Pore size 53 µm).

3. Extraction of Nematodes:

Details on extraction, killing, fixing and mounting techniques have been provided by several authors (Baermann, 1917; Cobb, 1918; Christie and Perry, 1951; Oostenbrink, 1960; Thorne, 1961; De Maeseneer & d'Herde, 1963; Steinhorst, 1966; Taylor, 1971;; Hooper, 1986; Shurtleff *et al*., 2000; Bohra & Baqri, 2004).

Extraction of Nematodes by the Baermann Funnel Technique

The sieve along with the filtered nematodes is kept on Baermann`s funnel filled with water, just touching the bottom of the sieve. Extra care is taken, while placing the sieve on the funnel to avoid air bubbles in between the bottom of the sieve and water level.

The stem of the funnel is attached to a rubber tube provided with a stopper. This is kept undisturbed for 14 hours. Active nematodes migrate form the sieve through tissue paper in a clean water in the stem of the funnel where they settled down at the bottom,

By carefully opening the stopper, a small quantity of water is taken from the funnel in a test tube or small beaker.

The method may be modified by a shallow petri dish to overcome the problem of poor oxygenation by the rubber tube. The supporting coarse sieve with aliquot on the tissue paper is placed in petri dish filled with water touching the bottom of the coarse sieve.

Extraction of cyst from soil

For extraction of cyst nematodes , soil samples are dried in the laboratory . Air drying is important because the dried cysts float on water and can be easily collected. Recovery of cyst from the soil samples consists of following steps: Floatation of cyst from soil along with plant debris, separation of cyst from soil and plant debris, estimation of number of cyst present in the soil and identification of cyst.

Following methods are generally used for the extraction of cyst from soil by Fenwick Can (Fenwick, 1940) method and also by sieving and picking of cyst method, Sieving method. (Bohra & Baqri,2004)

Extraction from plant material

Direct Examination: The infested plant material (roots or aerial parts) is washed gently with water and placed in a shallow Syracuse watch glass with little water. The dish is placed under a stereoscopic binocular microscope. The material is carefully teased apart with the help of two dissecting needles. If nematodes are present, they come out in the water and can be collected with help of a hair brush in a separate cavity block.

(i) Root-knot Nematodes: If the tissue is suspected of containing *Meloidogyne* spp. Then look for egg masses clinging to outside of the root. These are white to brown in colour and roughly hemispherical in shape. They can easily be detached when picked apart with needles. They will be found containing eggs and all staged of larvae and sometimes males. Look for female by pulling apart the root tissue with the help of dissecting needles.

(ii) Heterocera spp.: Females of these species are attached to the root with their necks embedded in the tissue. They are easily seen and can be detached, as described above.

(iii) Migratory Endoparasites: nematodes like *pratylenchus* and *Radopholus*, if they are numerous and active, will be apparent at once. If they are few or sluggish, they may be found only after the dish with dissected plants material has set aside for an hour or two.

(iv) Citrus Nematode and Reniform Nematode: Females of the citrus nematodes (*Tylenchulus semipenetrans*) and reniform nematodes (*Rotylenchus reniformis*) have their neck embedded in root tissue and their body outside the root. Since the body is usually covered with eggs mass and soil particles, it is difficult to see the body. Hence, the egg masses are detached so that the body may become visible. The egg masses of *Tylenchulus* and *Rotylenchulus* contain hatched larvae, besides the egg in various stages of development. If the infected roots are placed in shallow water for a few hours or overnight, the larvae and the egg mass are collected at the bottom of the dish.

Sedentary endoparasitic nematodes which do not emerge from the infested plant tissue can be stained with different stains for demonstration as well as for studying host parasitic relationship. The widely used stain is lectophenol-acid fuchsin.

Lectophenol-acid fuchsin solution:

Phenol	500 ml
Lactic Acid	500 ml
Glycerine	100 ml
Distilled Water	500 ml

Infested roots are washed with water to remove soil particles. Heat the solution to boiling point (above 80° C) and immerse the plant tissue for one to three minutes. Both plant tissue and nematodes will be stained in this hot solution. Destained by washing the plant material with water, and stored in lactophenol.

4. Killing and Fixation of Nematodes

The suspension containing the nematodes is kept in a big test tube for 2-3 hours so that nematodes could settle down at the bottom. Using a fine dropper of suitable length, excess water is removed carefully. The nematodes isolated as above are ready for killing and fixing. Formalin – Acetic Acid fixative (FAA) gives satisfactory results for fixation of nematodes.

Fixation: in hot fixative

i. 10% Formalin

ii. FA Fixative:

40% Formaldehyde	08ml
Glycerol	02ml
Distilled H2O	90ml
Glacial Acetic acid	4-5 drops

The fixative (FAA) should be taken in a separate test tube at least twice the volume of the nematodes suspension. This FAA is boiled at 100°C and then poured into test tube containing nematodes suspension.

Dehydration: Nematodes are transferred in Glycerin-Alcohol (5 parts Glycerin & 95 parts of ethanol) to desiccator containing anhydrous Calcium Chloride for dehydration.

5. Permanent slide preparation

Preparation of slide is the final step to get the nematode specimen ready for identification and to study under microscope. It involves the following steps:

Transfer of the dehydrated specimens to slide:

1. After 2-3 weeks, the nematodes are fully dehydrated in the desiccator and are in pure anhydrous glycerin.

2. A single droplet of anhydrous glycerin is taken at the middle of a 1 mm – 1.35 mm ± 0.05 mm thick glass micro slide.

3. 4 – 5 dehydrated nematode specimens, preferably of same size, thickness and morphological shape are selected under a stereo zoom binocular and are transferred to the droplet of anhydrous glycerin on the slide with the help of a single soft bristle of camel-hair brush.

Mounting and Sealing:

Wax sealing method: (De Maeseneer & D'Herde, 1963).

1. After transferring and arranging the nematodes in the glycerin droplet, 3 – 5 minute blocks of paraffin wax are placed around the glycerin droplet on the slide.
2. The number and size of the paraffin blocks depend upon the thickness of the nematodes. Now a round cover slip of 18 mm diameter is gently warmed over a small flame to remove moisture and placed carefully on the paraffin blocks.
5. Now the slide is placed on a hot plate with 60oC temperature. The paraffin blocks melt by the heat and the slide is immediately removed and is allowed to cool.
6. In this case the paraffin itself acts as a supporting material of cover slip as well as a sealing device.
7. Finally, the slides were properly labeled with its collection data and can be studied under microscope to identify the specimens and can be preserved for long time.

Figure: The step by step illustration of processing of soil sample by Cobb's sieving and decantation method followed by extraction of nematodes by modified Baermann's funnel technique.

6. FRESH WATER PLANKTON

INTRODUCTION

Aquatic organisms can be classified as benthos, nekton, plankton or neuston. Benthos are those organisms that are attached to, crawl over or that burrow into the bottom or substratum. The nekton consists of animals only, which can move around in the water purposively and independently of the movements of water itself. The plankton or the neuston consists of free-floating organisms that remain essentially at the mercy of water current. However, neuston are found on the surface film while plankton are found below the surface film.

Plankton constitutes an important component in the fauna of any aquatic ecosystem and the collection, sorting and preservation are mostly same irrespective of from wherever the plankton is collected viz., lakes, rivers or seas. However, the qualitative nature of the plankton varies among different aquatic ecosystems.

Species with an entirely planktonic life history are referred to as holoplankton. Temporary plankton are known as meroplankton. On the basis of size they have been classified as ultra (0.5 to 10 um), nanno (10 to 50 um), micro (50 to 500 um) and macro (more than 500 um).

PHYTOPLANKTON

Phytoplankton are chlorophyll bearing suspended microscopic organisms consisting of algae belonging to Chlorophyceae, Cyanophyceae and Bacillariophyceae. Their unique ability to fix inorganic carbon to build up organic matter through primary production makes their study a subject of prime importance. The quality and quantity of phytoplankton and their seasonal successional pattern have been successfully utilized to assess the quality of water and its capacity to sustain heterotrophic communities.

I. Class CYANOPHYCEAE (Blue greens)

II. Class CHLOROPHYCEAE (Greens)

III. Class BACILLARIOPHYCEAE (Diatoms)

IV. Class DINOFLAGELLATA

ZOOPLANKTON

Zooplankton are the microscopic free swimming animal component of aquatic systems represented by wide array of taxonomic groups; of which the members belonging to Protozoa, Rotifera, Cladocera and Copepoda are most common and often dominate the entire consumer communities. They are endowed with many remarkable features and are often armoured with spines, which hamper their predation, by higher organisms. The ability of movement not only provide them an effective defence measure but also enable them to actively search and feed upon the phytoplankton. Their high and rapid rate of parthenogenetic reproduction usually overcome the predation lossess and enables them to exploit algal blooms. They constitute an important link between primary producers and consumers of higher order in aquatic food webs. Therefore zooplankton studies provide key information for the management practices of aquatic systems.

The qualitative nature of zooplanktons varies to a great extent from freshwater ecosystem to that of marine ecosystem and the important faunal constituents of zooplankton from freshwater are dealt below:

I. PROTOZOA

Ia. RHIZOPODA

Ib. CILIATA

II. ROTIFERA

IIIa. CRUSTACEA: CLADOCERA

IIIb. CRUSTACEA: OSTRACODA (Mussel or seed shrimps).

IIIc.CRUSTACEA: COPEPODA

CYCLOPOIDA

CALANOIDA

HARPACTICOIDA

IV. OTHER RARE CRUSTACEANS

ANOSTRACA

NOTOSTRACA

CONCHOSTRACA

COLLECTION METHODS

The choice of field sampling instruments and distribution of samples will be determined by the kind of information sought and by the size of the water being investigated. Infact zooplanktons are not distributed evenly or at random but tend to be patchy. Further there is usually a strong vertical gradient in abundance which may change greatly because of diurnal migration by some species. The planktonic suspension is characterized by a great diversity of sizes of suspended particles from small protozoa to large entomostraca. The spatial distribution is far from being uniform vertically or horizontally. Usually plankton is more abundant in the upper layers, but often there are exceptions. The pelagial normally has fewer free swimming animals than the littoral area. Further, in certain conditions and situations the individuals of a given species are distributed in a thin, continuous layer at a certain depth, being practically absent elsewhere. The methods available can be divided into two basic categories:

1. Collection of samples of water followed by concentration of the planktonic organisms in the field or in the laboratory by various techniques. These methods are adapted mostly to the collection of organisms too small to be caught by a net (Protozoa, Rotifera). For this method water samplers are used and zooplankton can be removed by filtration. Also for phytoplankton this method is most suited. Further, Lugol's Iodine is used for sedimentation and if necessary centrifuge is also used.

2. Collection with towed instruments which are equipped with a net for the *in situ* filtration of the planktonic organisms from the water. These methods have been devised primarily for the collection of the larger zooplankton (Copepods, Cladocera, including their developmental forms and the larger Rotifers). A number of cyclopoid copepods have been found to have resting stage (diapause) as copepodids on the bottom. Hence microbenthos also has to be collected in such situation. Among the various methods for collection of plankton, the use of plankton net is most common. For quantitative studies this formula is used: V=II r2d where V= volume of water filtered, r= the radius of the mouth of the net, d= the length of the course of the net through the water. Further many quantitative samplers like Clarke-Bumpus sampler are also widely used. Pumps also are in use.

For some purposes it may be desirable to take a composite sample which consists of several samples poured together and homogenised, this allows for one count to give mean values, when it is necessary to give variance.

For benthos sampling, the basic process is to thrust a container (box or tube) into the sediment and close it to the extent that is necessary to keep the sample inside while it is raised to the surface. Core type samplers, Ekman type samplers, Peterson type samplers, and box type samplers are used for benthos collection. For running waters insect emergence traps are also used. For Ostracods a small cone net attached to a pole is useful for dragging through the vegetation.

PRESERVATION

Formalin or alcohol are the preservatives used for most of the groups. 5% formalin is used as a preservative for most of the planktonic animals. But it distorts some of the soft bodied species. For such animals 95% alcohol gives better preservation. A few drops of glycerine are added for further observation of inner parts. For benthic animals alcohol is preferred than formalin. Catches from emrgence traps are preserved in 70% alcohol.

7. CRUSTACEA

INTODUCTION

Crustacean habitats include benthic environments of the coastal, deep oceans and estuarine regions. In addition they also inhabit semiterrestrial, and freshwater environments of the ponds and lakes. They also live in pelagic environments of the temporary ponds and permantent lakes and in the epipelagic zones of the oceans.

Crustaceans have adapted themselves to live in a variety of ecological conditions. They can be broadly grouped under marine, freshwater and terrestrial crustaceans.

Marine

Crustaceans are more abundant in marine environment than in freshwater or on land. Majority of the crustaceans occur in the littoral and shallow waters. Most crustaceans found in this habitat are either sedentary forms which live permanently fixed to the ground or burrowing forms. Various species of isopods, crabs, anomurans and alpheids live on and under the rocks and boulders as well in the crevices. The diversity and abundance is more on the rocky intertidal zone along the coastal and in the coral reef ecosystems. Most of the burrowing forms live in the sandy substratum. The sandy coasts also support a variety of small microscopic interstitial fauna. In addition, large number of the brachyurans, macruarans and anomurans occur between the lowest low tide level to 100 m depth of various substrata. Crustceans also occur in the pelagic region. However, copepods are the major groups in this region.

Freshwater

In fresh water the crustaceans are more abundant in the littoral regions with variety of macropytes. Temporary ponds harbour a variety of small sized zooplankton community in which Cladocera is the dominant group. Freshwater crabs and prawns are found in the hillstreams, rivers, ponds, tanks and also in paddy fields. In addition, amphipods and isopods also live in this aquatic habitat.

Terrestrial

Despite great diversity in morphological features, only a few terrestrial forms have also so far evolved and among them the isopod group, *viz*. Oniscidae and Porcellionidae, have become completely terrestrial. It is found under the leaves, flower pots bricks and stones, in the garden and among the decaying vegetable matter.

COLLECTION

The usual instruments like hammer, chisel, scalpel, large wooden forceps, sieves, shovels, spades and hand net are required to collect the intertidal organisms. Two kinds of collecting gear i.e. push-nets for collecting shrimps and other organisms from grass flats and a yabby pump for borrowing decapods are used in the intertidal region. For collecting shallow and deepwater benthic forms, various types of dredges, trawls and nets are used. The floating planktonic crustaceans can be collected by different types of plankton nets and recorders. These equipment's are to be operated from mechanized boats with hauling facilities.

Terrestrial and parasitic crustaceans can be collected with simple instruments like forceps, mounting needle and brush.

Table 1. Habitats of Crustacea

GROUP	HABITAT
BRANCHIPODA	
Notostraca	Freshwater
Cladocera	Freshwater (95%) Brackishwater (3%) Marine (2%)
Conchostraca	Freshwater
Anostraca	Freshwater and Inland Salt Water
Copepoda	Marine & Freshwater
Cirripedia	Marine & Brackish water
Ostracoda	Marine & Freshwater
MALACOSTRACA	
Stomatopoda	Marine
Amphipoda	Marine, Freshwater and Terrestrial
Isopoda	Marine, Freshwater and Terrestrial
Cumacea	Marine
Decapoda	Marine, Freshwater and Terrestrial

SAMPLING

Aquatic

Most of the marine forms inhabit the littoral region in the sea. During low tide many slow moving intertidal forms can be collected easily with a forceps. A flat stone or a bunch of weeds suddenly turned over will generally reveal a variety of crustaceans. Vigorous rinsing of sea weed tuft in a tray containing a few drops of formalin will make these animals to come out and they can be easily picked out with a small forceps or by a pipette. Rockpools harbour rich fauna of decapoods and stomatopods and these are collected with the help of a small hand net. Isopods and other small decapods live in narrow crevices of rocks and these can be made to come out of their hideouts by squirting in a weak solution of formalin or by dropping a little bleaching powder. Small shrimps and other crustaceans living on attached vegetation in shallow water are collected with a push net. Many borrowing forms can be collected by digging out the sand. The sand is put into a sieve and filtered leaving the animals in the sieves. Small burrowing macrurans are collected with the help of a yabby pump. By this pump, the deep burrowing animals are removed without much damage. Most of the crawling animals like prawns, crabs and stomatopods which live near or at the bottom in shallow waters are collected by various type of soft dredges and trawls. For collecting planktonic organisms different types of plankton nets are used. These equipment's are to be operated from mechanised boats with winch facilities.

A small type of naturalist dredge is used to collect the bottom fauna present in lakes and rivers. This can easily be operated from a small boat. If it is a small stream, the bottom samples can be obtained with a shovel and by sieving it benthic animals are collected. For collecting the planktonic forms, an ordinary plankton net of small mesh size is used. The weak surface inhabiting cladocera and ostracoda may be collected with a hand net by sweeping through the weeds.

Terrestrial

Terrestrial isopods which live in damp places in the soil, under decaying vegetation are collected by digging the upper layers of the soil with a shovel and by examining the under surface of stones. The isopods may be picked individually with a forceps or with the help of a brush moistened with alcohol.

Parasitic

These are found mostly on the eyelieds, nasalcavities, buccal cavity, body surface and fins of fishes. These parasites can be removed from their host's body with the help of fine forceps, camel hair brush or by a needle. For porper identification it is better to collect the parsites with the hosts.

PRESERVATION

Before preservation, the animals should be washed thoroughly to remove the dirt and unwanted material attached to it. Preservation of specimens are carried out in three stages: Narcotisation, killing and preservation.

Narcotisation makes the animals less sensitive so that they will not be damaged or distorted by violent contraction, when killed. Some of the standard narcotising agents are Magnesium chloride. Menthol, Chloral hydrate and Alcohol. The animals are kept in a container with seawater and a small quantity of any of these

narcotising agent is added gradually at frequent intervals. The time taken and quantity of the narcotising agent to be used, depend on the size of the animal and quantity of sea water.

Once the animal become unresponsive, a proper killing agent is added, after draining out the narcotising agent. Formaldehyde is the best fixative for most crustaceans. The time for which they must remain in the solution varies with their size.

8. CRUSTACEA: BRANCHIOPODA

INTRODUCTION

The Cladocera comprise a group of small, mostly microscopic crustaceans included under the class Branchiopoda. They represent one of the most primitive group of lower crustaceans to which the general name of "entomostraca" was formerly applied. They are commonly termed as "Water-fleas" because of their characteristic "jerky" swimming action. During their nearly three-century old taxonomic history, this group was invariably treated as a distinct order under the Branchiopoda.

The cladocerans primarily inhabit almost all sorts of freshwater ecosystems and frequently occur in the littoral, limnetic, benthic, interstitial and ground-water environs. On the contrary, species of three genera namely *Podon*, *Evadne* and *Penilia* are known to be truly marine. They depict greater qualitative diversity and population densities in lentic environs. Planktonic cladocerans, in general, are capable of hundred-fold variations in population size, with peaks generally coinciding with periods of algal blooms. They are, however, usually rare in lotic biotopes and are primarily restricted to pool zones but the significance of their occurrence and associations in these biotopes is highlighted by Vila (1989). A majority of members of this group particularly those belonging to the families Chydoridae and Macrothricidae colonize littoral weedy margins of lakes and ponds. The genera *Ilocryptus* and *Monospilus* are structurally adjusted to the benthic zone. On the other hand, a few taxa like *Alona quadrangularis* and *Drepanothrix* live near the bottom, although not specially adapted to this mode of life. The species of Moina are usually noticed in muddy pools and eutrophic ponds and some species of this genus are also reported to occure in saline lakes. *Daphnia* species are invariably noticed in ephemeral pols, small ponds and lakes. Limnetic cladoceran communities generally include species of *Daphnia, Diaphanosoma, Ceriodaphnia, Bosmina* and *Moina*. Certain cladocerns i.e., *Sida crystallina* and *Ophryoxus gracilis* exhibit intermeidate status between the littoral and limnetic forms. The cladocerans are, therefore, found in most inland waters, including rivers, lakes (fresh- and brackishwater), pools both ephemeral and permanent, in the hyporehic zone of rivers and in caves. In addition, these organisms are reported to occure in dampened mosses, subterranean groundwaters, tree-holes and even wet tree trunks covered with Bryophytes.

COLLECTION AND PRESERVATION

The Cladocera can be collected principally by any using fine mesh net; preferred mesh size between 50-100 µm ensures collection of majority of species and even of all instars, although larger species have been successfully sampled in sweep nets of up to 250 µm. The members of this group, therefore, can be coveniently collected by standard nylobolt plankton net (No. 25), Birge cone net or by Frey's pole net. The last two are particularly useful for collecting specimens from the weed-infested littoral regions of lakes or large ponds, wetlands or benthic regions without having large items like aquatic vegetation or detritus clogging the net aperture. Modified quatrefoil light traps (Secor *etal*. 1992) have also been used to sample chydorids but they capture only those species which are attracted to light at night. Benthic samplers may be obtained by using a core sampler while pump sampler is usuful to obtain the specimens from the hyporehic zone. In addition, the cladocerans may be sampled from deeper layers of lakes or reservoirs by using Ruttner-sampler or Schindler-trap.

Formalin (4-5%) is usually used to preserve the collected samples. In addition, alcohol (70%) is another suitable preservative but its evaporation can roil the surface of sample and, hence, makes observation and extraction of specimens difficult. In both the cases, addition of 5% glycerol to the storage vials is, however, highly recommended to protect against any possible future desicciation or accidental drying of the materials. The field samples are initially screened with a dissecting binocular microscope to prepare preliminary inventories of various taxa. Individuals for identification may be picked out from unsorted collections with a fine tungsten wire loop or pipette and then placed in a drop of water/10% glycerol on a slide and further identified under a microscope. Larger specimens (>1 mm) may be identified without mounting or use of a compound microscope, but smaller taxa require higher magnification. As cover-slips cause distortion of the carapace, any measurements, drawings or photos of whole animals are usually done at this stage.

Permanent mounts of whole animals can be made in 100% glycerol, glycerin-jelly, Buparil, Polyvinyl alcohol-lectophenol mixture (mixed with few drops of lignin pink or India ink). These may eventually be dried and the coverslip edges are subsequently sealed with lacquer (nail varnish). The mounted specimens may be distorted under pressure of the coverslip in certain cases but due practice may be required to circumvent this problem. In addition, Canada Balsam is required as the best mountant for museum specimens and this process requires essential dehydration of specimens through an alcohol series.

Dissections of various body parts and appendages such postabdomen, trunk-limbs etc. may preferably be made from whole exuviae with fine tungsten wire needles and some can be mounted in Polyvinyl alcohol-lectophenol mixture. If exuviae are not available, these may be disarticulated by boiling the specimens in 5% solution of Sodium hypochlorite (NaOCl). In addition, chemical disarticulation of body parts/head shields may be undertaken by heating whole specimens in 1 ml of concentrated hydrochloric acid, carefully monitoring the process and degree of hydrolysis as described by megard (1965) and mounting the parts as mentioned above.

In light of the modern concepts of cladoceran systematics and increasing emphasis for observations of diagnostic morphological attributes and that of specialized parts/appendages at higher resolutions, the detailed examination using fine optical microscopic systems as well as Scanning Electron Microscope (SEM) are increasingly and essentially desired in this group.

9. CRUSTACEA: OSTRACODA

INTRODUCTION

The systematics of Ostracods have been studied extensively from a few areas of the globe, especially from the temperate zones. However, the studies on freshwater Ostracods from tropical countries are poorly known. Victor and Fernando (1979) gave an account of freshwater Ostracods of India. Thereafter, a major work in this field is lacking. The work of two authors is largely on their collection, and thus they have brought the up to date taxonomy of Ostracods of India.

The name of this group is properly given for the presence of a bean shaped shell. The entire body is enclosed in a shell, except the antennae and the legs. When the animals is disturbed, these parts are quickly withdrawn and the shell is closed; it stops swimming or any other type of movement, and ultimately sinks to the bottom of water. The shell is of one piece and it functions like a bivalve mussel. In some species, the shell is transparent and one can see the internal structure of the body. There are two pairs of antennae, and three pairs of limbs, modified into jaws. Two pairs of legs are used for walking and as a cleansing organs. A pointed structure furca may be seen protruding out of the shell. A simple eye is present near the top of head. The animals swim backward and forwards by the movements of antennae. They are also found on water plants, using their first pair of legs for scrambling. The food of Ostracods is decaying organic matter. Smaller particles passively come inside the body alongwith the water current. Both the sexes are seen. However, population without the males is also seen, and for this, probably, parthenogenetic eggs are laid. Eggs can tolerate dormant stage. An instance is on record that eggs of the Genus *Cypria* hatched after remaining buried in dried mud for about 30 years. Nauplius larvae comes out from the shells.

The ostracods are found in both running and standing waters, and are not exclusively planktonic forms. The main suborder Podocopa is exclusively freshwater. There are four groups under order Ostracoda.

A: Exclusively marine

B: Few freshwater forms

C: Well presented in marine and Freshwater habitats

D: Exclusively freshwater forms

They are free living except the members of one genus are commensals on the gills of Crayfishes. The size - range of animals is 0.5 to 2.0 mm in length.

COLLECTION METHODS

Collection of invertebrates made by limnologists and other workers, for plankton studies, by using plankton net also bring some Ostracod material. Several sweeps are made just above the substratum of the habitat and also among weeds. Sometimes, the benthic areas have to be disturbed to get Ostracod collection from the bottom. The collection is stored in a polythene container with small quantity of water and glycerine. Small quantities of alcohol is slowly added into the samples, for a duration of 20 minutes at regular intervals. The Ostracods subjected to this procedure die with their valves open. Chlorotene is also effective for relaxing the muscles and valves of Ostracods, exposing the soft part open, which makes dissection

easier, and the valves can be separated without any trouble or damage to the soft parts. A few drops of concentrated chlorotene is sufficient to preserve the material. After preservation, the material is separated by Tyler sieves of different mesh sizes to separate the small and large Ostracods and the coarse debries. The sieves are carefully washed into petri dishes and the Ostracods are separated. 70% methanol can also be used as a preservative. They can be separated under a stereoscopic dissection microscope, using a pair of needles and micropipettes. While dissecting under a microscope, in the medium of 70% alcohol in a slide cavity, the valves can be separated using a pair of fine tungston needles set in metal holders. The valves may be mounted on micropaleontological slides. The material preserved in formalin has to be transferred first into glycerine to relax, otherwise, the valves tend to curl up.

The soft parts on a clean slide may be teased out in a drop of polyvinyl lactophenol, stained with lignin pink and allowed to dry, otherwise slide mounting in glycerine and sealing of slide-coverslip by nailpolish also do better work for specimen mounting.

10. ANNELIDA

INTRODUCTION

Annelids are elongated and cylindrical worms, with their body formed of ringlike, true segments. Most of them are free living in fresh and salt waters. While a few are terrestrial forms found in moist soil, some live in the tubes made by themselves. Leeches are ectoparasites. More than 840 species of annelids are known from India, which are grouped under four classes viz. Polychaeta, Oligochaeta, Hirudinea and Archiannelida.

COLLECTION

POLYCHAETA

These marine/freshwater worms usually found in sand or mud are collected during low tide with spade, without damaging the delicate small worms. From cervices of rocks crow bar is used to reveal the worms. Bleaching Powder also help to induce them to leave the burrow. From their harbouring vegetations these worms can be taken out by washing vegetation in sea water, 75 : 25 seawater and freshwater have been found useful for collecting the worms. Dredge, anchor dredge, Peterson grab and scuba are used for collecting the polychaets. At night, pelagic forms are attracted to light and can be collected by townet.

OLIGOCHAETA

While collecting oligochaets shovel or spade is used for digging soil. Hand sorting is also useful. They can be collected from dung, moss, decaying vegetation. Diluted solution of 0.55% solution of formalin (25 ml formalin in 4.5 litres water), or potassium permangnate solution is used for extracting earthworms from soil. For potworms, extracting funnel (Wet funnel extractor, Nielson extractor) is found useful for the soil-sample collections.

HIRUDINEA

Forcep can be used for unattached terrestrial forms and from vegetation by a hand net. Both land and aquatic leeches are collected from bodies of animals; But a difficult process. Therefore, an application of little alcohol, formalin or common salt usually induces them to release their hold very quickly.

NARCOTIZATION

Polychaeta

Animals can be narcotized by adding small amount of 5 to 10% alcohol very slowly. Care should be taken to add not more than tenth of alcohol of total volume of sea water. Cocaine, Chloralhydrate and Menthol may also be used for this purpose. To avoid contraction, first they may be kept in 30-40% alcohol and, later transfer them to 70-90% alcohol.

OLIGOCHAETA

Same procedure as done for polychaeta but, by using freshwater as well as by pouring formalin on extended forms or dropping them in 70% ethyl alcohol. Removie them later on to a blotting paper, in straight position for fixation.

HIRUDINEA

For relaxing add 5-10% ethyl alcohol gradually or chloroform, chloral hydrate or weak nicotine or magnessium sulphate or carbon dioxide or weak acid like lemon juice. They are then transferred to shallow dish and straightened out before fixation.

FIXING AND PRESERVATION

POLYCHAETA

70-80% alcohol is the best fixative and preservative. The specimen must be properly submerged in alcohol. The pelagic forms can be killed and preserved in 5% buffered formalin.

OLIGOCHAETA

10-15% formalin is sufficient for the fixation, for a period of 24 hours. Specimen should not be curled or twisted, and may be preserved in 70% ethyl alcohol or 10-15% formalin, depending upon the size of specimen.

HIRUDINEA

After narcotization, the leechs are rapidly passed through a piece of cloth or finger to remove excess of mucous. The specimen are kept in between two glass slides, lightly bound together by rubber band or thread to prevent distortion and then kept in fixative i.e. 50% alcohol or 4% formalin. When completely hardened, they are preserved in 70-90% alcohol or 4% formalin.

11. ROTIFERA

INTRODUCTION

Aquatic ecosystems are colonized by diversified array of micro- and macro- organisms. Among these, Phylum Rotifera represents one of the oldest group of invertebrates which are presumed to be a product of the aerobic phase in the development of our planet. These interesting organisms were firstly studied and described by Leeuwenhoek. Due to their conspicuous ciliation and microscopic size, the rotifers were originally treated as Infusoria and not distinguished from monocular organisms. The members of this group are also commonly termed as "Wheel-Animalcules" because of their characteristic "Wheel-organ" or "corona" that bears close resemblance to a pair of revolving wheels.

Rotifers comprise a very small group of animal kingdom but are often the most abundant metazoans in inland waters, both qualitatively and quantitatively. They exhibit world-wide occurrence from the Arctic and the Antarctic regions to the Tropics. About 95% of the known species of rotifers are found in freshwater which is regarded as their original habitat, while only a small group (less than 5% species) of haliphiles are recorded from athallasic saline waters of brackish and marine environs. During the course of evolution to their present multiplicity, these organisms have successfully colonised a wide variety of aquatic and semi-aquatic environs and occur as creeping, planktonic, semi-planktonic, sessile and semi-sessile forms. The rotifers are, therefore, found in open waters of lakes, ponds, pools, canals, slow flowing rivers and also comprise an integral component of biocoenosis in pristine groundwaters, dampened mosses, psammo-littoral and soil micro-invertebrate communities. Habitat preferences are indicated for different species, but many species of Rotifera, in particular the planktonic ones, are widespread and ubiquitous.

COLLECTIONAND PRESERVATION

As the rotifers belong to different communities namely plankton, periphyton, benthos, psammon (interstitial) and moss-dwelling, specific attention is required to obtain collections of these categories. Planktonic and semi-planktonic species can be sampled by towing or dragging a nylobolt plankton net (No. 25) in open-waters of lakes, ponds and other water bodies. planktonic taxa from different depths may be collected by drawing water with the help of the Ruttner-, Clarke-Bumper-, Schindler-, or Kremmers samplers or else by any locally designed vertical sampler and filtering the water through plankton or any fine-meshed net/sieve.

There is so far no standard method to collect periphytic rotifers. A large number of members of this community may be collected from the littoral regions of various aquatic biotopes with a nylobolt plankton net. In such cases, aquatic vegetation is firstly disturbed to dislodge the associated biota and susequently the samples are taken from these environs. However, more effective collection of the littoral forms can be ensured by careful scanning of aquatic plants and their leaves and manual extraction of these animals under a stereoscopic dissecting binocular. The latter procedure is also effective for collecting the moss-dwelling and sessile rotifers. Besides, an alternating series of horizontal and vertical glass slides may be hung in water bodies for colonizing various periphytic or sessile individuals.

Hess sampler or Ruttner sampler can be used to collect benthic taxa from the uppermost layers of the bottom sediments. The sand-pipe traps or sediment corers are used to collect individuals from the deeper interstitial habitats. The rotifers may be extracted by stirring the sediments into a suspension and decanting

the water through a fine mesh net. This method, however, may not detach Cretan soft-bodied rotifers which, in turn, can be extracted by repeated washing of a part of the sediment with 4% aquaous solution of MgCl2 or else by manual collection with a pipette under a stereomicroscope. The groundwater rotifers can be sampled by drawing water from the deep groundwaters and filtering it through fine mesh net or by scooping a plankton net through deeper waters of domestic wells or subterranean waters.

The ideal, and the often the most easiest, method is to examine the rotifers alive to determine body shape, general morphology, coronae, locomotion, presence or absence of foot, internal organs and other salient characters. Their very hyaline, almost invisible, mucous tubes or nests can be made more perceptive by the addition of a little India ink. The observations on live specimens are necessary in soft-bodied rotifers (bdelloids in particular) which contract to amorphous 'blobs' on preservation. The animals can usually be examined in a small drop of water on a glass slide under low magnification. Cotton wool stands or methyl cellulose can be added to slow down rapid swimmers. Where enough rotifers are present, the gentle placing of the coverslip usually results in specimens being trapped in dorsal, ventral or lateral views to allow the study of the desired characters.

Examination of preserved material can be a useful and sometimes essential adjunct to a study of living forms. Extended rotifer specimens can be obtained by certain relaxing agents or narcotics such as Cocaine, Neosynephrin, Strychnine nitrate (1/10,000 or weaker), solution of benzamine (2%), tricaine methane sulphonate or even very dilute formalin (1%). However, a much easier and best method involving hot water acts as a remarkable relaxing agent but requires a careful practice to master this technique. Neutralized formalin (5%) is a suitable long-term preservative, especially for the loricate rotifers; a few drops of glycerol may be added to each sample to prevent any accidental drying of the material. Alcohol (70%) is also a suitable preservative but its evaporation can roil the surface of the sample and, hence, makes observation and extraction of specimens difficult.

Different samples are screened with a stereoscopic binocular microscope to prepare preliminary inventories of the various rotifers. The specimens belonging to different taxa are then isolated, rinsed in distilled water and are mounted in Polyvinyl alcohol- lectophenol mixture (with few drops of lignin pink or eosin or India ink). Semi-loricate, illoricate and other soft - bodied forms are mounted in glycerol or glycerin-jelly. The permanent mounts are often sealed with a ring of nail polish. Taxonomic characters of the different rotifers may be examined with an ordinary or phase contrast microscope and the illustrations may be made using camera lucida or a drawing -tube attachment. The measurements are generally expressed in micrometers (µm). The detailed structures, wherever necessary, may be observed with SEM following suitable methods of preparation of the material and its observation.

Trophi appear to be species-specific and, hence, are valuable taxonomic discriminator. These are also often used in classification of this group. One of the most important feature of trophi i.e., the number of uncal teeth are difficult to count even at highest magnifications using a compound microscope. SEM permits finer resolutions of structures and has the potential to clarify much of the present systematic confusion in Rotifera. Fresh 3% Sodium hypochlorite solution effectively dissolves soft tissues of various individuals between 1-2 hours leaving only the trophi remaining; over-exposure may digest parts of trophi as well. The degree of disintegration is monitored periodically using a compound microscope. The trophi are cleaned in distilled water and placed in a vacuum dessicator for overnight to dry. These are subsequently prepared for SEM studies.

12. MOLLUSCA

INTRODUCTION

A mollusc is defined as a soft bodied, non-metameric, triploblastic, coelomate, basically a bilaterally symmetrical invertebrate, typically having an anterior head, a ventral muscular foot, the visceral mass enclosed in a thin fleshy mantle, well protected by an external calcareous shell formed from its own secretion from the mantle, and also with a "viliger" or free swimming trochopore larva normally present in the embryonic development.

The estimated number of molluscs in the Indian subcontinent is around 5205 species, out of the estimated around 84978 species of the world, falling under the families: 242 marine, 22 freshwater and 26 land forms. At the species level, about 6.12% are known from the world are represented in India. Out of the seven classes present in the world, India is represented by five classes of Mollusca. There are herbivores, carnivores, scavengers, deposit feeders, commensals and parasite molluscs. However, all the freshwater forms are either herbivores or suspension feeders. It is the marine ecosytems, molluscsa occupy and exhibit comparatively more diverse habitats and feeding habits.

The members belonging to phylum Mollusca have been evolving in both time and space leaving behind a rich and continuous record since Cambrian time. They are widely distributed on land, in freshwater as well as in the sea. Although molluscs occur in all possible habitats except in aerial environment, they are abundant in marine habitat, which accounts for more than half of the known species. Of the seven recognized classes, five *viz.*, Aplacophora, Polyplacophora, Monoplacophora, Scaphopoda and Cepahalopoda are exclusively marine. The other two large classes *viz.*, Gastropoda and Bivalvia comprise of the most successful molluscs adapted to different marine and freshwater habitats and include 94% of the species. The Gastropods have been able to colonize the terrestrial habitat successfully, whereas the Bivalves being unable to overcome their filter-feeding habitat, have not been able to invade to the land. The land forms are therefore, exclusively confined to the class Gastropoda which is divided into two subclasses *viz.*, Prosobranchia (527 species under 34 genera) and Pulmonata (960 species under 106 genera).

Molluscs are more abundant in marine environment than in freshwater or on land. Only a few groups such as cephalopods exhibit free swimming or pelagic habitat, the benthic forms are more abundant as infauna burrowing into substratum, rocks and corals or the epifauna, the attached forms on rocks, algae, corals etc. Freshwater forms are either benthic (Bivalves) or littoral (Gastropods) in their habit. The land forms (snails and slugs) prefer moist environment and mostly active dunging monsoon. The semi slug and slug mostly prefers moist habitat and occurring under leaves, stones, litter, log and on tree surface, whereas land snails is an ubiquitous component of environment and found all the possible habitat *viz.* dumping areas, under rock and stone, on leaves, under the bark of the tree, on tree or occasionally few snails group also found inside soil (within 10 cm under soil surface) and lime stone areas.

Molluscs exhibit diverse form, size, shape sculpture, and colouration. Among the Indian land forms genus *Vallonia, Pyramidula, Kaliella, Carychium* etc. measure length about 1 mm to 4 mm, whereas in freshwater form, *Gyraulus* measure 4.00 mm while the *Tridacna*, a marine form measures more than 100 cm and weighs more than 100 kg. The largest mollusc however is the giant squid found in the Pacific Ocean.

Collection Equipment

Forceps, scalpel, shovel or spade, chisel, brush, assorted petridishes, specimen tubes, polythene or glass jar sieves of different mesh sizes, enamel tray, plastic buckets, metallic frame for quantitative estimation, hand nets, water net or scoopnet, dredges, cloth sheet, thermometer, chemicals (alcohol, narcotising agent), field note book, field labels, pencil etc.

COLLECTION

Prior to making collection of molluscs, the field data of relevant ecological parameters like hydrographic information *viz*. type of water body (lentic/lotic), temperature, intensity of light, nature of bottom sediment, periphytes, macrophytes, and other data such as number, size, colouration are recorded in the field notebook. For qualitative estimation of molluscs, quadrate method using metallic frame of $1m^2$, standard scoop or dredge method for benthic forms, palm leaf method i.e., by placing palm leaves at regular interval along the shore or on the bank, after exposing for certain period time, and to count the snails attached on it are to be employed.

For the collection of freshwater molluscs, hand nets made of round metallic ring with fine polyester mosquito nets are dragged over the aquatic vegetation. The contents of the net are emptied into an enamel tray. The specimens are collected with the help of brush or with forceps. Scoop nets are also employed similarly, for the collection of freshwater forms, made up of 30 cm x 30 cm metallic frame of steel bars fitted with wire netting. A 10 cm deep scoop is soldered to the frame with 8 cm wide blade. Land dwelling forms are collected from among the litter, under debris, bark and vegetation or from their place of occurrence with the help of brush, forceps or even manually by handpicking, however in dryer place like central India, the gunnysack trapping methods can be an effective for collection of snails, including slugs, especially when large numbers of samples are required. Gunnysack should be wet and folded 2-3 times and placed on each 20 x 20 m plot for one or two days. This sort of arrangement provides air circulation, cooling and moist environment for suitable congregation of snails, whereas one should also sample the leaf litter and soil for enrich the collection or for ecological studies. This samplings are the most effective methods for the minute land molluscs. Since, snails require moisture for survival, there is possibility of more number of snails inside the soil and leaf litter. Therefore, field survey team should collect the soil and litter from 1 x 1 m sampling plotfrom the field for a proper assessment of population in the area. Marine forms are collected with the help of chisel, hammer of with forceps for those which inhabits the crevices of rocks or corals. Sand dwelling forms are collected with the help of shovels and filtered with sieves of suitable mesh sizes. Wood boring forms are collected with the help of hammer and chisel.

Narcotisation

Land molluscs after collection are immersed in water in a glass jar enclosed with lid for some time, for asphyxiation. Adding a few drops of absolute alcohol or rectified spirit effects rapid killing. Duration of 12-24 hours may be required for the complete narcotisation. Freshwater and marine forms after its collection are placed in an enamel tray containing freshwater or marine water respectively. Narcotising agents such as Menthol, Chloral hydrate N or Magnesium sulphate are sprinkled over the animals in the tray, allowing them to relax gradually. Narcotisation is not needed for cephalopods, as they die, once removed from sea water. The duration for narcotisation varies depending on the size of the specimen and the nature of the chemicals, magnesium sulphate for e.g., may take 2-6 hours while with menthol, the duration for

narcotisation is 12-24 hours. Solutions of propylene phenoxetol (0.5%) is a good relaxing agent. It is important to observe that the specimens should not undergo desiccation during narcotisation. When these chemicals are not available 4% formaldehyde can be used adding 3-4 drops to the water repeating every half an hour, until the specimens are fully narcotised. The method is useful for narcotising the marine nudibranchs.

Fixation & Preservation

The narcotised animals are fixed and preserved in 70% alcohol. Formalin is not used for molluscan fixation. Screw capped plastic tubes, glass jar, drums of suitable sizes etc. are used for keeping the collected material. Specimens of bigger sizes are to be wrapped in cheese- cloth (Guaze cloth) and to be preserved in sufficiently big drums. Smaller tubes are to be packed in 1-5 li, screw capped, wide mouth jar, with cotton padding all around. For histological preparation, aquatic animals are fixed in either hot Bouins fluid or Absolute Alcohol-Formalin-Acetic Acid (AFA). The duration of fixation varies from 20-24 hours specimens are then washed in running tap water to remove the mucus secreted and preserved in ascending grades of alcohol.

A dry molluscan shell is preserved after removing the soft body parts of the animal using a bent forceps and the empty shells are boiled in water for about 30-60 minutes to clear off the remnant tissues. The operculum, when present should be retained along with the shell. The operculum glued to a cotton plug can be snuggly fitted to the shell in its proper position. The material either placed in tubes/jars/drums are to be labelled using lables either printed or hand written with water proof, black Indian ink or with soft lead pencil. Tracing paper may be used for writing lables, since normal paper lables are likely to become soft and disintegrate in the preservative, in due course of time. Dry shells are to be packed with sufficient cotton padding to avoid damage and the boxes are to be labelled. Containers of suitable size should be selected for preservation of specimens, keeping in view of their size. There should be enough space around the specimen in the container so that the specimens remain immersed always in the preservative.

13. INSECTA

INTRODUCTION

The Class Arthropoda, which includes insects, spiders, mites, and their relatives, is without question the most successful group of organisms on earth. Insects alone account for nearly 73% of all fauna known to science. The study of insects and their relatives is of increasing importance as society faces increased challenges to preserve and enhance environmental quality, reduce pesticide usage, increase crop productivity, control food costs, and increase trade in the global community. Because of the damage inflicted by pest species, increased knowledge of these organisms has the potential to save lives and money. Correct identification of a newly detected pest or disease vector is of utmost importance because the scientific name of an organism is the key to all known information about its morphology, its behavior and life history, and its potential threat to human welfare. However, many species, especially the smaller ones, must be collected and properly preserved before they can be identified. Because correct identification seldom is easy, it is important that specimens be preserved in the best condition possible. The identification of a particular insect or mite usually requires examination of minute details of its anatomy with the aid of a hand lens or microscope. Some specimens may require dissection or even a study with the electron microscope. If these details on a specimen are concealed, missing, or destroyed because of improper handling or preservation, identification is difficult or impossible, and information about the species to which it belongs cannot be made available. Therefore, adequate preservation and proper labeling of specimens are essential to their identification. A summary of the methods and techniques used by professionals and amateurs alike to collect and preserve specimens for study are provided here. While many of the methods covered here, such as pinning, have changed very little in the last hundred years, other techniques have become available only in the last few years or decades with advancing technologies.

WHAT TO COLLECT

Because of their incredible diversity, insects, mites, and other related groups vary widely as to their proper collecting requirements and methods. In the following sections, we will explore in brief some of the many recommended techniques and look at the varied equipments used by collectors. Which species and how many specimens to collect depend on the purpose for which the material is intended. For hobbyists and students, small samples are usually adequate. However, when important pest insects and mites need to be identified, they should be collected in series if at all feasible. A sample of 20 specimens should be considered the minimum, and even larger numbers may be desirable. If adults and immature are present, specimens should be collected of all life stages. Excess specimens can be discarded or exchanged, but it is not always possible to collect additional specimens when needed. Frequently insects and mites cannot be identified accurately from immature stages, and it is then necessary to rear them to the adult stage to obtain a precise identification. Many persons starting a collection attempt to collect every specimen they find. The experience and knowledge gained in making a general collection are of value in helping the collector to decide on a speciality.

Basic Equipments

Collecting methods may be divided into two broad categories. In the first the collector actively searches out the insects, using nets, aspirators, beating sheets, or whatever apparatus suits his or her particular needs. In the second, the collector participates passively and employs traps. Both approaches may be used simultaneously. Using a variety of collecting methods will help to maximize the representation of fauna present, especially when briefly visiting an interesting area. While picking up insects by hand is simple and sometimes effective, their size, mobility, and the possibility of being bitten or stung usually dictates that various kinds of equipments and special methods are needed. In fact, as experience collecting increases, or the target group becomes more focussed, the use of specialized techniques increases.

Collecting Insects

Collecting nets, Dip nets, Beating trays, Aspirators, Camel hair brushes, Forceps, Killing bottles, Glass vials, Field storage boxes, Paper data labels and pencils, Collecting bags are the basic tools necessary (Plate1 & 2).

Collecting nets come in three basic forms: Aerial, sweeping, and aquatic. The first is designed especially for collecting butterflies and other flying insects. Both the bag and handle are relatively lightweight. The sweeping net is similar to the aerial net but is stronger and has a more durable bag to withstand being dragged through dense vegetation. Aquatic nets are used for gathering insects from water and are usually made of metal screening or heavy scrim with a canvas band affixed to a metal rim. A metal handle is advisable because wooden ones may deteriorate when repeatedly wet. The insect collecting net is the basic tool of the insect collector. The net should be light and the handle may be made from an aluminium pipe, a broom handle or a sturdy dowel stick. The length may vary between 50-120 cm. A metal opening or hoop, no less than 30cm in diameter, can be made from heavy steel wire, about 5 mm in diameter. The net bag should be about 90 cm deep tapered at the bottom, and made from nylon netting which does not disintegrate or tear easily. Around the hoop the net should be reinforced with a strong binding material. The ends should be left open so that the metal ring can be passed through the binding. One way to attach the ring to the handle is to use hose clamps. The insect net can be used to collect individual specimens when they are in flight or at rest on flowers or shrubs. Once an insect is caught in the net, the handle is given a quick twist to fold the bag and prevent escape. The insect is then transferred into a suitable container or a killing jar. General collecting can be done by sweeping the net back and forth through foliage and grasses.Net sweeping results in the collection of a variety of insects that may be removed by hand, forceps or an aspirator. Another way of dealing with the sweep-net catch is to shake the contents of the net to the bottom and invert the net into a killing bottle. Insects can then be sorted later. This technique may result in the excessive collection of unwanted specimens.

Dip net

The dip net is used to collect aquatic insects. It has a short bag and a fairly long handle. Some are designed with a flat side opposite the handle to allow the net to scrape along the bottom of a pool. The contents of the dip net can be emptied into a shallow white plastic tray of water and the required insects can then be sorted easily. Surplus insects should be returned to the habitat from which they were collected.

Beating tray

The object of beating is to capture crawling insects that do not fly readily. The beating tray can be made from strong white cloth, rectangular or square, about 100cm x 60cm, with pockets at the corners into which the ends of two diagonal bracing sticks are fitted. The tray is held beneath the branches of trees and shrubs and the branches beaten with a strong stick. The insects are dislodged and fall into the beating tray. The specimens are then collected by hand, forceps or aspirator. With the sticks removed, the beating tray can be rolled into a neat parcel for easy transport.

Aspirator

The aspirator bottle is a device for collecting small delicate insects individually. It is used to collect insects directly from the ground, foliage, the net or from the beating tray. An aspirator bottle consists of a bottle(chemical resistant) (7cm x 2,5cm) fitted with a rubber stopper. Two holes are drilled through the stopper to take two pieces of hard plastic tubing, one about 7cm long and about 5 mm in diameter. The tubes are pushed through the holes in the stopper and the other tube of 20-30 cm long and 10 mm diameter ,show below and above the stopper. The end of the longer pieces, which will be inside the bottom when the stopper is inserted, should be covered with a piece of mosquito netting/gauze(cotton) to prevent insects being sucked into the mouth of the collector. A rubber tube 30-45cm is attached with at least 2 cm to both plastic tubes on the outside of the stopper. To use the aspirator, air is drawn through the apparatus by sucking on the longer tube with the mosquito netting. The insect is sucked into the bottom through the smaller tube that is pointed towards the insect.

Camelhair brushes

Delicate insects like termites that are best stored or preserved in alcohol can be picked up on the tip of a fine brush moistened with alcohol and then transferred to a glass vial containing alcohol.

Forceps

The best forceps are those with prongs that are rounded or come to a point and the inside surfaces milled. The prongs should make contact at the tips only, so that an insect can be gripped firmly without slipping out. Soft stamp collectors forceps are useful for soft-bodied insects.

Killing bottles

Once the insect has been captured, it should be killed quickly and in a way that leaves it relaxed. This is usually achieved by placing the specimen in a killing bottle moistened with a killing agent. The agent must be relatively harmless to humans e.g. ethyl acetate. Only one or two drops are needed to charge the jar with vapour sufficiently concentrated to effect rapid death. Excess ethyl acetate causes condensation on the inner walls of the jar that will wet and discolour specimens. Any wide-mouthed glass jar can be used with a simple modification. Cover the bottom of the jar with a thin layer of sawdust. A sheet of paper, cut to the shape of the jar, is placed on top of the sawdust, which is then covered with a mixture of plaster of Paris and water to a depth of about 10mm. The plaster of Paris is made by adding powdered plaster of Paris to water, little by little,stirring continuously, until the mixture has the consistency of relaxing box thick cream. It is then poured into the middle of the paper in the jar and allowed to spread out to the sides. The jar is then placed outside in the shade to allow the contents to dry completely. Bottles and jars of different sizes

can be prepared in the same way. It is advisable to have at least three killing jars – one for grasshoppers which kick and dribble, one for moths which shed scales all over the jar, and the third for other insects. Never put delicate insects like butterflies with hard insects like beetles into the same jar.

Glass vials

A collecting kit should include several glass/polycarbonate vials, preferably in a range of sizes, some clean and dry and others containing liquid preservatives. Soft-bodied insects and larvae are placed directly into preserving solutions. This kills the insect as well as preserving and storing it. The dry bottles are used for transporting live specimens for rearing or a study.

Field storage box

Another important item of field equipment is a box or tin containing layers of soft tissue paper, cut size, which fit snugly into the corners of the box. When the first batch of insects is ready to be taken from the killing jar, the top layers are removed in order to start at the bottom of the box. The dead insects are emptied into the bottom layer and a data label is placed with them. The rest of the tissue paper layers are then placed on top. The same procedure is followed with the second batch of insects; each batch must be separated from the previous one by a layer of tissue. Each layer must receive its own data label and specimens from separate localities should not be put together. The insects should be pinned before they dry out, but if that is not possible they could be placed in a relaxing box for a day or two.

Relaxing box

A relaxing box is a dampened airtight box with a drop of ethyl acetate to prevent fungal infection (Plate-1).

Paper data labels and pencil

Each specimen must eventually be labelled with the information relating to its capture. This must be recorded at the time of capture and it is convenient to have some small paper labels ready (about 50 x 15 mm). The basic information required for every specimen should include the following: locality, date of capture and the name and initials of the collector. A grid reference would also be useful for more accurate location. The label information must be recorded in the field; never trust your memory when it comes to label data. Specimens from different localities and dates should always be kept separate from one another and labelled separately. It is often useful to have additional information, like host plant, found under rock, trapping methods etc. Writing in the field is important and it is best to use a HB pencil for this. Ballpoint type pens are usually not satisfactory.

Collecting bag

A canvas bag with partitions for sorting items is useful, and should be slung from the shoulder by means of a strap to leave the hands free. A bag slung on the back has the disadvantage that it must be taken off each time it is needed.

1. The equipment used to assemble a general insect or mite collection need not be elaborate or expensive. In many instances, a collecting net and several killing bottles will suffice; however, additional items will permit more effective sampling of a particular fauna. Many collectors carry a bag or wear a vest in which they store equipment.

The following items usually are included in the general collector's bag :

2. **Forceps:** Fine, lightweight forceps are recommended
3. **Vials:** containing alcohol or other preservatives.
4. **Killing bottles:** of various sizes.
5. **Small boxes or containers:** for storing specimens after their removal from killing bottles. These may be made of cardboard, plastic, or metal and should be partly filled with soft tissue or cloth to keep specimens from rolling about. Do not use cotton because specimens become entangled in the fibres and may become virtually impossible to extricate without damage.
6. **Small envelops:** for temporary storage of delicate specimens and/or gelcaps for tiny specimens.
7. One or more aspirators.
8. **Absorbent tissue:** for use in killing bottles and aspirators.
9. **Notebook:** and writing equipment for jotting down notes and label data.
10. **A strong knife:** for opening galls, seedpods, twigs, etc and a pair of scissors for cutting labels.
11. A small, **fine brush** (camel's hair is best) for picking up minute specimens.
12. **Bags**: for storing plant material, rearing material, or Berlese samples
13. A **hand lens**.

Methods

1. Active Sampling:

1.1. Hand Picking:

Arthropods are searched visually in the selected habitat and captured using for nets, aspirators, beating try/sheet, forceps, brush etc. This is an intensive method of sampling and very effective for several groups of insects. Within the sampling unit, all potential habitats of arthropods are to be visually searched. Preferred habitats such as under the stones, logs, tree holes, rock cracks, crevices are to the thoroughly searched. Vegetation, especially those in flowering and fruiting stage attract several insects and are ideal places for sampling. Similarly host plants and animals attract herbivorous insects and ectoparasites and provide a easy sampling ground. In aquatic habitats arthropods can be effectively sampled using pond or hand held dip nets of different sizes.

Passive Sampling:

Arthropods can be effectively sampled using a variety of passive sampling methods. In passive sampling methods, target taxa is lured to the traps, baits or other devices manipulating the behavioural or habitat preferences of the species. Since several of the arthropod species are cryptic and elusive they may not be encountered by active sampling methods but by using passive methods they may be captured. Hence, passive sampling methods compliments active sampling methods in documenting species diversity. Passive methods can be broadly categorized as traps, baits and other methods.

1.2. Traps:

1.2.1. Light Trap: Several species of insects are attracted to light especially during dawn and dusk hours. Light traps are used all over the world as a standard methodology for trapping and sampling insects. Since insects are attracted to various wave lengths of light especially in the UV range, selection of the light source is very important. Mercury Vapour lamps used for street lighting (120-150W) operated with 2mX2m white thick cloth as screen is found to be very effective in attracting diverse insect orders. Mercury vapour lamps attract more insects (Plate-1) than Compact Fluorescent Lamps (CFL) and can be operated with a portable diesel generator. Along with this UV lamp (Blacklight blue) may also be used to attract more insects, especially moths and flies.

1.2.2. Pitfall Trap: Several ground dwelling insects, crustaceans, arachnids, centipedes and millipedes can be efficiently sampled using pitfall traps of different sizes. Plastic traps of different sizes and depths can be made with discarded water bottles, cups, dust bins or small buckets. The pit fall trap is to be covered with a small lid in such a way to allow entry of arthropods and exclude small vertebrates such as lizards, rodents and frogs. The trap may be baited with meat, fruit or dung to attract a specific group of arthropod. The trap may be also filled with dilute ethanol to kill trapped arthropods. The rim of the trap is to smeared with few inches of petroleum jelly (Vaseline) to prevent the escape of trapped arthropods. Within a plot or transect few traps are to be kept for effective sampling. The trap need to be examined daily to remove trapped arthropods.

1.2.3. Flight Interception Trap: Several flying insects can be trapped using this method. A piece of netting about 2m or more in width can be stretched across three poles or trees to form a wide "V" the pointed end of the "V" is to be directed towards maximum light or direction of the wind. The colour of netting may be light blue and dark green so as to merge with the background. A gently sloping triangular roof is to placed over the "V" shaped trap. Two or more nets may be placed in zigzag manner or in opposite direction to increase the sampling efficiency.

1.2.4. Malaise Trap: This is a modified non directional flight interception trap with cross baffles and collection device in the center. The cross baffle made of fine dark netting material is covered on top with slopping roof of same netting material. The collecting device in the center may contain solid or liquid killing agent or attractant. This is a very effective trap for many flying insects (Diptera, Hymenoptera, Hemiptera, Coleoptera etc.) and can capture species which are not normally encountered in routine methods. The trap can be set at different vegetation layers (under story, mid canopy and canopy level) to sample insects of different strata.

1.2.5. **Colour Trap:** Several species of insects are attracted to a particular wavelength of light or colour. Using this behavioural bias traps can be made to attract insects. Commonly used trap is "Yellow Pan Trap". This can be easily made with medium sized yellow food grade/ microwave safe plastic bowls available in super markets. The bowl can be filled with dilute alcohol or dilute fruit essence. Several species of bees, wasps, bugs and beetles can be trapped by this. Yellow pan traps can also placed under flight interception or Malaise traps. Many blood sucking dipterans such as mosquitoes, midges, tabinds etc are attracted to slowly moving black object. Sheet of thick black cloth or black umbrella can be used for attracting such insects.

1.2.6. **Sticky Trap:** Several minute flying insects (dipterans, aphids, plant hoppers etc) can be captured using non-toxic, non drying yellow or blue sticky traps. These traps are hung in vegetation or near flowering trees and plants to collect floral visitors. However, they are not suitable for lepidopterans

as they spoil the specimen. These traps are commercially available and widely used in pest control.

1.3. Baits: Baited traps are lures where the target taxa is trapped using appropriate baits. Baits are generally food, oviposition attractants or sex pheromones.

1.3.1. Food Baits: A variety of food baits are used to attract insects. Commonly used food baits are rotten fruits, meat, fish, oil etc. Fermented mixed fruit attracts several species of butterflies and moths. Many species of lepidopterans can be trapped using fermenting fruits. Cow dung pats are effective baits for sampling dung beetles.

1.3.2. Host Baits: Ectoparasites such as dipterans, ticks, mites, fleas, lice etc are highly host specific and they can be sampled only by locating or collecting the host. Some domesticated hosts such as poultry, cattle, goat, sheep and pets attracts several species of ectoparasites and can be easily collected by hand picking. It is difficult to sample wild vertebrates for ectoparasites. However, roosting spots, nests, den or freshly dead animals offer unlimited opportunity to sample larval and adult stages of ectoparasites.

1.3.3. Oviposition attractants: Many insects have specific oviposition sites and attracted to such sites through chemical cues. Oviposition attractants such as decaying meat, fruits, animal scats attract several insect species. Oviposition attractants can be combined with pit fall traps to collect a particular group of insects.

1.3.4. Pheromone Traps: Pheromones are behavioral altering chemicals used for communication between members of same species. Sex, trail and aggregation pheromones are examples of such chemicals. Many pheromone traps are commercially available to sample agriculturally important insects. Since pheromones are highly species specific, it is used for collection of targeted species only.

1.4. Other Methods

2.3.1. Rearing: During surveys, several immature stages of arthropods such as egg, larvae, pupae and nymphs or nests will be encountered. Since it is difficult to identify species in immature stages, they may be rared to adult stage and identified. This technique is especially useful for lepidopterans, hymenopterans and dipterans. Further, this technique provides other useful information on host species, parasites and parasitoides. However, raring is laborious procedure and out most care is to be taken to maintain temperature, humidity, light and food. Since some larval stages such as that of aquatic insects are difficult to rare under laboratory conditions, emergence traps are set under field conditions to collect emerging adults.

2.3.2. Soil and Leaf litter Sampling: Several species of small insects, mites, ticks and other arthropods live in the soil and they are difficult collect with hand. One of the successful ways to collect such arthropods is to collect the soil and leaf litter and place them in Burlese Funnel or Winkler's bag. Before placing the leaf litter, it can be sifted using a coarse sieve to remove twigs, leaf, stones and other large objects. As the leaf litter or soil dries under the heat of sunlight or electric bulb, the insects move towards to bottom where collection jar with killing agent is kept. The samples collected in the killing jar are to be emptied from time to time and samples sorted into different taxonomic groups.

2.3.3. Aquatic Sampling: Diversity of arthropods, especially crustaceans and insects are very high in aquatic habitats. The crustacean diversity is high in marine and estuarine habitats. Conversely, freshwater ecosystems are dominated by insects. Aquatic arthropods occupy different strata, substrate and

vegetation in the aquatic habitat. Sampling methods are designed in such a way to collect arthropods from different microhabitats in an aquatic ecosystem.

Other trapping methods

For trapping ground dwelling insects, a jam jar can be sunk into the ground with its opening level with the surface. Many insects, especially beetles, fall into this and cannot escape due to the smoothness of the sidewalls of jar. Some preservative may be placed in the jar. A few traps depend on colour to attract insects. A shallow yellow or white tray, about 3 cm deep and as large as possible, may be filled with water and a detergent. Place this in the open. Insects, especially aphids and other small flying species, will be attracted to the colour and fall into the water.

Hand collecting: Insects are found in a variety of places where the use of a net is not possible - under logs or stones or loose bark. Here collecting is done by hand.

Under bark: Interesting insects are found under loose bark. An insect net is useful for catching insects that drop from under bark on dead logs or branches.

Dung: Fresh droppings of animals, especially mammals, attract insects. Flies are usually the first to arrive and lay their eggs in the dung. As the droppings dry out and are changed in other ways, other types of insect arrive, some to feed on the dung itself, and others on those species already present. An aspirator can be used to collect the scattering insects while scraping the dung.

Rocks: Turning over stones of almost any size will yield insects. Stones should be replaced to encourage repopulating. Large rocks, especially in damp places, have a fauna all of their own.

Flowers: Many small insects may be found inside flowers. To obtain the small habitual flower inhabitants, the individual flowers should be shaken over a white sheet or cardboard. The insects that fall out can be collected by aspirator. A net is also useful for collecting visitors to flowers.

Collection of insect from forest canopy: Approximately more than 30 % of the insects found in the forest ecosystems dwell in the different layers of the forest canopy. Majority of these cannot be collected through conventional means. Even ropes and ladders mused to make collections from the canopy yield limited results. Representative insect fauna even from a dense generally inaccessible canopy cover can be collected by fogging the selected trees with a biodegradable insecticide. Hundreds of species of insects, spiders and other arthropods that fall to the ground can be collected on plastic sheets spread below.

Collecting Aquatic Insects: Aquatic insects may be collected by the same means as terrestrial insects, but specialised equipments is required.

KILLING AND PRESERVATION KILLING AND PRESERVATION

Liquid Agents for Killing and Preserving

Insects and mites of all kinds may be killed and preserved in liquid agents. Some kinds of insects are best kept dry; it may not be advisable to allow others to become dry. Ethanol or ethyl alcohol mixed

with water (70 to 80 percent alcohol) is usually the best general killing and preserving agent. For some kinds of insects and mites, other preservatives or higher or lower concentrations of alcohol may be better. Because pure ethanol is often difficult to obtain, some collectors use isopropanol (isopropyl alcohol) with generally satisfactory results. Parasitic Hymenoptera are best killed and preserved in 95 percent alcohol. This high concentration prevents the membranous wings from becoming twisted and folded, hairs from matting, and soft body parts from shrivelling. This concentration may also be desirable if large numbers of insects are to be killed in a single container. Adult bees should not be collected in alcohol because their usually abundant body hairs become badly matted. On the other hand, soft- bodied insects, such as aphids and thrips, small flies, and mites, become stiff and distorted if preserved in 95 percent alcohol and should be preserved in alcohol of a lower concentration. Adult moths, butterflies, mosquitoes, moth flies, and other groups with scales and long, fine hairs on the wings or body may be worthless if collected in alcohol regardless of the concentration. Formalin (formaldehyde) solutions should not be used because the tissues become excessively hardened and the specimens then become difficult to handle. Larvae of most insects should be collected in alcohol and subsequently killed in boiling water to "fix" their proteins and prevent them from turning black. Large specimens or small specimens that have been crowded into one vial should be transferred to fresh alcohol within a day or two to reduce the danger of diluting the alcohol with body fluids. If the alcohol becomes too diluted, the specimens will begin to decompose. For some groups, preservation is better if certain substances are added to the alcohol solution. Thrips and most mites, for example, are best collected in an alcohol-glycerin-acetic acid (AGA) solution, and for many larvae a kerosene-acetic acid-dioxane (KAAD) solution is preferred. If KAAD is used, larvae need not be killed in boiling water. Larvae and most soft-bodied adult insects and mites can be kept almost indefinitely in liquid preservatives; however, for a permanent collection, mites, aphids, thrips, whiteflies, fleas, and lice usually are mounted on microscope slides. Many insects collected in alcohol are later pinned for placement in a permanent collection. Hard bodied insects such as beetles can be pinned directly after removal from alcohol.

Dry preservation

The requirements for dry preservation will vary with the types of insects collected the list below covers most general collecting equipment (Plate 1 & 2).

Pins: Stainless steel pins must be used, because they do not corrode or become brittle with age. The entomological pins 3.8cm long come in various thicknesses and numbers (0,1,2,3,4,5). The most useful pins are a no. 5 (large insects) a no. 3 (medium) and a no. 1 or 2 for small insects and 0 for micro insects.

Steels\minutens: These are minute headless pins used for small insects like mosquitoes and midgets.

Polyporous pith: Used for staging small insects mounted on minutens. Usually purchase in strips which may be cut to size.

Points, cards, glue: A small card or card triangles on which to mount small insects may also be used. Use a soluble glue to stick the insects to the card or onto the bent point of a card triangle.

Setting/stretching board: A setting board is used to hold the wings in position while the insects are drying. A simple board can be made from polystyrene foam, cork or wood. Grooves are cut in the board to house the body of insects. Because it is best to have the walls of the groove close to the body to support the wings, grooves should be of few different widths. A good size for the board is about 30 x 25 x 5 cm with grooves

ranging between 5, 10, 15 and 20 mm. The setting board should be kept in a cardboard box with a lid, containing some naphthalene, to prevent damage by other live insects.

Pinning block (Plate-1).: A pinning block allows insects and labels to be positioned at standard heights on the pin. The cheapest and easiest way to make the block is to use wood. It can either be a solid block with holes drilled to different depths or a set of pinning steps with a hole drilled through each level

Storage box (Plate-1): Large collections are kept in glass-topped drawers in cabinets, but this can be very expensive. Temporary wooden boxes with lids and bottoms made from polystyrene foam or cork are a good alternative. The box should be airtight, to keep the fumes of any repellent in, and to keep pest insects out. It is standard practice to place many kinds of insects in small boxes, paper tubes, triangles, or envelopes for an indefinite period, allowing them to become dry. It is not advisable to store soft-bodied insects by such methods because they become badly shrivelled and very subject to breakage. Diptera should never be dried in this manner because the head, legs, and most of all the antennae become detached very easily. Almost any kind of container may be used for dry storage; however, tightly closed, impervious containers of metal, glass, or plastic should be avoided because mould may develop on specimens if even a small amount of moisture is entrapped. Nothing can be done to restore a mouldy specimen. Dry-stored specimens must be labelled with complete collection data in or on each container Some insects, such as small beetles, should be glued to points directly from the layers for permanent preservation, but if they are to be pinned or otherwise treated, they must be relaxed.

Papering: Although pinning specimens when they are fresh is preferable, the storage method known as papering has long been used successfully for larger specimens of Lepidoptera, Trichoptera, Neuroptera, Odonata, and some other groups. It is a traditional way of storing unmounted butterflies and is satisfactory for some moths, although moths too often will have their relatively soft bodies flattened, legs or palpi broken, and the vestiture of the body partly rubbed off. To save space in most large collections, file Odonata permanently in clear plastic envelopes instead of pinning them. Papering consists of placing specimens with the wings folded together dorsally (upper sides together) in folded triangles or in small rectangular envelopes of glassine paper, which are the translucent envelopes. Glassine envelopes have become almost universally used in recent years because of the obvious advantages of transparency and ready availability.

Liquid Preservation: Preservation of insects in alcohol is a complex subject and like many things, it varies somewhat from one group to another. For example, spiders preserve well in ethanol, but tend to become too flaccid in isopropyl. The opposite is true for many myriapods. If one specializes in an insect group suited to preservation in one or another kind or concentration of alcohol, consult specialists in that group or experiment to find what yields the best results.

In general, ethanol and isopropanol mixed with water is the most widely used preservation fluids. Most commonly, a mixture of 75% alcohol to 25% water is used. The water should be distilled to ensure a neutral pH and the solution should be thoroughly mixed since alcohol and water do not mix easily by themselves. Additives should be avoided. Special care should be taken with labels placed in alcohol. Paper should be high quality rag or linen and acid-free. The ink should contain vegetable gum (such as India inks) as these seem to withstand the constant exposure to the alcohol the best. Typewritten labels and computer generated (laser printed) labels are generally unacceptable. The best system is still professionally printed labels. Shell vials plugged by cotton or with polyethylene stoppers are recommended. Avoid stoppers made from cork, rubber, or neoprene, as they tend to degrade and/or leach chemicals into the alcohol. Shell vials are preferred over necked vials as it is easier to remove the specimen and the chance of damage

is reduced. Each vial should be individually labelled with complete collection data. The shell vials are kept in wide mouthed, gasketed bail-top jars with straight sides. Avoid metal screw caps, Bakelite lids, greased glass, and ground glass as they may rust, warp, crack, leak, or allow the alcohol to evaporate. Generally it is recommended that each jar contain between 10 and 40 vials. Avoid glass-glass contact by placing a folded paper towel in the bottom of each jar. Keep vials upright within jars. Each jar should be filled with alcohol to just below the level of the gasket. If material is going to be stored for long periods of time, the jars should be checked periodically and the alcohol topped off. Labels may also be placed on the outside of the jars to indicate the enclosed contents. Light is the chief enemy of alcohol preserved material, and as a result, jars should be stored inside cabinets. Fire safety is always an important consideration when storing or working with alcohol collections. Concentrations of vapours can be very hazardous so care should be taken that work areas are properly ventilated and that there is no source of open flame nearby. In larger collections, special cabinets, exits, and other precautions may be necessary to meet the fire code.

Plate-1: Different type of Collection Methods

1. Insect collecting net

2. Aspirator

3. Light trap

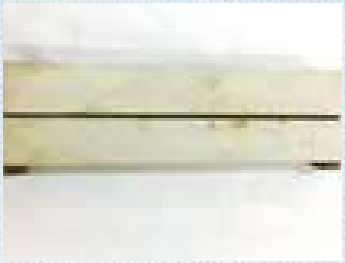
4. Killing bottle

5. Relaxing box/jar

6. Foreceps

7. Scissors

8. Pinning block

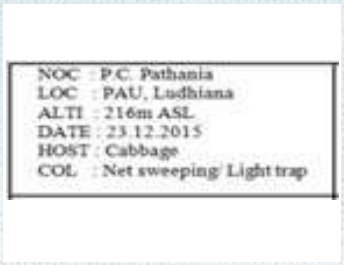


9. Stretching board

10 Insect collection box

11.Insect collection box (Naphthalene balls fixed)

12. Insect preservation box



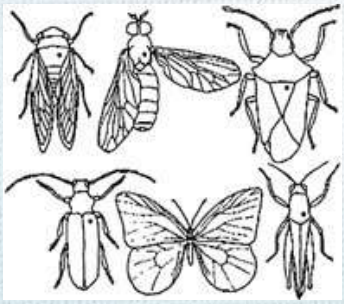
13. Label

14. Naphthalene balls

15. Insect pins (entomological)

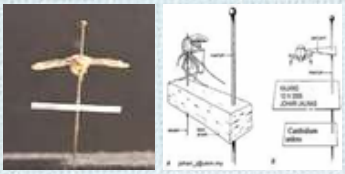
Plate-2: Methods of pinning and preservation

i) Insect collection with insect collecting net



ii) Methods of pinning the insects of different insect orders for their dry collection (Line diagrams)

iii) Methods of pinning the insects of different insect orders for their dry collection (Photographs)



iv) Different methods of mounting and double mounting the insects(Line diagrams)

v) Different methods of mounting and double mounting the insects (Photographs)

vi) Double mounting the insects (Photographs)

vii) Preservation of insects in the insect collection box/show cases

viii) Preservation of insects in the insect storage cabinet

ix) Preservation of insects in the insect mobile rack system

Table-1: Different method of preservation

GROUP	STAGE, FORM OF SEX	HABITAT	METHOD OF PRESERVATION	APPROPRIATE CONTAINER OR TREATMENT
Acarina	Adult, both sexes desirable	All habitat types including terrestrial, freshwater and marine environments.	70-80% alcohol	In vials, or clear and mount on microscope slides
Collembola	Adult	Soil, leaf litter and organic mulches, under bark or decaying wood.	70-80% alcohol	Preserved in vials
Protura	Adult	Moist habitat usually in the humus and Leaf mold of temperate deciduous forests.	70-80% alcohol	Preserved in vials
Diplura	Adult	Moist soil, leaf litter and humus	70-80% alcohol	Preserved in vials
Thysanura	Adult	Hide under stones or leaves	70-80% alcohol	Preserved in vials
Ephemeroptera	Adult; males desirable	Herbivorous, immature stages are aquatic. Lives in unpolluted habitats with fresh, flowing water. Some sp. active swimmers, flattened and cling to the underside of stones, a few are burrowers, tunnels in the sand or mud.	70-80% alcohol	Preserved in vials
Odonata	Adult; males desirable	Aquatic, predaceous both immatures and adults	Dry	In paper envelops/folders or cellulose wadding

GROUP	STAGE, FORM OF SEX	HABITAT	METHOD OF PRESERVATION	APPROPRIATE CONTAINER OR TREATMENT
Orthoptera	Adult	Terrestrial, herbivores, plant feeding insects	Dry	Pin through the near of prothorax slightly to the right of the mid-line and with wings spread on the left side only pack in cellulose wadding Dry the specimens thoroughly and rapidly to prevent decay.
Phasmida	Adult	Wet and dry forests, grasslands and gardens	Dry	Pin through the thorax or pack in cellulose wadding. Dry the specimens thoroughly and rapidly to prevent decay
Embioptera	Adult; males desirable	Humid habitats or underneath bark, stones, or leaf litter	70-80% alcohol	Preserved in vials
Plecoptera	Adult; males desirable	Nymphs are aquatic habitats, mainly in cool, clean, flowing waters with high oxygen. They prefer streams, rocky, stony, or gravel substrate, and in sandy areas	70-80% alcohol	In vials

GROUP	STAGE, FORM OF SEX	HABITAT	METHOD OF PRESERVATION	APPROPRIATE CONTAINER OR TREATMENT
Dermaptera	Adult	Dark, sheltered environments and under rocks, logs and the bark of trees.	Dry	Pin through the right tegmen, or in vials
Zoraptera	Adult	Under the bark of dead and fallen timber. Are most common in rotting wood and logs with loose bark but with the cambium relatively intact.	70-80% alcohol	Not allow to dry
Mantoidea	Adult	Warmer regions, particularly tropical and subtropical latitudes. Many species present in the tropical rainforest, although others can be found in grasslands, desert and meadowlands.	70-80% alcohol	Pinned
Blattodea	Adult	Tropical or subtropical climates, but they also inhabit temperate and boreal regions	70-80% alcohol	Preserved in vials
Psocoptera	Adult; males desirable	Wide variety of habitats throughout most of Australia, preferred moist environments. The majority can be found on vegetation, plant foliage, branches and bar and leaf litter. Some species present in association with humans and in side books or in food products.	70-80% alcohol or (if scaly winged) dry	In vials or in layers of cellulose wadding

GROUP	STAGE, FORM OF SEX	HABITAT	METHOD OF PRESERVATION	APPROPRIATE CONTAINER OR TREATMENT
Phthiraptera	Adult	Body of the host mammals, chewing and sucking lice are ectoparasites	70-80% alcohol	Preserved in vials
Thysanoptera	Adult	Flower blossoms, under leaves and hiding in bark. This insect infest the vegetables, flowers, flowering plants, fruit crops and trees as they feed.	60% alcohol (preferably mixed with glycerine and acetic acid in the ratio of 10:1:1)	Preserved in vials
Hemiptera	Adult; both sexes desirable	Terrestrial, including a number of important agricultural pests, but some are found in freshwater habitats.	70-80 % alcohol (mixed with glycerine and acetic acid in the ratio of 20:1)	Preserved in vials
Hemipteran: Siphunculata	Adult	Blood feeding ectoparasites	70-80% alcohol	Preserved in vials
Hemiptera: Auchenor-rhyncha	Adult; males desirable	Terrestrial	Dry; in 70-80% alcohol (mixed with glycerine in the ratio 20:1)if small	Pinned through the prothorax, or in vails with a few pieces of twisted paper to prevent movement in transit. Smaller specimens may be micro-pinned or mounted on card points. Leafhoppers may be preserved dry in glass vials plugged with cotton wool.

GROUP	STAGE, FORM OF SEX	HABITAT	METHOD OF PRESERVATION	APPROPRIATE CONTAINER OR TREATMENT
Hemiptera: Sternorrhyncha Psylloidea	Adult; males desirable	Terrestrial	95% alcohol; galls dry	In vials or mounted on card points; gall dry in paper or cellulose wadding
Hemiptera: Aleyrodoidea	Pupal cases (with associated adults,if possible)	Terrestrial	95% alcohol (pupal cases in situ on host plant)	In vials or macerate, clear and mount on microscope slides. Winged adult cannot be indentified at present but may be retained for further study)
Hemiptera: Aphidoidea	Adult(alates and or apterae)	Terrestrial	95% alcohol	In vials or macerate, clear and correctly mount on microscope slides

GROUP	STAGE, FORM OF SEX	HABITAT	METHOD OF PRESERVATION	APPROPRIATE CONTAINER OR TREATMENT
Hemiptera: Coccoidea	adult females (not old)	Terrestrial	70-80% alcohol (preferably attached to parts of the host plant)	In vials or macerate clear and mount on microscope slides. Well-prepared specimens on microscope slides can be identified quickly.Older adult females sometimes become too heavily sclerotized for satisfactory slide preparation and winged adult males cannot be identified, but may be retain for futher study. Do not scrape scale insects off plant material as it damages them.
Heteroptera (other)	Adult; males desirable	Terrestrial	Dry, in 70-80% alcohol alcohol if small	Pin through the scutellum slightly to right of the mid-line; or in vails if small
Hymenoptera	Adult	Tropical and subtropical regions except polar region	Dry/70-80% alcohol	Pin through the anterior thorax; small specimens may be micro-pined

GROUP	STAGE, FORM OF SEX	HABITAT	METHOD OF PRESERVATION	APPROPRIATE CONTAINER OR TREATMENT
Parasitic Hymenoptera	Adult; females desirable	Tropical and subtropical regions except polar region	Dry; 70-80% alcohol (90-95% for large ichenumonids)	Small adults (up to about 8 mm long) in 80% alcohol in vials. Alternatively small chalcids may be mounted on cards on their side. Medium sized adult may carefully glued on card points with wings free and both dorsal and lateral surface visible. Larger adults should be pinned through the anterior thorax. Medium to large adults can also be stored and dispatched in 80-90%calcohol in vials.
Strepsiptera	Adult	Tropical habitat	Pinned /70-80% alcohol (immature)	Pinned

GROUP	STAGE, FORM OF SEX	HABITAT	METHOD OF PRESERVATION	APPROPRIATE CONTAINER OR TREATMENT
Coleoptera	Adult; both sexes and especially males desirable males desirable (with associated larvae of wood and other plant boring forms if possible.	Soil, humus and leaf litter, under the bark of living and dead trees, decomposing wood, under stones and logs, in dung, carrion and the fruiting bodies of many types of fungi.	Dry; larvae or very small adult in 70-80% alcohol	Adults to be pinned through the anterior third of the right wing base, if small double mounted with a micropin or carefully glued to standard sized cards or card points so that both upper and lower surface are visible. Use minimum quantities of water or alcohol soluble glue to enable removal for dissection, if necessary. Small adult and all immature stages should be kept in 80% alcohol in vials. A series of specimens are generally required as secondary sexual characters are important for the accurate identification of beetles.

GROUP	STAGE, FORM OF SEX	HABITAT	METHOD OF PRESERVATION	APPROPRIATE CONTAINER OR TREATMENT
Neuroptera	Adult	Soil types, freshwater habitats, or other locations that guarantee the availability of certain kinds of prey. The larvae Some larvae prefer drier regions.	Dry, in 70-80% alcohol if small	Pin through the thorax, Or to be kept in vials if small
Megaloptera	Adult	Freshwater streams	70-80% alcohol	
Trichoptera	Adult	Streams, lakes and marshes, and permanent and temporary ponds.	Dry/in 70-80% alcohol if small	Pin through the thorax or to be kept in vials
Lepidoptera	Adult, males usually desirable	All terrestrial habitats ranging from desert to rainforest, from lowland grasslands to mountain plateaus, but almost always associated with higher plants, especially angiosperms	Dry	Pin through the meso-thorax with wings spread out. Never preserve adult Lepidoptera in alcohol, because it destroys taxonomically important wing patterns. Larvae and pupae preserved in 80% alcohol in vials, and whenever possible.

GROUP	STAGE, FORM OF SEX	HABITAT	METHOD OF PRESERVATION	APPROPRIATE CONTAINER OR TREATMENT
Diptera	Adult; males desirable	Everywhere, humid, moist environments, but can also be found in deserts, forests, mountains, and even polar regions.	Dry if large; in 70-80% alcohol if small	Adult flies to be pinned laterally through the thorax with the pin at right angle so that enters just below and in front of the other wing base. Specimens which are pinned dorsally through the thorax slightly to one side of the mid- line are acceptable provided that vital chararcters are not damaged. Micro pins should be used for all the large specimens. Small sized adults (less than 3 mm long) to be kept in 70-80% alcohol in vials. Please note that Tachinidae should be labeled with the full scientific name of their host, or with data cross-referencing them to host specimens included in the same collection.

GROUP	STAGE, FORM OF SEX	HABITAT	METHOD OF PRESERVATION	APPROPRIATE CONTAINER OR TREATMENT
Siphonaptera	Adult	survive on a wide range of host species. The larvae of all fleas appear grub-like and are usually found in the nests of their host or other areas where they commonly rest.	In 70-80% alcohol	In vials, or carefully clear and mount on microscope slides.
Mecoptera	Adult; males desirable	terrestrial insects that usually live in moist sylvan habitats	Dry, 70-80% alcohol if small	Pin through the thorax or kept in alcohol vials
Strepsiptera	Adult; males	a wide variety of habitats wherever their hosts live, especially in tropical habitats.	70-80% alcohol	Preserved in vials

14. MYRIAPODA (Millipedes and Centipedes)

INTRODUCTION

Myriapods are wingless terrestrial arthropods with at least nine pairs of walking legs, in adults, and a trunk not distinctly sub-divided into thorax and abdomen. They form an important group of soil animals comprising centipedes and millipedes, which occur in association with soil mites, termites, isopods, earthworms, pseudoscorpions, cockroaches, nematodes and molluscs. They have a tendency to remain near the moist surroundings, although excessive moisture may lead them to desication and death due to osmosis. Chilopoda, Diplopoda and Pauropoda were formerly included under a single class Myriapoda (Myriad-meaning multiple; pedes meaning legs) on the basis of their possessing many legs and body presenting two major subdivisions, the head and the trunk. These have now been recognized as widely divergent phylogenetically and have been assigned the status of separate classes, which along with hexapod, are placed in the subphylum Mandibulata, possessed with a pair of jaw like trophic appendages, the mandibles. They are tracheate i.e., air breathing animals through trachea.

COLLECTION AND PRESERVATION

Niches: Collecting centipedes and millipedes and locating their possible hide-outs/ niches requires a lot of skill coupled with experience. They are found singly under stones, logs, leaf litters, cow dung, flowerpots, bark of trees, sheath of banana stems, inside termite mounds, etc. The most suitable habitat is an area with optimum moisture and temperatures conditions near a forest patch, around ponds, lakes or rivers or on the hill slopes, etc. They are nocturnal and the most appropriate time for their collection is either early in the morning or in the evening hours. However, in hills it can be collected throughout the day especially on the hill slopes, which receive filtered sunlight through tall trees, and the floor is covered with leaf litters and the soil is gravel.

The stones or the other objects when upturned or removed exposes the animal and the light and the sound disturb it. The animal being photonegative and nocturnal tries to escape in loose damp soil or leaf litter or retreats underground showing positive geotaxis. The animal runs very fast in a zigzag manner using pebbles, grass roots or humus as a cover for defense and once lost to the vision of the collector, it vanishes. The species *Cormocephalus dentipes* Pocock, which is very common, in Western Himalaya, Uttaranchal, makes a network of fine galleries (Khanna and Tripathi, 1984) under stones for its asylum, defense and efficient escape, in case of the onset of danger.

Conventional Methods of Preservation

For Taxonomical Studies: While collecting centipedes, extreme care is required to be taken to hold the animal gently through its trunk but firmly, with the help of a pair long forceps to avoid damage to its body. If not properly handled, its walking legs, anal legs or antennae may be damaged or broken; thereby render it unfit for taxonomic studies. The centipedes are preserved in 70 per cent alcohol or in solution of 2% formalin. The size of the container should be big enough so that the captured animals can move freely within the killing reagent before it dies, and it should not get curled up or coiled. The curled up or coiled

animals when drawn-out for taxonomic studies get damaged. The specimens preserved in 2% formalin solution should be washed with running water immediately after they are brought back to the laboratory from the fields. Since the specimens preserved in formalin for a long time turns hard and become brittle also at some stage, which makes their taxonomic examination troublesome. The washed animals, from different localities, should then be transferred to separate collection tubes containing 70% alcohol, along with collection data labels for permanent preservation.

Calcium Chloride	10 cc
Commercial formalin	10 cc
Distilled water	80 cc
Calcium carbonate	1 gm. Approx.

Collection for the Study in Captivity: For behavioral studies un-damaged live material is required. The live material collected should be kept in a container or large glass trough, almost half filled with damp earth and humus, also collected from the actual site of collection of specimen. A wire mesh or a perforated plastic lid or a sieve or a black linen cloth tied around the rim of the container may be used to prevent escape. The soil is periodically moistened to its optimal requirement. It may be noted that excessive moisture leads to fungal infection and desiccation. It is also advisable to replace the soil periodically to clear it from any possible infection. Termites are the best food that the centipedes may prefer, the earthworms comes next. The centipedes exhibit extreme cannibalism in captivity. Due care is, therefore, required to be given while selecting the size of the glass trough as well as the number of specimens, their sizes as also the proper amount of food.

15. ARACHNIDA

INTRODUCTION

Arachnids are one of the oldest habitants on this plants, 'THE EARTH' Million years of their existence have given them enough opportunities to acquire the most suitable habitats to lead a unique and unparalleled life styles. Some of them are appropriately termed 'The Living Fossils' as because they have not changed their morphological look even today. Many of them are poisonous thus creating fear psychosis, misunderstandings, superstitions and misconceptions about them. Evolved from Trilobites, about 450 millions years ago and believed to be the first arhropods to lead a terrestrial life are 'The Eurypteries'. The fore most change they acquired was the aerial respiration through '*Booklungs*' in place of '*Gill book*' and secondly the able '*podomeres*' to move easily through terrestrial habitats. The Arachnids alone have been put into 10 different orders *viz*., Scorpionida, Uropygi, Plapigrads Schizomida, amblypygi, Araneae, Phalangida, Ricinule, Chelonethi (Pseudoscorpionida), and solifugi. To recognize an arachnid among Arthropods is simple; one must verify I). The presence of 4 pairs of walking legs, II). The first pair of chelate appendages III). The cephalic and thoracic regions joined together to form unsegmented cephalothorax, IV). The body is divisible in to two parts as cephalothorax (prosoma) and abdomen (opisthosoma). So far, Arachnids are known by about 90,000 species from the World, whereas approximately 3,600 species are reported from Indian sub-continent i.e. about 4% of the world total.

Habitats

Aracnids invariably inabit in all the three habitats namely *Terrestrial* (*soil*), *Aquatic* and *Arboreal*. In the terrestrial habitats, exhibiting diverse features, one can always encounter with these animals. However, these are common residents of temperate and trpoical landmasses; at the same time, they are not uncommon in forests. In fact, recent studies have shown and proved that their biodiversity of this group is richer and versatile in the Tropical rain forests. Desert ecosystem has the most venomous species of Arachnids this may be due to the habitat influence. Forest premises with profuse litter are the common habitat most suitable for some of the arachnid groups e.g. Phalangids, Schizomids, Cheloniths and Ricinuleids etc. In the desrt ecosystem, Scorpions, Solifugid and Spiders flourish. Where as, the soil ecosystem rich in Acarina and Palpigrade fauna. Some of the arachnids have chosen aquatic habitats e.g. Xyphosura inhabiting marine waters, coastal creeks and estuaries, Pygnogonids (marine forms), Mites and Tardigrades (Fresh waterforms). The Pentastomids (in mammals) are the only internal parastic arachnids. In a limited sense to the arborial habitat dwellers, inhabting on trees above the height of more than 608', and leading a sectarian life (parthenogenetic propagation) are the scorpions of the family Buthidae found under tree barks and on Palm trees. Some inhabit deep caves e.g. Scorpions, Schizomids and few Spiders, which are generally blind and without body pigments. Araneae, the spiders are omnipresent in all three habitats.

Scorpions

Scorpions comprise a diverse and highly successful order of Arachnida. They are the oldest known terrestrial metazoan, occurring in Silurian fossils, and evolved 400 millions of years ago. With all the evolutionary changes in the terrestrial exosystems, it is interesting to note, that they have not under gone much change in their external morphology, hence they are called "*living fossils*" Scorpion fauna of the world comprises

of a little over 1500 species under 110 genera and 9 families. In Indian subcontinent 108 species under 5 families are known.

Scorpions are nocturanal in habit, hide under rocks, and logs of wood, between the crevices in the rocks and dark corners. They are distributed in tropical and subtropical forests. Abundant in arid and semi arid zones of the world. They occur in all ecological niches like, grass lands, savannas, deserts, intertidal zone and even in the high altitude zone like Himalayas and alps. They are absent in Antarctic zone.

According to their habitat scorpions are classified into three groups : 1. Burrowing forms (Psammophilos), 2. rock dwelling (Liothophilus) and 3. Arborial Species genus *Orthochirus*, *Odontibuthus* and *Cosmobuthus*) and species under the families Ischnuridae and heterometridae dig pits like burrows. Expect species of Ischnuridae, other species dig small pits of 6-8 inches depth. Usually rocks or boulders cover these pits. In the case of Ischnuridae the burrows are deep, nearly 1-2 feet in depth with a flask like chamber at the end of the burrow species belonging to the genus *Lychus* live under loose rocks in most terrain. *Mesobuthus* species recorded to occur on coconut and palmyra trees. They usually occur under leaf sheaths and in inflorescence.

COLLECTION

As scorpions are nocturnal in habit they are best collected during the nights. The best time to collect them is on new moon days and 15 days before the full moon day (on well illuminated rights the number emerging from the burrows are noted to be less). During the nights by using UV light, one can easily collect the specimens. During daytime, by turning the rocks or boulders, and searching between the crevices the specimens are collected.

Can trap method

Usually small pits are dug in the field and cans or jars are put in the pits half filled with 50% formalin and 2% glacial acetic acid. By this method some specimens could certainly be collected though they may be few in number. Instruments needed are very simple viz a long forceps, glass bottles, and plastic bags. Specimens collecteld from the field should be segregated immediately and kept in separate containers to avoid cannibalism.

Precautions suggested

While using UV lights care should to be taken in the areas infested with poisonous snakes since this will attract these snakes also. Some scorpions could sting through the plastic bags. It is preferable to wear thick boots and gloves during collection. Also a first-aid kit should be kept handy.

PRESERVATION

The old method of preserving the specimen is to drop the live specimens in alcohol. This method does not give good results. As the specimen shrinks, a better method suggested by williams (1968) is as follws :

1. Specimens should be killed by heat shock, by dropping the specimen in hot boiling water until the metasoma stretch straight. (usually it take 5 sec.)

2. The specimen then should be fixed in the following fixative for 5-10 hours for normal sized specimen.

Formalin	12 parts
Glacial acetic acid	2 parts
Isopropyl or n-propyl alcohol	36 parts
Distilled water	56 parts

In the case of large sized specimens fixative should be injected. The scorpion should be rinsed in 50% Isopropyl alcohol for one hour and then preserve the specimen in 70% Isopropyl alcohol.

Spiders

Spiders are fascinating creatures with a unique life style. All spiders are carnivores, predating on variety of insects. They are aboundant and can be found all most anywhere from households to the crevices, in rocks and vegetation up to 22,000 ft. altitude. The current worldwide taxonomical tally of spider is about 37,000 species accommodated under 108 families. In India, approximately 1500 species accommodate in 43 families.

Most of the spiders are small except the tarantulas. A spider body consists of two main parts an anterior prosoma or cephalothorax and a posterior opisthosoma or abdomen. A narrow stalk, the pedicel, connects these. The prosoma mainly serves the purpose of locomotion food uptake and nervous integration, whereas the opisthosomais concerned with digestion, circulation, respiration, excretion, reproduction and silk production. A dorsal and a ventral plate, the carapace and the sternum cover the prosoma. It serves as a place of attachment for six pairs of extremities. One pair of biting chelicera and one pair of leg like pedipalp, situated in front are four pairs of walking legs. In mature male spiders the pedipalps are modiffied into copulatory organs- a unique feature, not found in any other arthropod. The abdomen in soft carries the spinnerest on its posterior end.

All spiders possess spinning glands that secrete silk. Spinning ability has made the spiders become a part of folk myth and legend. Family Aracnidae (orb-weavers) has a variety of spinning gland and the ground dwelling forms has four spinning glands. Morphologically & histologically, one distinguishes ampullate, acinate, tubuliform, agregate piriform and flagelliform or coronate gland. Each type of gland secretes a different kind of silk with its own specific characteristics. Some are true orb-weavers, some make irregular webs, and some are unable to make any web. The transition form liquid silk into a solid thread has nothing to do with exposure to air but simply brought about by tension that orient molecules of the fibroin so that they are parallel to each other. Spiders are carnivorous and empoly different strategies for capturing the prey.

COLLECTING TECHNIQUES

The easiest way to capture and collect spiders is to scare them into a dry container and then transfer them into a container with alcohol. The container can be placed in a freezer for a few minutes. In the freezer the spider will enter torpor and die relatively quick and may experience less trauma. Carbon dioxide gas can also be used to anesthetize spiders before collecting. The following are a few basic methods used while collecting spiders.

1. Visual search

Walk through the habitat and search visually for spiders, their webs or retreats (curled leaves, silken cases) walls of houses, building and basement, which are spider haunting grounds.

2. Sweeping

Using a heavy insect net sweep through the soft vegetation or tall grass with vigor. After a few sweeps, dump the content of the net onto a flat sheet and capture the spiders. This is one of the best methods for capturing active hunters such as jumping (salticidae), lynx nursery-web, and sac or ghost spider (oxyopidae, Lycosidae, Clubionidae). Small web-building species are also frequently captured.

3. Beating

This method is similar to sweeping. In this case spread the cloth sheet under a bush or the low branches of tree. Grab the branches and give them a vigorous shaking, alternatively strike them with a stick or stiff branch. Spiders will be dislodged from their location and fall on to the sheet.

Beating and sweeping technique do not work well in wet conditions. If there is heavy dew or if it has rained recently the net and the spiders will stick to the wet cloth and are often damaged or killed during the sweeping. Avoid sweeping and beating in such conditions.

4. Pitfall trapping

This method is effective for capturing ground-living spiders. Any smooth sided container buried within the ground surface will work. Some prefer to have a funnel at the top of the container. Inside the pit, place a second cup so that the contents can be removed without disturbing the edge of the pit. This edge is the crucial key to success. If a spider detects a lip or a ridge, it is likely to walk around rather then tumble into the trap. To avoid the problem of rain, place a cover over the pit. The smaller the gap between trap and lid will, reduce accidental captures of small vertebrates. Sometimes pits are left “dry” but normally filled with auto antifreeze in the pits. This will kill and preserve the captives with minimal evaporation. In either case the problem encountered are dry pits-small spiders will be killed or eaten by centipedes and other organisms. In wet pits the fluid may attract wildlife, which could be poisoned. Antifreeze is available in two forms: ethylene glycol-based and propylene glycol-based. Propylene glycol is preferred because it is not that toxic.

5. Litter Sampling

With help of gloves, collect a large amount of leaf litter and place them over a large funnel that is fitted with ¼ to ½ inch hardware-cloth or wire screen. The funnel should be placed in a rack with the

opening over a cup with alcohol. Suspend a bright light over the top of the leaf litter. Ensure bulb is at lest 3 inches away from the top of the litter but *never let it touch the leaves*. As the pile of leaves dries out the small organisms will migrate down and eventually fall through the funnel into the alcohol-filled cup.

The two tools that render the best result in the shortest amount of time are kick nets and sweep nets.

PRESERVATION METHODS

There are two basic methods of preservation, they are: the dry method and wet method. The dry method is employed for preserving hard-bodied invertebrates but **never for spiders**.

The wet preservation technique involves storing specimens in alcohol, formalin or some other liquid preservatives. The wet method has the advantage of being free of damage from mold or pests.

PRESERVATION OF SPIDERS

Live spiders are placed in a clean, lid container with a small volume of 1% propylene phenoxylol and swirl gently. This will anaesthetise the spider and it will go limp with its legs extended. The aquatic spiders can walk on water, so keep swirling until they drown, When the spider is limp, use a small paintbrush to carefuly transfer the spider (and its data slip) to a glass vial filled with 70% ethanol, and seal with a lid.

PRESERAVTION

The standard Preservative is 70% ethyl alcohol. This is not easy to obtain and it is expensive. The best substitute is 70% isopropyl alcohol. This is easy to obtain from the local pharmacy sold as “rubbing” alcohol. Some spider collectors use 80% alcohol in the field because the water in the bodies of the spiders will dilute the solution somewhat, and it kills the spiders a little faster. In this case; transfer the spiders to 70% alcohol on return from field. Do not use denatured alcohol because the agent may have unpredictable effects on the long-term stability of the specimens and render the specimen unfit for DNA analysis. Another fluid to avoid is “ethyl rubbing alcohol”. This is denatured with other solvents such as xylene, toluene, or acetone that will damage the spider specimens.

16. ACARI

INTRODUCTION

The phylum Arthropoda includes the group of animals, which unlike the insects or myriapods, have neither antennae nor mandibles. These are known as Chelicerata, of which the class Arachnida makes up the largest part. These are predacious in habit and are either oviparous or viviparous. The living members of Arachnida are grouped into nine orders : 1. Scorpionida, 2. Pedipalpida, 3. Microthelyphonidae, 4. Salupigida, 5. Ricinulei, 6. Opiliones, 7. Pseudoscorpionida, 8. Acari and 9. Araneae. The order Microthelyphonida and Ricinulei are so far not recorded from India.

Plant feeding mites damage plants by desaping and inoculating viruses to plants; stored product mites infest stored food grains and flour; parasitic mites attack domestic animals, poultry, apiary, fish and other aquatic animals; inoculate cestodes to cattle; causes dermatitis, respiratory troubles and other allergic troubles to humans. Ticks do harm directly by feeding on blood or causing injury to the skin of the host. A few of them acts as efficient predator of plant feeding mites.

Why do we collect arachnida? All the members of arachnids are of no economic importance. Only the ticks and mites of the order acari and spiders belonging to araneae are of great importance as most of them are either beneficial or harmful to man.

Plant feeding mites damage plants desaping and inoculating viruses to plant; stored product mites infect stored food grains and flour; parasitic mites attack domestic animals, poultry animals, honey bees in apiary, fish and other aquatic animals; inoculate cestodes to cattle; causing dermatitis, respiratory trouble and other allergic problems to man. The ticks do harm directly by feeding on blood or by making injury to the skin of the hosts and also causing viral and other diseases to animals and human beings. There are also mites which are beneficial to man. Some mites act as efficient predator of plant feeding mites. Few aquatic mites feed on mosquito larvae and check their population. Soil inhabiting mites play an important role in humification of organic matter resulting in increase of soil fertility. Some mites are also being used as biological control agent and bioindicator of ecosystem health.

The spiders are considered to be one of the best friends of mankind, because they feed on insects which are generally harmful to man and domestic animals. Spiders are now used as resource material for homeopathic and allopathic medicines. Spider venom is used in preparation of several drugs. The thread made of spider silk is used in surgery. Except a few spiders like black widow spider and tarantula, others are practically harmless. Besides the importance discussed above all the arachnids play directly and indirectly a vital role in ecosystem regulation. Considering the many fold importance of arachnids the quantitative and qualitative analysis of arachnid fauna in the study area is to be done, for which collection of arachnids is of first and foremost need.

Habitat

Acari (ticks and mites): Both the ixodid (hard bodied) and argasid (soft bodied) ticks live as external parasite on reptiles, birds and mammals including man. Some argasid ticks are found under bark. The ticks also live on vegetation in forests where animals move and in grassland.

Mites inhabit almost all possible habitats from the lowest intertidal zones to the highest mountains including the deserts, arctic and antarctic regions and oceans. Depending on the nature of habit and habitats seven broad groups of mites are recognised as mentioned below:

1. **Soil mites:** This group of mite occur abundantly in any type of soil and litter rich in organic matter and humus. They are common in arable land, forest soil, pastures, etc. Their population is rich on the upper 5 cm of soil strata because the layer contains a good quality of organic matter. They are cosmopolitan in distribution.

2. **Plant mites:** These mites on plants are usually found on the under surface of leaves, either in colonies or in solitary condition, in the angles formed by the midribs and other veins, on upper surface of leaves, on twigs, crevices of stems, flower buds, within galls, under bark, inside any deformed plant parts and on fruits. There are two types of plant mites *viz.*, plant feeding and predators. Some plant mites are fungivorous. They are distributed all over the world.

3. **Water mites:** They are usual inhabitants of freshwater lakes and ponds, swamps, marshes, creeks, springs, rivers, streams and ditches. Many species are known to occur in hot springs as well as in frozen tundra pools. Some groups of water mites are permanent inhabitants of marine environment. The subterranean water mites are found in sandy or gravel soil near river bank. The larvae of these mites are parasitic on various groups of aquatic insects and on some vertebrates mainly chelonia and fish. The adults of some are parasitic on the gills of freshwater mussels and snails. They are also cosmopolitan in distribution.

4. **Nest mites:** These mites occur in nests and nest debris of birds and mammals. They live there as blood suckers, predators, scavengers, coprophagous, mycitophagous, etc. They are found all over the world.

5. **Dust mites:** A varied groups of mites occur in house dust. Most of them are parasitic in habit and thereby causing allergic diseases. They are cosmopolitan in distribution.

6. **Stored product mites:** A diverse group of mites are found to attack stored products. They are most abundant in tropical countries.

7. **Parasitic mites:** A large number of mites live as parasite of invertebrates and vertebrates. Some occur on external parts of body of animals including man; as parasites of insects; occur at the bases of hair follicles, and fur; on feather of birds; respiratory tracts of reptiles and birds; within nasal cavities of seal; occur within skin; within air sacs of birds; urinary and intestinal and respiratory tracts of man; respiratory tracts of honey bees, etc.

COLLECTION OF ACARI

A number of methods are in practice for collection of arachnid specimens occurring in different habitats. But the choice of method of collection depends upon the habit and habitat of the specimens to be collected, time and place of collection and the nature of investigation to be done.

Collection for community study

The following methods are commonly used for collection of different groups of acari for community study. In community approach the main emphasis is given on the qualitative analysis of the community involving comparisons between species lists.

Collection of mite

i) **Soil mites:** The methods for collection of soil mites owe their origin in principle to methods of 'hand-sorting' or to devices developed by Berlese (1905) - the 'Berlese Funnel' and also a simple 'flotation device' described by Balogh (1948).

Sampling: The soil samples rich in organic matter and litter or plant material are collected with shovel and kept in polythene bags and tied with rubber band. It is desirable not to take too much of samples in one bag. The mouth of each bag containing samples supersaturated with moisture should be kept open intermittently. Otherwise, the mites may get trapped in the accumulated water droplets on the wall of the bag and die.

Different kind of extraction methods are clasified into two categories, *viz.*, *mechanical method* and *dynamic* or *behavioral method*.

Mechanical method: In this method animal's role is passive and separation of animal depends on nature and density of the substrate, the size, shape and density of the animal and properties of its integument.

The following two mechanical-extraction methods are commonly used for collection of mites :

a) **Direct examination:** This method is often referred to as hand-sorting. The soil and litter gently crumbled by hand and spread out on a sheet of paper or in a white enamel tray and examined with open eye or 10 x lens or under a stereo binocular microscope. The mites are picked up by a fine sable hair brush. This method is generally followed for instant collection of mites in the field. All other mechanical and dynamic methods also involve hand-sorting of animals.

b) **Flotation method:** In this method two operations are followed. First, the mineral soil is separated from the organic matter (animals + plant debris) and then the mites are separated from plant material by differential wetting. The sequence of steps is as follows:

i) The thoroughly crumbled soil sample is washed by jets of water and passed though a set of sieves from higher to lower mesh size. The larger soil particles are removed and mites along with small soil particles and plant debris are settled in the "settling can".

ii) The contents of the settling can are then transferred into "Ladell can" fitted with fine phosphor-bronze gauze.

iii) After all the water is drained out, the drain hole is plugged.

iv) A concentrated solution of magnesium sulphate (MgSO4) of a specific gravity 1.2 is then pushed into the Ladell through the drain plug (Fig.) and at the same time a stream of air is bubbled into the suspension from below. This will cause the liberated animals and the organic material to float to the surface, leaving the denser mineral particles behind.

v) The float is then filtered and collected on filter paper or on a sieve.

vi) The residue collected after filtration is thoroughly washed into beaker or flask with a jet of water. The suspension is then boiled.

vii) After the suspension is cooled, xylene or benzene is added and the mixture is shaken vigorously. The animals then accumulate above the plant material on the benzol-water interface.

The floatation method helps to recover inactive or resting stages, eggs and dead animals but it sometimes damages the minute specimens and it becomes difficult to separate the small ones particularly hairy mites from other floating plant material. The method involves a very lengthy and time consuming process.

Dynamic method: In this method active participation of the animal is involved. The behavioral response of the animals to stimulus like heat, illumination, desiccation are utilized.

The most popular kind of dynamic method for extraction of soil mite is dry funnel method.

Dry funnel method: In this method the movement of animals out of the funnel depends upon three important factors like light, temperature, desiccation and the effect of gravity. The dry funnel method was originally devised by Berlese (1905). He used hot water jacket as a source of heat. This has been modified by various workers depending upon the need and circumstances and to improve the efficiency. The most popular extractor is the Berlese-Tullgren funnel.

The funnel made of glass or galvanized sheet is fitted on a stand and a fine gauged (1.5-2.5 mm) sieve is placed in the funnel. A 40 w electric bulb with reflector is fitted over the funnel as a source of light and heat. A collecting tube containing 70 % alcohol is fitted with the stem of the funnel. The thoroughly crumbled soil samples is put into the funnel and the light is switched on. The duration of extraction period is 2-4 days depending upon the amount of moisture present in the sample. After the examination is over, a large number of mites are collected in the collecting tube. For extraction of a number of samples at a time the Tullgren's apparatus containing a battery of funnels with light source is used.

To minimize the problems in using Tullgren's apparatus which causes losses through the trapping of animals in water condensed on the sides of funnels, rapid heating and desiccation resulting less time available to the animals for moving down, etc., several workers modified the apparatus in various ways. In one apparatus a narrow space was left around the periphery of the sieve free of sample material. It was provided means for adjusting the amount of heat. Later high gradient cylinder extractor and air conditioned extractor were developed. In the first one the temperature and humidity are controlled by an electric heater and a cold water-bath is maintained beneath the samples. In the second extractor cool air is circulated around the funnels and the upper part of the apparatus is heated in the usual way.

Sometimes extraction of samples in the field is needed. In such cases Expedition funnel or portable funnel is the most convenient one. The apparatus is specially designed to fold into small piece and paraffin stove or hot water placed over the funnel is the source of heat.

ii) **Plant mites:** The following methods are usually used for collection of plant mites.

a) **Hand-sorting:** By observing the colour and deformities of the leaves the infested ones are identified and directly examined the niches of the mite on the leave under 10x lens in the field and under stereo bionocular microscope in the laboratory and mites are collected with a fine sable hair brush moistened with alcohol. Atleast 30 leaves or plants are to be thoroughly examined to have representative collection.

b) **Sweeping:** The Insect collection net is swept through vegetation and a large number of mites are trapped on the inner wall of the net. The mites are then collected by brush.

c) **Beating:** Beating is usually employed to dislodge mites from foliages. Usually a white enamel tray covered with cotton pad on the inner surface is placed below the portion of the plant from where the mites are to be collected. The plant parts are betted with a stick for 15-20 times. The mites are dislodged and after falling on the cotton they get themselves entangled. The mites are picked up by a fine brush.

d) **Brushing:** The affected leaves are brushed by brushing machine at least for twice. The mites are dislodged into vaseline coated plastic discs from where the mites are collected with the help of fine brush.

e) **Aspirating:** Small active mites are collected by a sucking tube or aspirator, straight from the plant into the collecting tube containing alcohol.

f) **Scrapping and Teasing:** The inflorescence and other infected plant parts are scrapped or galls are teased gently, the mites come out and can be collected by fine brush.

g) **Flotation method:** The infected leaves are kept in a jar containing water and teepol (a detergent) and shaken vigorously for several times. The mites are dislodged from the leaves and float on the surface of the mixture which is then filtered. The residue containing mites is kept in watch glass and washed with alcohol for separating the mites. The mites are then sorted out and collected by a fine brush.

h) **Heat desiccation method:** Mites from infected leaves are collected by using Tullgren's Funnel apparatus. The details of the procedure is discussed earlier for soil mites.

i) **Special methods for collecting eriophyids and other gall forming mites:** The galls are gently opened out and put in an open glass jar and kept it in an airy room away from direct sunlight. The galls are gradually dried, the mites come out and start crawling on the inner wall of the jar, which is previously wiped with glycerol 5 cm below the mouth the jar to avoid escape of mites. Later warm chloropicric acid is poured in the jar and shaken vigorously for sometimes. The jar is kept until the plant parts get settled. The liquid is decanted and filtered and mites are collected from the residue on the filter paper with fine brush.

iii) **Water mites:** The following methods are commonly used for collection of water mites.

a) **Handpicking:** The parasitic aquatic mites attached to the body of aquatic host is located with hand lens and are collected with fine brush.

b) **Dipping:** In most cases water mites are collected by this method. The collecting tools like white enamel bowl and wide mouthed pan with long handle are immersed in water and lifted with

water with a quick movement. The water sample is then observed under binocular microscope and the mites are collected with fine brush.

c) **Netting:** The free living water mites are collected with great success with nylon nets of narrow mesh fitted with an iron ring having a long handle. The visible water mites can be scooped up with the help of this net. Another type of net, the Birge net, is also used for collection. A fine mesh nylon net fitted with a fine sieve at the wider mouth of a funnel, the narrow end of the funnel is open and a long wire is attached to this end which helps to put the net into deep water. The contents of the Birge net are examined with hand lens and mites are collected with a fine brush.

iv) **Stored product mites, Nest mites, Dung mites, Lichen mites and Moss mites:** Heat desiccation method as discussed earlier for soil mites is the most suitable way to extract mites infecting stored products, birds and mammals in nest and living in dung, lichen and moss.

v) **Dust mites:** The dust mites are collected in the following steps :

First Step- A small quantity (2-3 gms) of dust sample is mixed with kerosene oil in a beaker and the solution is stirred slowly with a magnetic stirrer for about 15 minutes. The mixture is filtered through a 500 micron sieve.

Second step- The material on the sieve is washed in a beaker by kerosene oil jet and filtered with a 500 micron sieve.

Third step- The filtrate recovered from first and second steps are mixed and is filtered through Buchner funnel. The mites are collected from filter paper with fine brush.

Fourth step- The sediment formed during third step is washed with kerosene oil and carbon tetrachloride (1 : 3) (sp. gr. 1.4) and centrifuged and the mites are collected with fine brush. Lastly the sediments are mixed with para carbon tetrachloride (sp. gr. 1.6) and the process is repeated. However, most of the mites are recovered in third and fourth steps.

vi) **Parasitic mites:** The following methods are in use:

a) **Direct examination method:**

1) The infested invertebrates are directly examined by hand lens or under a stereo binocular microscope and mites are collected by fine forceps or brush.

2) The host may be kept for 2-4 days in a screen cage over a pan of water. The parasites will drop from the host into water after feeding.

3) The living or dead animals are combed or brushed over a tray or paper. The mites, if present, fall down and are collected with a moistened brush.

b) **Light trap:** The light trap is placed on the ground. The free unengorged larvae of trombiculid mites are collected with the trap.

c) **Application of detergents/repellants:**

1) The carcasses of birds, small rodents, etc. are submerged in soap solution and then shaken vigorously. The ectoparasitic mites are detached from the hosts and can be collected from decanted washing with brush.

2) The host animal particularly bird is kept in a cylinder with its head and neck protruding through a hole in the upper bid. The repellents like chloroform fumes or dry dye or pyrethrum is applied in the cylinder and the bird is encouraged to flutter. The mites from the host's body drop off on the cloth or paper kept at the bottom of the cylinder. The mites are collected with fine brush moistened with alcohol.

d) **Flushing:** The mites infesting nasal passages of birds, reptiles and mammals can be collected by flushing water or soap water through one of the nostrils. A 20 gauge needle is cut off 2 mm from the base and the end is grounded to a smooth rounded tip and fitted to a 5 ml syringe. The dead animal is firmly grasped by the throat in order to close the oesophagus or trachea. Plane water or soap water is introduced with high pressure into one of the nasal passages with the syringe. The water comes out from the other nostril contains mites which are collected by fine brush.

e) **Autopsy:**

1) The lung infecting mites can be collected by dissecting the organ of the dead host and the parasites are picked up with the help of fine forceps or brush.

2) Spliting of the bills of dead host between nares also facilitates the recovery of parasitic mites.

f) **Application of acaricides:** The skin parasitic mites may be recovered by Hopkin's dissolution method. Fresh or dry skin pieces are kept in 5-10% KOH or NaOH solution over a water bath till hairs dissolve completely. The contents of the beaker is immediately filtered through fine stainless steel gauge. The residue on the gauge is then washed well in a petridish and the mites are collected by brush. To make the collection more easy the residue is treated well with ZnSO4 solution and the mites float on the surface of solution.

Collection of Tick: The following two methods are usually followed for collection of ticks.

a) **Hand picking method:** Tick specimens are directly removed from the host body with the help of fine forceps.

b) **Flag dragging method:** A white or yellow lint cloth about one meter square attached with a long stick is dragged repeatedly on forest vegetation, humus and grassland. The questing ticks get attached with the fibres of the lint cloth. Flag is examined at frequent intervals and specimens are carefully picked up by fine forcep or brush and kept in vial containing 70% alcohol.

COLLECTION FOR TROPHIC STUDY

In trophic study a greater range of related species is encountered and distinctions between them are often critically important in detecting changes in community composition. This study requires more accuracy in sampling and extraction.

Sampling and extraction of Soil mites:

Soil samples are collected by a stainless steel soil corer having core size of 7 cm diameter and 7 cm depth, which helps to maintain a definite sample size. The core containing soil is placed inverted i.e. upside down in the Tullgren funnel apparatus for extraction of soil mites . The floation method is also effective in

extraction of soil mites for quantitative study. For collection of mites from litter a wooden rectangle is placed and litter is collected from the area surrounded by the rectangle. The surface area and depth also should be recorded.

Sampling of Plant Mites:

a) **Direct counting method:** The leaves (30-40/plants) are sampled and put into a petridish containing cotton wool soaked in chloroform to immobilize the mites. The leaves are then examined under stereo binocular microscope one by one and mites are counted and collected from the entire leaf or from a definite leaf area (basal, middle and upper part) depending upon the intensity of population and dispersion pattern. According to some workers comparing the plotted values based upon actual counting of mites with the number of leaves having no mite, one can have a rough estimate of mite population.

b) **Imprinting method:** The under surface of leaf placed on a Whatman filter paper No. 1 and a wooden roller is rolled rapidly with pressure sufficient enough to crush the specimen and characteristic stains are formed on the filter paper making permanent record of the population. Later the spots are counted and analyzed. The stains of adults and eggs are also possible to differentiate.

Sampling of Ticks: The population study of ticks can be done by employing flag dragging method for a definite period (1-2 hrs.) covering a unit area.

PRESERVATION OF ACARI

Acari: All mites and ticks excepting eriophyid mites and water mites can be preserved in vial containing 70% ethyl alcohol. A few drops of glycerine may be added to alcohol to avoid of drying of specimens. For prolonged preservation Oudeman's fluid (Glycerol-5 parts, Ethyl alcohol (70%) - 87 parts, glacial acetic acid - 8 parts) is used.

Special method for Eriophyids: This mites are better preserved in dry condition by wrapping the infested leaves/galls in tissue paper. These leaves or galls when simmered in Kiefer's preparatory solution (Resorcinol - 50 gms, Diglycolic acid - 20 gms, Glycerol - 25 ml, some iodine, water - 10 ml.) the mites are brought to normal condition.

Water mites: Alcohol and formalin make the water mites brittle. To avoid this problem the water mites are preserved in the Koenike's fluid (Glacial acetic acid - 10 parts, Glycerine - 45 parts, water - 45 parts).

The vials containing the specimens are to be plugged with cotton wool and are kept in inverted position in a jar. The jar should always contain enough alcohol so that the specimen vials remain in fully immersed condition. The vials and the jars should be properly labelled giving necessary collection data.

17. ECHINODERMATA

INTRODUCTION

Echinoderms are a fascinating group of animals. They are exclusively marine and occure in every habitat from the intertidal zone to the bottom of deep sea trenches. As a rule echinoderms are pentamerous. They have two unique features which are not found in any group of animals. The first one is the presence of water vascular system and the other is the possession of tentacle-like structures called tube-feet. Both of these help the animals in locomotion and food gathering. They have no heart, brain or eyes. Various forms may be carnivorous, omnivorous, herbivorous, detrital foragers and planktonic and suspended sediment feeders. Although they are most highly evolved among the invertebrates, yet they show primitive characters like radial symmetry, regeneration and a sexual reproduction. Because of their hard parts their fossil history is well documented. Sexes are separate. Larval forms are interesting. Some of them show marked similarly to the larvae of Protochordates. There are no parasitic echinoderms and no microscopic animals in the group. At present more than 6000 species of echinoderms are living in various seas of the world. In the seas around India about 800 species are known, most of them are collected from deeper waters.

COLLECTION

Echinoderms chiefly live in association with the coral reefs. The best way to collect them is to look for them during low tide in the interdial region by turning over the coral stones. Most of them are fugitive and cryptic in nature. Very rarely we get smaller forms in intertidal region. As they grow older they move on to the coral reefs. Some of them live in the coral crevices. Some species of sea urchins make neat holes in the rocks by mechanical means. Collections can be made with added advantage during the night with a petromax lamp since all the echinoderms are nocturnal in habit. They can be collected by snorkelling in the shallow waters. Deeper water forms can be collected by using SCUBA. Good collections can be made by examining the cod end material of the trawlers. Night trawling is likely to yield more number of interesting forms. Algal collections can be repeatedly washed in fresh sea water to collect small brittle stars and sea cucumbers. Scrapings of boat bottoms and other structures installed in the sea will yield very interesting material. Dredge collections are extremely useful since some of the species never occur in the intertidal region.

PRESERVATION

Proper preservation of the material collected is most important otherwise all the efforts taken to collect them will go as a waste. All echinoderms have calcareous plates and sea cucumbers have microscopic calcareous spicules. Therefore they should never be preserved in formalin which is always slightly acidic in nature. If they are preserved in formalin the spicules will dissolve rendering the identification of the sea cucumbers impossible. The calcareous plates of asteroids, ophiuroids, echinoids and crinoids will also drop off if they are kept in formalin for a long time. It is most essential to keep a proper record of the exact place of collection, depth and habitat of the animals which will help in the identification of the species. Without precise locality label the specimens will lose their scientific value. It is often possible to identify the animal if we know the place of collection. Echinoderms should always be preserved in rectified spirit

only. Asteroids, ophiuroids, echinoids and crinoids should first be immersed in fresh water to kill them. It will just take a few minutes to kill echinoderms in fresh water. Those killed in the fresh water also retain the colour for a long time. After they are killed they are taken out of water and put on a towel to get the water absorbed. When specimens are free from moisture content they are transferred to rectified spirit with proper labels. In case of echinoids the peristomial membrane can be punctured to allow the coelomic fluid to run out of the body. When once they are completely drained of the water, rectified spirit is injected into the body cavity of the echinoids with the help of a hypodermic syringe. This will help to preserve the viscera and gonads. If the rectified spirit gets diluted due to the presence of the water in the animals they can be transferred to fresh rectified spirit. If this is not done and the rectified spirit gets too diluted the animals will putrify. They are put in containers with firm lids which will not allow the rectified spirit to evaporate. Sea cucumbers should not be killed in fresh water like the other groups. They should first be narcotised by putting them in large bottles with fresh sea water. To this menthol crystals are added. The bottle is covered with black cloth and put in a dark place and left in that way overnight. This way the sea cucumbers will expand completely putting out the introvert and tentacles in the extended condition. The nature and number of tentacles are essential in the identification of the sea cucumbers. The next day the sea cucumbers are taken out of the bottle and put on a clean towel for the water to be absorbed. A small cut is made at the posterior end of the body to drain out the coelomic fluid. Normally during narcotization the animals will eviscerate. The details of the respiratory trees should be noted. Rectified spirit should be injected into the body cavity of the sea cucumber, otherwise they start decomposing. The specimens so preserved are kept in safe custody for later identification.

VERTEBRATES

18. PISCES

INTRODUCTION

Fishes are the most numerous of the vertebrates, comprising, more than half the number of recognized living vertebrate species. Ranging from a few mm to about 70', from typical torpedo shape to flat and bizarre shapes, from free living, commensal to parasitic mode of life, they are known to occur everywhere from below freezing Antarctic waters to hot springs of more than 1000 F and from soft freshwaters to very salty waters, in sunlit mountain streams to dark deep abyssal depths, their vertical range of population exceeding that of any other higher forms of animal life, from approximately 3 miles above sea level to nearly 7 miles beneath it; it is really no wonder that there are so many different kinds of fishes taking into account their antiquity (their presence on earth outdates all other vertebrates by more than 100 million years).

The prime task of any such studies is the collection, preservation and identification of voucher specimens from all habitats using all possible collection methods. Some knowledge of the natural history and ecology of the study organisms is required when looking for a particular species. The field crew should understand the general physical and biological principles that affect fish aggregation, activity and habitat preference etc.

India harbours about 11.5% of the fish fauna known so far in the world. Being the staple food of the masses, fishes are of great economic value and several indigenous boats and fishing gears are employed in their capture. Basically two categories of gear types are employed viz. the active and the passive, the former is moved through the water body while the latter is left stationary for a time along the water course. Fishing nets that are employed are basically of the scooping, enclosing or entangling type. The active gears commonly used in inventory surveys include seine nets, trawl nets, cast nets, dip nets, and the hook and line. The commonly used passive gears include gill and enmeshing nets and trap nets.

Before collecting fish from any region a worker should obtain written permission from the competent authority. Since the collection methods given here are mainly intended for a fishery biologist or systematist or a research student, the various collecting crafts and gears are not dealt in detail.

MARINE FISHING CRAFTS AND GEARS

Marine fishing craft

Crafts or boats used in the east and west coasts of the Indian seas are varied. On the East Coast are found the *catamarans, marula boats, dinghis, nanku* and *Tuticorin boats*.

Catamaran is a primitive raft constructed by tying together several (3-7) curved logs and are shaped like a canoe with a cone shaped end rising above water level and forms the point from which the rudder is controlled. In different areas along the coast, according to the number of logs tied together, they are variously known as the *Coromandel type* (probably the original type), the *Kolamaram* (used in Nagapatinam), the *Orissa* and *Ganjam* type, the *Andhra* type and the *Boat-Catamaran* used around Mandapam areas.

The ***marula*** boat is made of planks sewn together with coir ropes and usually without frames or ribs. The variations of this boat include the *Bar boat* in Orissa, *Padava*, or *Padugam* in Andhra and a ribbed type used in Kakinada and Masulipatnam. *Najka* and *dinghi* are carved and well designed large boats of Orissa and West Bengal. *Tuticorin boat* is also a carved model employed in inshore waters, more as cargo boats. On the West Coast, the types of boats are different, to suit the different conditions. Chief among them are the *dug-out canoes, plank-built canoes, outrigger canoes* and *built up boats*.

The large sized *dugout canoes* are known as *Vanchi* or *Odam* and the smaller ones are known as *Thonies*, employed for gill net or drift net and for seining.

Outrigger canoes, locally called *Rampani* since they are used for the casting of the *Rampani* net used for mackerel fishing in the Kanara and Konkan coasts.

Built-up boats are the most highly evolved of the indigenous fishing crafts and deviations from the typical structure are variously known as *Machwa* with a broad hull, pointed bow and straight keel and the *Satpati* or *Galbati* with a medium pointed bow and broad beam.

MARINE FISHING GEARS

This includes mostly nets of various sizes and shapes and are basically of two types, the stationary or fixed and the active gears. They are the bagnets, boat seines, shore seines, dragnets, driftnets, gill nets and cast nets.

Cast Nets are circular nets manually thrown to fall flat upon the surface of shallow waters. When the net sinks with the weight of the sinkers around the periphery, the fishes get trapped under it and the central string connected with the bottom line along the circumference pulls them up.

Drift Nets and Gill Nets are wall like rectangular passive gears of various sizes and meshes. The yarn may be of hemp and is held vertical by floats and sinkers.

Stationary Nets are widely employed along the tidal zones of inshore waters. They are kept in position by wooden poles or stakes and floats or sinkers are used to keep the net stretched. Nets are usually rectangular or conical in shape and vary in size. With the high tide fishes swim into the net and are trapped behind when the water recedes with the low tide. Stationary structures or barriers made of brushwood, poles or planks trap a variety of species.

Bag Nets are conical in shape, without flanks or wings. They are used in waterways with currents strong and high enough to keep the net in an expanded position. The mesh size usually increases from the bag position towards the flanks. Those with long tapering flanks are known as *Iraga valai* in Andhra coast or *Thuri vala* in Tamilnadu and *Kolli vala* in Kerala, specialised for oil sardine fishing. The nets are operated from two boats or catamarans while one canoe drives the shoal into the wide gap of the net. Along the Bombay and Gujarat coasts, a bagnet known as *Dol* is extensively used. It is a long conical net with a wide mouth fixed by bamboo poles or stakes and the tapering end is held on to a boat. The net is kept straight and expanded during strong and high currents and fishes are trapped in it.

Shore Seine is basically a conical bag with two wings or rectangular in shape and are called *Berjal* in Orissa, Pedda or *Alivi vala* in the Andhra coast, *Periya vala* or *Mada valai* in the Coromandal coast and *Kara valai* in the Gulf of Mannar. The *Rampani* of the Konkan and Malabar coasts extensively employed in mackerel fishing is the biggest shore seine net of the Indian seas. While one end of the net is fastened to

a heavy stake on the shore, the other end is attached to a boat which makes a semicircular sweep and is brought back to the shore filled with trapped fish pulled by the ends by a group of men. The Rampani of the Konkan and Malabar Coast extensively employed in mackerel fishery is the biggest shore seine of the Indian seas.

Hooks and Lines are employed from boats in the sea using suitable baits on hooks. Chain hooks are used for catching large fishes like sharks, perch, tuna etc.

INLAND FISHING CRAFTS AND GEARS

Crafts include **rafts** and **boats**

Rafts are made from various indigenous material like stems of banana tied together to form a floating platform and is a primitive type of boat used in Tamilnadu and West Bengal. In Bihar and Tamilnadu earthern pots are tied together to support a light platform of bamboos. Early men used buffalo hides to make simple rafts. In the upper reaches of Ganges and in Kaveri and Tungabhadra a light raft made of cane or bamboo is covered with animal hide and used as a boat. *Dongas* are small dugout canoes made by hollowing out the stem of a Palmyra palm and is used in shallow waters and paddy fields. Regular boats are strong and sturdy built from planks and is used in large lakes and backwaters and rivers with strong currents. *Dinghi* of varying size are employed to operate purse nets and dip nets. These boats have no keels but are narrow and have a tapering bow and stern. The *Chandi nanka* is wider and used in operating drift nets.

INLAND FISHING GEARS

Owing to the vast and varied freshwater habitats, diverse nets are employed of which a few are mentioned here The most useful tool for collecting small-sized species in most waters is a dip net with a very fine mesh-size, 1.5-2.0 mm and a convenient frame size of 50-70 x 40-50 cm. This net is very useful for collecting benthic fishes from streams and also to make collections from dense vegetation among roots of floating and marginal plants. However for collecting species from larger, open water bodies the following nets are used.

Cast Nets: Most suited for fishing for surface dwellers and shallow water species. Large mesh sized nets used for capturing large fish, sink down faster and trap more fish. The only disadvantage is the presence of detritus, vegetation, and large boulders, which hampers the collections, causing destruction to nets also.

Seines: Used for collecting fishes from still or slow flowing open waters. It is a wall of net with floats and sinks for encircling a known area of water. *Tied bag seine* is usually 25 or 35 ft long, 6 ft deep, sides or wings of 0.5 in sq. mesh the bag trailing is 6 to 8 ft of 0.25 sq in. mesh, with float and float line with sufficient buoyancy to assure that it will not submerge when the net is being drawn through water, lead line “double leaded” to make certain that it will stay on the bottom when the seine is being pulled. In soft bottomed quiet waters extra leads may be omitted. Perishable parts of the gear should be protected from decay by treatment with suitable twine preservative (*e.g.* Copper napthenate). *Tied straight seine* is 10 ft long, 6 ft deep with a mesh size of 0.25 sq in. Hauling is done carefully to prevent fishes escaping from the mouth of bottom of the net. The advantages are that seines can be designed to suit the water body of known depths. It also can be made in detachable sections and with different mesh size. The disadvantages

are the disturbance to the habitat sampled and the sediments, gravel and macrophytes which gets swept in the net, causing injury and damage to the fish fauna.

Traps: Some kinds of traps are useful for sampling stream fishes and the success of the traps depends on fish movements or migrations. They range in size and portability from small minnow traps to semi-permanent weirs. *Been Jal* is a bagnet set against tidal current. It is tied to bamboo poles or stakes to keep it open, floats are tied to the lateral wings and is operated in deltas of rivers to capture small sized fishes. Estuarine gears include *Khal Patta Jal* of the Sunderbans, *Janos* of the Chilka lake and *thattuvala* of Kerala backwaters, wherein portions of lagoons are enclosed by bamboo screens and when water recedes at low tides, cast nets or dipnets are employed to capture the trapped fishes. *Shanglo Jal* is a pursenet operated from dugout canoes in the upper reaches of estuaries. *Karal* or *Katla Jal* is a drift net made of thick cotton with a mesh size of 13.7 – 15.2 cm. A series of bamboo floats (about 12) are suspended by means of ropes and operated from a canoe.

Gill Nets are passive traps to entangle mobile fish in fairly calm waters, the nets are kept in a vertical position by floats and sinks. Fish caught by this method often die while being caught. *Chhandi Jal* is a drift gill net in vogue in estuaries in West Bengal especially employed in Hilsafishing.

Electrofishing: Electro fishing is a means for collecting fish that is of particular value in streams and to a limited extent in standing waters. It is however not detailed here since this is prohibited in India.

Poisoning: Poisons are sometimes used in collecting fish. The ichthyocide most commonly used are powdered roots of *Derris* sp., the seeds, leaves or other plant parts with rotenone content in a concentration lethal to fish i.e.in a concentration on one part per million parts of water (*e.g.*1 lb. of powder containing 5% of rotenone to each million pounds of water). The mixture is harmless to warm blooded animals, but kills fish in a matter of minutes at 700 F and up, requiring longer times at lower temperatures. It must be used with extreme caution in running waters because the toxic wave will sweep downstream for some distance before becoming diluted below the danger point.

Some of the indigenous plants employed as poison agents are the fruits of *Randia brandisii* (Tam.Kadukkai), 5-6 kg used for killing medium sized fish when water level is low in summer. The climber *Acacia torta* (Thodan valli) when crushed in water develops a surf and when mixed with water destabilises the fish in half an hour. The tender shoots of bamboo (Mula or Illy) (50-70 shoots) are crushed, mixed and added. The bark of the tree *Zyzigium caryophyllaeum* powdered and mixed into the stream, the fish are quickly sedated and can be collected using traditional methods. Also the sap of *Euphorbia* (Thirukalli) plant is poisonous to fish. The plants are cut and put on the surface of stagnant water to poison fish.

Other Methods: Besides the foregoing commonly employed means there are many other ways to collect fish including the diversion of streams, forming temporary bunds and from under stones in the partly dry area, small loaches and balitorids can be collected. Sometimes small impoundments are made and the fishes present in the small area with a fairly good representative species could be collected with small hand nets. Also by the drainage of ponds or pools fishes can be collected. Fishing with small scoop nets, sieving with cloth or mosquito nets in shallow littoral zones are sure to yield good results. In some pools explosives are also used which act should be refrained from. The primitive bow and arrow, spear and harpoon, each has its place, as has a variety of devices such as "Arbalete" an underwater spear gun for use while diving. For capturing fry, fine mesh nets have been used; to take fry and eggs from bottom nests, a suction tube is used.

However it is imperative to remember that while making collections, permissions need to be sought from the competent authority and only one or two representative specimens of the species should be collected. In the case of other species that do not fall into this category of threatened species a few more specimens can be collected. The threatened or endangered species are mostly the large carps viz. the *Hypselobarbus* spp., Tor spp., *Barbodes* spp. etcand the hill stream fishes found in the upper reaches viz. the Homalopterids and those species known to be endemic to a particular stretch of a single river system.

PRESERVATION

To ensure the quality of the collected fish or tissue, careful and correct preservation procedures are very crucial as also of importance is the correct containers and waterproof data labels.

Anaesthetizing to Kill

All fish must be killed prior to fixation which can be done by leaving the fish in high doses of the anaesthetizing solution. This is not only an ethical treatment of a live animal but is also to our advantage in that an anaesthetized fish relax and hence can be preserved in a more natural state.

Fixatives

Whole fish

Formalin is widely used as preservative and is available in liquid or powder form and the most ideal concentration is a 10% solution. Marine fish, on the other hand, are preserved in 2 to 4% formalin in filtered seawater. For larger fish a slit is made along the right side of the body to allow penetration of formalin into the body to preserve the internal organs. In some cases formalin is injected into the body. Commercial formalin may contain dissolved impurities, such as iron and formic acid, which may cause disintegration of parts of the fish. When impure formalin is used, iron comes out as a brown flocculent precipitate, which can be prevented by adding Rochelle salt (sodium potassium tartrate) at the rate of 10g salt to 1 litre of commercial formalin. A 10% solution of buffered formalin is prepared by combining one part full strength formalin with 9 parts distilled water and adding approximately 3 ml of borax (Sodium borate, buffering solution) per litre of solution.

Formalin is slightly acidic and will decalcify and soften bony structures, which process can be slowed down by the addition of a buffering agent. Fish also tend to change in weight and length and undergo discolouration, over time.

Alcohols such as ethanol and iso-propanol are poor fixatives and not recommended for preservation but are better suited for skeletal structures such as otoliths.

Another alternative for preserving specimens especially to preserve the natural colour and for tissue studies and genetic sampling, is to quickly freeze them in dry ice or liquid nitrogen. However specimens frozen without initial preservatives tend to fall apart, when thawed and hard to accomplish and maintain on long field surveys.

Disposal of Formalin

Formalin must be oxidised to formic acid prior to disposal in the sanitary sewer as an aqueous waste. For each 10 ml of formalin (10% solution) 25 ml of household bleach should be added slowly while

stirring and for at least 20 minutes before pouring into the drain with at least 50 times its volume of water. Protective gloves, clothes and eye protection is mandatory when working with formalin. (Procedure: from Armour, Browne, and Weir in "Hazardous Chemical Information and Disposal Guide, Dept. of Chemistry, University of Alberta).

Precaution

Formalin has a powerful and irritating smell, is carcinogenic, causes allergic reactions on prolonged and repeated exposure and is extremely painful on cuts and wounds. Specimens stored in formalin or alcohol which are classified as toxic and dangerous substances must be identified and labeled with Workplace Hazardous Materials and Information System (WHMIS) labels, an up-to-date Material Safety Data Sheet (MSDS) for each substance should accompany field crews. This precaution has so far not been made mandatory in India.

19. AMPHIBIA

INTRODUCTION

Amphibians are cold blooded (poikilothermic) vertebrates, living both on land and in water. The word 'amphibia' is derived from two Greek words namely amphi meaning 'both and bios meaning 'life'. They have soft glandular skin, devoid of scales, hair and feathers as seen in the higher vertebrates. The amphibians originated from primitive fishes and they were the first vertebrates to terrestrials about two hundred and fifty million years ago.

Amphibians exhibit a wide range of adaptive radiation suited to their habitats and modes of life viz, burrowing, aquatic, semi-aquatic, terrestrial, arboreal and sub-terrain. Being cold-blooded animals amphibians thrive well in temperatures ranging from 20-30 degree centigrade and relative humidity 50-70%, hence they are considered indicators of pollution and disturbance to the habitat (since they are the first to disappear from a deteriorating habitat). Unlike other warm blooded animals, the amphibians are incapable of regulating the body temperature internally, as a result they spend most of the time near cool, moist and shadowy areas. They lay eggs either in water or in moist surroundings. In the adult, respiration takes place either through lungs, through gills, through the skin or mucous membrane lining the mouth and pharynx or a combination of these. As for the physiological adaptation and the habitat suitability like water bodies (ponds, lakes, streams, nallas, rivers, marshy areas) and land. Most of the distribution of amphibian species is restricted to the warm and humid tropical parts of the world, with some exceptions to life in dry deserts and high latitudinal ranges.

The most important characteristic of amphibians is that they undergo complete metamorphosis from egg to adult stages. With the exception of caecilians all amphibians have four distinct following stages in their life histories.

An aquatic or terrestrial egg stage,

A free-living aquatic tadpole or larval stage (with a few exceptions)

A terrestrial or partially aquatic young frog, toad or salamander stage and finally

The adult stage that can reproduce.

Except the worm-like caecilians the amphibians are known to produce six different type of calls *viz*. Release calls, Warning sounds, Rain calls Screams, Territorial calls and the Mating calls, the last to attract the mate is the commonest.

COLLECTION

Knowledge about the habit and habitat of the amphibians will be very handy to collect them since, their place of occurrence is limited depending on their living patterns.

The day active (diurnal) aquatic forms are collected by an ordinary aquatic net fixed on a long handle, the nets should have a sturdy wooden handle. The terrestrial forms are caught by hand or net. Arboreal forms are difficult to locate because of their camouflaging nature. Tree trunks, leaves and tree holes and bushes

should be searched. Burrowing forms have to be dug out from under the soil using garden trident scrapers. They could be located by the help of faint telltale marks left on the soil surface.

Nocturnal forms are easier to be caught by using head lamps, torchlight, searchlight or petromax light. Adult male frogs and toads will be easily located in the field by listening to the calls they make.

Tadpoles and smaller forms are collected with the help of aquatic dip net. Tadpoles are found in stagnant or running water, and some species are found only on the moist surface of rocks where they grow and metamorphose. Care should be taken to collect the various stages of tadpoles including two-legged, four-legged and metamrphosised ones. This will help in fixing the identity of the tadpole.

Since the colour of the specimen is lost by preservation it is important to note the colour in live state onsite. Shape of the pupil and the exact habitat should also be recorded in a filed notebook. Colour photos of the live specimen will be very useful for identification.

Equipment for collection

A 30 cm. and 12 cm. Long forceps, a pair of scissors, syringe with needle, polythene bag, absorbent cotton wool, mulmul cloth, an aquatic dip net with a long handle, chloroform or Benzocaine, ethyl alcohol, formaldehyde solution, plastic containers of various sizes, a torch or petromax light, a good single-lens reflex camera with macro, close-up lens and flash, unidirectional mike with recorder, thread, labels and field note book is the basic necessary equipment and field note book in the basic necessary equipment.

Preservation

Small specimens can be killed in 4% formaldehyde solution. A small incision on the abdominal wall would ensure a better fixation of viscera. Larger specimens should be anesthetized by using saturated solution of Benzocaine or Chloroform and 6% formaldehyde solution injected into the visceral cavity. Individual specimen should be wrapped in cotton wool in a desired position for setting. The specimens may be transferred to fresh 4% formalin solution and rinsed well. The preservation will be made in 70% ethyl alcohol. Stronger alcohol would make the specimen shrink considerably.

Caecilians are better preserved in straight position. Since the number and position of various rows of teeth in the buccal cavity is of taxonomic significance for species conformation, it would be desirable to keep the mouth open by the help of the cotton plug or cork piece.

Packing for dispatch

If a live adult frog is to be sent to a laboratory, place it in a plastic zip lock bag with a damp paper towel. Ensure to fill the bag with air, the preserved one after fixation may be wrapped in cotton and mulmul cloth soaked in 4% formalin. These may be transported in polythene bag tied with thread in lightweight plastic containers.

20. REPTILIA

INTRODUCTION

The Reptiles we see today represent the remnants of the group that engaged in the most successful adventure in the history of the emergence from the waters in which animals originated and the invasion of dry land. Reptiles are clearly distinguished from fishes and amphibians by the development of the amniotic egg. Reptiles can be defined as ectotherm amniotes, as they cannot maintain their body temperature like that of birds and mammals. The invasion on the dry land and the somewhat later development of the amniotic egg opened up a whole new environment to the earliest Reptiles. Certainly everyone knows dinosaurs. The Reptiles as dolphin like ichthyosaurs and turtle like Plesiosaurs, bird like Pterosaurs flew or at least glided and may have approached endothermy. We know that these various fossil reptiles fed on plants, vertebrates, fishes and on each other. Great indeed has been the decadence amongst reptiles since the Jurassic and Cretaceous epochs, which lasted from ten to fifteen million years, when much of the earth's surface was covered with steamy jungle and warm shallow seas. Twelve whole orders and very many more suborders, families and genera have become extinct. Only four orders exist today.

1. LORICATA or CROCODYLIA: exs. Crocodile, Alligator and Gharial.
2. TESTUDINES: exs. Turtles, Tortoise and Terrapin.
3. RHYNCHOCEPHALIA: Only one living species restricted to New Zealand alone (Tautara: Sphenodon punctatus)
4. SQUAMATA: Suborder (i) LACERTILIA exs. Lizards
   (ii) Serpentes or Ophidia exs. Snakes.

COLLECTION

Reptiles are highly seasonal in appearance and behavior. Therefore, collection should be done only when they are active and accessible. Before proceeding for collection of Reptiles one must have a generalized idea about their varied habits and habitats. Since most of the reptiles are under Wild life Protection Act, one must be very careful before handling the animal. He/she must have permission from the authority to handle the animals.

Types of body forms in reptiles

1. Tetrapod in gait: exs. Crocodile, Lizard, Monitor lizard etc.
2. Trunk or middle part of body becoming hard and enclosed in body shell. ex. Turtle and Tortoise.
3. Limbless with elongated body: ex. Snake.

Locomotion in reptiles

1. Walk high or low on legs - exs. crocodile, turtle, lizard, etc.
2. Arboreal or climbing on trees – ex. Chameleon.

3. Swimming – exs. crocodile, turtles, snakes, etc.
4. Gliding through air – exs. flying lizard and snake.
5. Serpentine or snake like – exs. snakes and skinks.

Preferred types of habitats

1. Aquatic or living in water or Amphibious - exs. crocodile, turtle, marine and freshwater snakes.
2. Arboreal living on trees - exs. Chameleon, flying lizard, flying snake, Tree snake and lizards.
3. Living on barren or waste land or under boulders or in rock crevices (Dry habitats) - exs. Geckonid lizard, monitor lizard, snake etc.
4. Living in burrows- exs. monitor lizard, snakes, etc.
5. Living in dense forest - exs. Forest lizards, python, king cobra and other snakes.
6. Living in dry ecosystem like hot and cold deserts - exs. Desert lizard, desert monitor lizard, vipers, etc.

EQUIPMENTS REQUIRED

Long forceps, tongs, flexible long canes or sticks, various type of Snake hooks, Snake Tongs (Grabbers), Snake traps, Snake bags, hammer and chisel, scalpels, bone cutters, pair of scissors with long blades, strong canvas bags with string around the mouths, high top leather or gum boots, hand gloves, different types of nets, measuring tape, pair of divider, field note book, pictorial hand book for reference, glass bottles with wide mouth, tin container, absorbent cotton wool, mulmul cloth, chloroform, formalin, rectified spirit, arsenic soap, common salt, torch, hand lens etc.

COLLECTING METHODS

Snakes:

Snakes can be collected by various types of hooks. If permissible, a smart blow on the spine just below the head may be delivered to break the spinal cord and wait until it is motionless (Acharji, 1990). Head portion of the snake should not be damaged as its characteristics are important in specific determinations. Handle the snake with a forceps carefully avoiding its head. Catching a live shake is always risky. Putting the live snake in a collection bag needs extra care. After releasing the snake in the bag, the bag should be tied with strong cord. Extra precaution should be taken over the tied end of the bag and body contact should be avoided. Live snakes can be killed by exposure to chloroform vapour.

Lizards:

Those with diurnal habitats can be trapped as well as netted. In case of fast runners, shooting by means of dust shot may be resorted to. Raiding their haunts can collect cryptic and nocturnal lizards by hand.

Tortoises and Turtles:

Smaller forms can easily be collected by means of hand net; whereas the larger ones are collected either by large nets or by harpooning. Killing a turtle or a tortoise the following methods can be adopted.

(i) A blow of a hammer delivered on to the neck to break the cervical vertebrae.

(ii) By injection of strong formalin or strong solution of chloral hydrate in the body cavity by a hypodermic syringe, or

(iii) By introducing a wad of cotton soaked in Prussic acid (Hydrocyanic acid) in to the retracted cloaca of the animal.

Crocodiles:

Crocodiles come on land for basking or catching their prey like Turtles and Tortoises; smaller crocodiles can be easily netted and by harpooning we can collect the larger ones. Trapping is hardly possible.

PRESERVATION

The most important criteria for good preservation are that the fixative or preservative should reach each organ of the animal. The scale-covered, hard skin of reptiles is impermeable to preservatives. So the animals are fixed by injection in the hypodermis. Fixatives: Formalin, FAA (Formalin -Alcohol -Acetic acid) and Alcohol.

Snakes:

Small specimens of snakes are preserved directly in 70% alcohol. For medium sized specimens, an incision is given in the belly before immersion in ethyl alcohol. For large specimens, is immersed in 4-5% formaldehyde solution or 90% ethyl alcohol. Snakes are injected at 4-5 cm intervals along the whole length of belly and tail. Moderate pressure at the base of the tail of a freshly killed snake everts its hemipenes (Khan, 1996). Fixing a snake involves coiling its body after injection according to the size of the container. Sometimes large specimens are skinned and preserved dry.

Lizards:

Small lizards can preserve in direct 70% ethyl alcohol. For medium size an incision can be given in the belly and for large lizards required injection in the limbs, and removal of gut from the body or a cut in the mid abdomen of lizard helps penetration of 4-5% formaldehyde or 70% alcohol. Sometimes the large specimens are skinned and preserved dry. Dead lizard is placed belly down, in the fixing tray. Its legs are folded in natural position, soles facing down and digits are evenly spread. The tail is bent on the left side. The cloth is folded over the animal and sprinkled copiously with formalin and left for 6-12 hours.

Tortoises and Turtles:

For smaller specimens 8% formaldehyde solution is injected in to the body in sufficient quantity till it starts oozing out of the mouth. Then the specimens are dipped in 4-5% formaldehyde solution for two or three days. For larger specimens incision may be given in the axilla and groin region to allow the fluid to gain access to the organs. Soft shelled turtles may first be preserved in formalin by the above method and

after a few hours washed in water and then transferred to 70% ethyl alcohol. By this procedure the colour pattern of the body will be retained. Large specimens are skinned, treated with arsenic soap and preserved dry.

Crocodiles:

Juvenile specimens not exceeding 30 cm. may be preserved in 70% ethyl alcohol, after making an incision on the belly. Larger specimens are preserved dry. For this body of the animal is placed upon its back and an incision is made on the skin of the belly in midline, backward to the tail tip. This is followed by four cross cuts from the median line to each of the limbs down to the toes. The skin is taken out from the body except the soles, which remain intact with the skin. The skin is cleaned of all fat and flesh and dried in sun for further processing in the laboratory. Before this, common salt is rubbed throughout its inner surface. The skull and skeleton are cleaned and preserved dry.

21. AVES

INTRODUCTION

Class Aves includes all birds and the study of birds is termed as 'Ornithology.' Study of comparative anatomy and fossil evidences lead to the generally accepted belief that birds are derived from primitive reptiles. The bird skeleton shows many reptilian characters. T. H. Huxley said that birds are "glorified reptiles". Therefore many zoologists have preferred to combine birds with reptiles in a single class known as Sauropsida. During 1861 a fossil of 150 million years old animal was discovered in Europe, which gave convincing clues about the ancestors of birds. The fossil was named *Archaeopteryx* that means primitive winged creature, as they share reptile-bird characters. Hence it is regarded as a connecting link between the two. Presence of scales, thumb bones on their legs and egg laying habit are the reptilian characters retained by the bird.

Important characters of a bird: Shape, size, weight and most of the internal body parts of a bird are adapted for the purpose of flying. The adaptations for flight are the Spindle- shaped body; mobile neck; short tail used as a steering organ and an organ of balance or rudder; fore limbs modified as wings and presence of hind legs only; presence of feathers, scales and claws as exoskeleton; endoskeleton fully ossified, spongy and light but equally strong; fusion of vertebrae; bones are rod-like, stout and pneumatic; absence of urinary bladder, hence excreta are in the form of semi-solid state; oviparous habit; well developed pectoral and all other muscles associated with flight; powerful heart with four chambers; higher percentage of haemoglobin; homoeothermic nature; presence of internal air sacs in the body; well developed sight and hearing.

Feathers and ability to fly are the essential characters of a bird but there are some birds like Emu, Ostrich and Penguin, have lost the power of flight through a long disuse of their wings. Penguins cannot fly in the air but they can '*fly under water*' with the help of paddle like wings. They can swim at a speed of 36 kms per hour, which is the normal speed of flight of many birds.

COLLECTION AND PRESERVATION

Bird identification up to subspecies level is difficult. In such cases, one has to collect the bird specimen for scientific study. For research, study and display in museum, birds are to be collected, with proper permission from the concerned authorities. Shooting of birds is strictly prohibited under Wild Life (Protection) Act 1972 (as amended up to 1991). Keeping the birds in captivity also warrants permission. Thus it is advisable only to 'shoot' the bird with a good camera and not with a gun.

Collection

i) **Mist Nets:** Various types of mist nets are available. The nets are fixed by taking in to account the wind direction, sunshine, location etc. The entangled bird should be removed carefully to prevent injury to the bird and damage to the net. This requires skill.

ii) **Firearms:** Shotguns of various bore sizes are used for collecting different sizes of birds. The bird captured by using firearm has to be treated very carefully. Throat and cloaca should be plugged with cotton wool. The blood from the plumage should be wiped off before the bird is wrapped in a

newspaper or tissue paper so that plumage is not disturbed. The collected bird's soft body parts like colour of eyes; iris and legs should be noted down.Use of Firearms to collect birds is not permitted in India now as per Indian Wildlife (Protection) Act, 1972.

PRESERVATION

Birds are preserved either wet or dry. For dry preparation, one has to be skilful and this is a technical job requires special training course commonly known as "Taxidermy."The following methods are used for bird preservation.

i) **Rolled skin:** A small cut has to be made on the abdomen, and then using various chemicals and proper instruments to free the skin from body and turning it completely inside out slowly remove all the body parts. Then chemicals are applied to the skin for permanent preservation. The skin is again brought back to the normal position.An artificial, conical shapedbody is made as per the natural size & shape of the bird by rolling inside with waste jute or cotton wool. A galvanized wire is inserted in to the legs. The rolled and preserved bird is brought back to its original shape. The abdominal incision is stitched. This type of preservation of skin is called rolled skin. This method is usually used for scientific study.

ii) **Mounting:** The method used for mounting a specimen for display. A similar method as described above is adopted for bird skinning. For mounting, an appropriate natural posture is to be given to the wings or legs or tail or to the head or combination of these. The specimen is then dried up for a week, then fixed to a suitable branch, twig or board for display.

iii) **Whole specimen:** Badly damaged birds or young ones are to be preserved as a whole specimen. For this 75-80% Ethyl Alcohol or Formalin solution of desired strength is generally used both as fixative and preservative. A proper dose of fixative has to be injected in to the abdominal cavity of the specimen.

22. MAMMALIA

INTRODUCTION

Mammals are found in all types of habitats, from snowy heights of the Himalaya to the plains, from thick rain forests to the arid zones and from terrestrial to aquatic ecosystems. Aquatic Indian mammals mainly belong to the orders Cetacea and Sirenia. Mammalian fauna is richest in NE India followed by Western Ghats, Eastern Ghats and the Desert. Indian mammalian fauna is influenced by the Indo-Chinese, Malayan, Ethiopian and Palaearctic elements.

Mammalian species vary in size from tiny shrew to elephant and giganticwhale. It is difficult to draw a clear-cut line of demarcation between small, medium and large sized mammals. However, for convenience, they can be separated on the basis of size in the following way.

MAMMALS

SMALL	MEDIUM	LARGE
Head and body length	Head and body length	Head and body length
Less than 30 cm	Between 30 cm-100 cm	above 100 cm

COLLECTION

Collection of specimens serves the following main purposes:

1. The specimens serve as a reference collection in identification of the species.
2. As an authentic evidence for recording distribution of fauna.
3. Study of body parts (organs) for various purposes.
4. To serve as an exhibit in museum for education, research and public awareness.

Mammal collections as a whole can be broadly divided into two types:

1. Macro-species collection for large and medium sized mammals
2. Micro-species collection for small mammals.

Macro-species collection now, largely done only for museum exhibits or to fill the gaps in the existing collections as it involves medium and large sized mammals where taxonomy has been fairly well known. Moreover, populations of these species, in most of the cases, are under severe threats of survival. Hence procedure for mammal collection records / reports can be categorized in following way:

Since in most cases mammalian species are recorded through reporting based on the authentic information, an observer must carry field diary with him and record as much information as possible. Such information can be recorded in Mammal collector's Field Diary in the following way:

FIELD DIARY

Name of Collector/ Recorder: _____ Locality: _____

Dist.: _____ State: _____ Country: _____

Latitude: _____ Longitude: _____ Altitude: _____

Collection/Record Station No.: _____

Date: _____ Time: _____ Temp. Recorded: _____

1. Habitat:
2. Climatological details:
3. Method of collection/SightingRecord:
4. Other observations if any, like sighting of other species:
5. No. of specimens collected/Sighted Coll. No. - Adult/Immature, Sex etc.:
6. Nature of Preservation Wet/Dry:
7. Details of organs or tissue samples collected:

Before proceeding for the actual sighting/collection of the mammalian species, one must possess a generalized information about their morphology, habit and habitats. Because, there is always an intimate relationship between the structure of an animal, its mode and habit of life and the conditions under which it lives. Adaptation to a particular kind of life which various groups of mammals chose to follow has led to the evolution of the amazing diversity in form and structures seen in today's mammalian species.

COLLECTION OF MACRO (LARGE/MEDIUM SIZED) MAMMAL SPECIES

Due to restrictions on collection of Large/Medium sized mammals under Indian Wildlife (Protection) Act, 1972, one has to heavily rely only on the records and reports based on direct or indirect evidences. For keeping records and also for updating them one can go through the following steps:

1. Gather maximum knowledge on distribution localities and range of distribution of a species under study.
2. Collect available information on past records from Gazetteer and detailed bibliographic references so as to know about the status of occurrence in the past.
3. Knowledge of past and present taxonomic status is essential.

4. Update the gathered information by sending questionnaires to Experts, Forest/Wildlife Departments, N.G.Os. and or local informants.
5. Try to record Direct/ Indirect evidences on the latest occurrence of a species concerned. For information on indirect evidence one must possess thorough knowledge on the morphology, habit and habitat of the species under study.
6. If collection of a specimen is must, knowledge of the jurisdictional limits of their Protected Areas and closed periods for hunting is essential. One must obtain special purpose permit for collection of such specimens for academic and research purposes from the Chief Wildlife Warden of the respective State.
7. Knowledge of conservation status of the species concerned is equally essential.

COLLECTION OF MICRO (SMALL) MAMMAL SPECIES

Articles required for collection and preservation

The main equipments required for the collection of mammalian species are fire arms, nets, traps, harpoons, crowbars, shovels, choppers and different types of poison baits. Weighing balances, vernier, tapes, chemicals, preservatives like formalin, anaesthetizing agents like chloroform, large forceps, scissors, absorbent cotton wool, muslin cloth, arsenic soap, common salt, hand lens etc. are also required for processing after collection.

Collection methods

Before proceeding for the collection one must thoroughly scan the area of operation. Each of the habitat types such as cultivated fields, orchards, waste lands, buildings, hillocks, bunds, caves, forests, river side catchment areas of the water bodies etc., should be inspected with the help of local guides/informants. Sampling is carried out during daytime for diurnal species and in the evening or at night for crepuscular species.

Traps: Numerous traps and trapping methods are available for collecting small mammalian species. The kind of trap and quality of a bait to be used are selected with care depending on the animals sought. Traps should neither be too small nor too big for the anticipated catch. In general, traps can be categorized into two types:

1. **Snap-break-back traps:** These traps are best suited for small mammals which develop trap shyness. These traps are good and handy. They are to be tied to a nearby object with a string to avoid loss. The only drawback in this type of a trap is that the skull of the animal is broken during the process of snap trapping.
2. **Live traps:** There are different types of live traps used in different parts of the Country. These traps are used for catching the live specimens. Some of the important ones are:
    1. **Single catch traps:** Only one animal can be trapped at a time. Example: Wooden traps, Sherman traps, Japanese wire trap, noose or snare trap, Wiesel trap etc.

2. **Multiple catch trap:** These traps can catch more than one animal at a time. Example under these categories are Wooden traps, Pitfall traps and Glue traps.

Success of any trapping operation rests with a suitable bait, selection of the site, number of traps used, trap line, trapping duration etc.

A list of some popular traps and the animals for which they are best suited is as follows:

NO.	TRAP	LIVE OR KILL	PREFERRED ANIMAL	TYPE OF ANIMALS
1.	Snap Break back trap	Kill	Shrew to small Squirrel	T, A, B
2.	Sherman trap	Live	Shrew to small Squirrel	T, A, B
3.	Pitfall trap	Live	Shrew to mouse or mole	T, B
4.	Have-a Hart trap	Live	Squirrel or bandicoots	T, A, B
5.	Snare trap	Live	Small squirrel	T, A
6.	Wonder trap	Live	Shrew to bandicoots	T, B
7.	Glue traps	Live/kill	Shrew to rats	T, B
8.	Wooden traps	Live	Shrew to bandicoot	T, B

Where A: Arboreal, T: Terrestrial, B: Burrowing

Nets: Use of mist nets has brought about revolution in techniques for bat and bird collections. These are made from nylon thread. Size of the most popularly used mist net is 6–20 m wide and 2–3 m in height with four to five tiers. Bats that are unable to detect the net, hit the net and fall in a pocket of the tier. The bats get entangled in the nylon thread as they struggle. A trapped bat sends signals to other fellow bats in the area which rush for help. During the process other bats also get entangled and trapped. One should be very careful while removing a trapped bat to avoid damage to the mist net. Prior to selection of the site, one is supposed to locate roosting places of bats and knowabout their activities in the area. Bats can also be collected with the help of insect nets by using them properly in the roosting sites. Some bats roost in hollow trees or loose bark of the trees. Banana plants, caves, crevices in rock, old buildings, roof rafters, ruined monuments, temples, tunnels, inter-nodal cavities of bamboo, palm fronds, corrugated metal roofs, culverts etc. also serve as favourite roosting sites for most of the bat species. The nets are generally tied on poles fixed at a selected place or on branches of the tall trees. Sometimes mist net fixed on bamboo

sticks is dragged with the help of a vehicle with lights on at a slow speed during evening time. The results are quite successful with the use of mist net in this way also.

MEASUREMENTS

It is always advantageous to take measurements of the specimen collected before initiating its preservation. Key characters vary in different groups. In mammals, in general, external measurements and other data like weight, sex, number and position of mammae, condition of gonads, number of planter pas, adult status etc. are recorded. Four standard measurements are generally taken in mammals:

1. **Head and Body length:** From tip of the nose to anus.
2. **Length of Tail:** From anus to the tip of the tail vertebrae, excluding pencil of hairs, if any.
3. **Length of Hind foot:** From heel to the tip of the longest toe excluding claw.
4. **Length of Ear:** From intertragal notch to the farthest edge of the pinna excluding hairs, if any.
5. In bats, length of forearm, tibia, metacarpals, phalanges, calcar, noseleaf, tragus etc. are also recorded.
6. In case of large and medium sized mammals, height and girth at various points are recorded.

PRESERVATION

There are two methods of preservations in Mammals, wet and dry. If due to some reason preservation is delayed, the specimen is converted into skeleton by digging and burying it under the soil for a prolonged period of time.

Wet preservation

Most of the small sized mammalian species can be preserved in liquid preservatives. Preservation includes two processes: fixation and preservation. Either one chemical or a combination of chemicals are used for both the processes. Normally 10% formaldehyde (1 part of 40% solution of formaldehyde and 3 parts of water) or 90% ethyl alcohol is used as fixative and 4% formaldehyde solution (1 part of 40% formaldehyde solution and 9 parts of water) or 70% ethyl alcohol is used as a long term preservative. Sometimes the specimens are fixed in 10% formaldehyde solution but preserved in 70% ethyl alcohol. Since formaldehyde solution is partially acidic in reaction, the solution is buffered with a little amount of common salt, chalk or borax before use.

The fixative of 10% solution is injected into the abdominal cavity or the abdomen is cut open along the mid-ventral line to expose viscera. Sometimes a piece of absorbent cotton soaked in the fixative is pushed inside the abdomen to facilitate proper inflow of the fixative.

The specimen injected with the fixative is given the desired shape in an enamel tray and immersed in the fixative for 24 hours or more depending on the size. The specimen is checked to see whether the body parts have become stiff after fixing. Finally, the specimen is transferred to the preservative solution of the above mentioned strength. If formaldehyde solution is the fixative, the specimen must be washed thoroughly in

running water and semi-dried, before it is preserved in 70% alcohol. Later, the preservative is changed periodically for proper maintenance.

Dry Preservation

In dry preservation, the skin of the animal is removed and treated with chemicals (Arsenic soap) to fix the tissues. Dry preservation is useful to preserve medium and large sized animals. It is also useful for the preparation of museum exhibits. Dry preservation is a part of taxidermy.

Small mammals: Preparation of study skins of small mammals involves two processes – skinning and rolling.

Skinning: The animal is placed on its back. The fur along the mid-line of the abdomen is parted. Skin of the animal is cut with a scalpel longitudinally for about 2-3 cm. Care is taken not to open the abdominal cavity. The skin is separated from the flesh over the abdomen and then on each of the hind limbs, with the help of fingers, until both the knee-joints are exposed. After loosening the skin around the knee, the joint is severed. The skin is separated all round upto the base of the tail so that it can be grasped with two fingers. Magnesium carbonate is sprinkled on the inner surface of the skin to facilitate gripping. The skin is held at the base of the tail by the left thumb and forefinger and the tail vertebrae are pulled out of the skin by the right hand. In some cases where the tail vertebrate cannot be pulled out easily due to injury, etc., the skin of the tail is cut length-wise at the place of obstruction and tail vertebrae freed. Freeing of the skin from the body is continued forward turning it inside out. On each of the forelegs, it is freed up to the elbow-joint and then the joint is severed, and freeing of skin is continued further forward. Ears are cut as close to the skull as possible. Eyelids are freed from the eyeballs, by the point of a scalpel. Further anteriorly, the skin is cut free at the nose by slicing it through the nasal cartilage. Extreme care is required to avoid damage to the cranium. Some bats have delicate protruding premaxillary bones and incisors. These bones as well as nasal bones should not be damaged. The skin is finally freed from the flesh by cutting it at the base of lips.

The skin over the forelimbs and the hindlimbs is inverted as far as possible and the flesh over the remaining portion of the bones cleaned off. The fat below the skin is scraped off. Soft tissue from the sole is removed as much as possible through a slit made in the middle of it.

The lips are sewed and the fur-side skin is turned out. The blood stains, if any, are cleaned with cotton soaked in cold water. If the skin is too dirty, it is washed in cold water with soft soap. The fur is dried by using magnesium carbonate. The skin is again inverted. Moist alum powder(alum pulverised) is rubbed throughout the inner surface of the skin. Some quantity of alum is also inserted inside the hollow tail-skin with the help of a wire. Soft parts like lips, base of ears, sole of feet, etc., are painted with arsenic soap paste. In case of small specimens like a mouse, the application of the arsenic soap paste on the inner surface of the skin serves the purpose; alum may not be used at all.

At present two methods are prevalent for keeping the processed skin. One is the traditional method which is termed as rolling in which shape is given to the skin by filling it.inside with cotton wool or sawdust. Another method to preserve the flat skin is by inserting a piece of cardboard of suitable size in the skin. Skins thus prepared are called study skins.

Rolling: A piece of galvanized wire of 18, 20 or 22 gauge (depending on the thickness of the tail) and slightly longer than the tail is taken and one of its tips filed to make it pointed. Some cotton wool is twisted around the wire to make the structure approximately equal to the original thickness of the tail. A little arsenic soap paste is then smeared on it. The wire with the cotton wool is now inserted into the tail-skin.

The limb muscles are prepared by wrapping cotton wool around the limb bones. To provide additional support, a wire is inserted in each limb so as to reach the longer toe. The fur-side of the skin is turned out. An artificial body somewhat similar in size and shape to the original body is prepared with common wool and inserted within the skin. Little wisps of cotton wool are inserted in the spaces between the outer skin and the artificial body, especially below the eyes, ears, nose, etc., to give the skin a proper shape. After this, the cut on the abdominal portion of the skin is sewed.

Specimens other than bats are set on a piece of thermocol placing the limbs close and parallel to the body, the forelimb pointing forwards and the hind limbs backwards. A thin layer of cotton wool is wrapped from the nose to the neck of the specimen so that the whiskers lie backwards along the sides, and the ears lie flat on the head. It should be mentioned that keeping the ears erect in rolled skins, no doubt, facilitate examination of both sides of the ear but is disadvantageous in storing them in this way, because there is every likelihood of the ears being damaged. The specimen is left to dry in a shady, airy place. Fleshy parts like the lips and soles are painted with carbolic acid.

The positioning and pinning of a bat specimen will determine its usefulness for research purposes. For example, the distal and proximal portions of the forearm, all the metacarapals and phalanges, calcar, attachment of wing membrane to hind foot, etc., should be exposed so that they can be measured even after preservation. The practice of positioning one of the wings extended is discouraged, as it occupies more storage space, and the wings are very likely to be damaged. The wing membranes should be tucked under the proximal end of the forearm. Similarly, the thumb of the bat should always be turned down and pinned beside the next digit. If left extended, it is likely to break. The ears and the nose-leaf are given extra support so that these may dry in extended position.

In the second method, instead of making an artificial body with cotton wool, a piece of thin cardboard is cut to the approximate size and shape of the head and body of the animal. A thin layer of clean, combed cotton is wrapped around the cardboard body; thread may be wrapped around it to hold the cotton in place. The cardboard body thus prepared is now inserted into the skin. The fore and the hind limbs are folded over the cardboard on the ventral side and the tail is set straight. Each limb and the tail are then fixed in position by a loop of thread. The dried skin is separately wrapped in a thin sheet of tissue paper or a piece of newspaper.

COLLECTION OF BIOLOGICAL SAMPLES

Biological materials (whole specimen, skeletons, skulls, horns, antlers, tusks, ivory, traces of tissue, blood, hair, bone, egg shells, faces, etc.) are the important samples that can be collected during field survey. These biological samples should collect appropriately by investigating team without any contamination or mixing of biological samples of other species. Here, the best practices of sample collection have been suggested based on the efficiency of the subsequent DNA analysis results carried out in the laboratory for various types of samples. A check list of the items required in the sample collection kit are as follows: Gloves, magnifying lenses, Compass, Mask, small note book, sterile Plastic viols, Ethanol/sprit bottle, forceps, scissor, Glass slides, scale, inch tape, silica, Envelops, white cloth, ink pad, cello tape, surgical blade, swab, FTA card and swab (Fig. 1).

Fig.1 Anessential wildlife sample collection kit

COLLECTION OF TISSUE AND SCAT SAMPLES

In field, it is rare to get whole animal body. Therefore, maximum efforts should be made to browse the chances to get small piece(s) of meat and or other body remains of the deceased animal. Here, a flow chart is given to illustrate the collection of tissue/scat or any other biological material in dry condition:

1. Use a screw capped sterile small vial (100 ml/gram capacity)
2. Sterile collection vial would be filled with (1:2) Silica Desiccant. Put a filter paper or normal paper on the silica gels.

   Note: Instead of Silica, ethanol can also be used for collecting samples in wet condition (in such cases 2/3rd volume of the container with 70% or absolute Ethanol/ ethyl alcohol which is chemically C_2H_5OH).
3. Place small meat (10-20 gms)/skin piece (3x3 cms)/fresh scat (15-20 gms.) over the filter paper (or directly dip the sample in ethanol) and make airtight with the cap. Please write the species and place, date of collection of the sample on the vial.
4. Paste the information related to the samples on the wall of the vial using some glue or cellotape (Fig. 2).

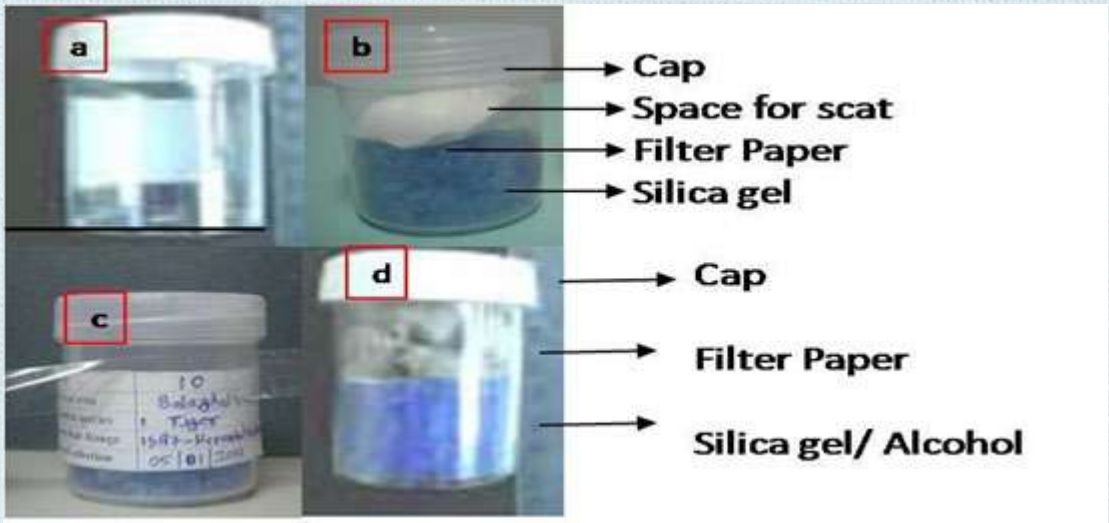


Fig. 2: Collection of tissue and scat samples

COLLECTION OF BLOOD SAMPLES

Finding fresh or un-coagulated blood in field is very rare and there would be maximum chances to get coagulated blood spot or blood stains at the crime scene. Here, an illustration are made to exhibit the ways to collect the fresh or un-coagulated blood and coagulated or blood stains.

Blood in vaccutainers (blood may be collected as usual for routine purposes) and stored at 4°C (lower compartment of refrigerator) until handover to the investigating lab. If, the vaccutainers are not available in field conditions, the examiners can collect blood spot or stains on FTA Classic Card, a commercial product, manufactured by Whatman which is suitable for isolation, purification and storage of nucleic acids. Simply apply the sample to the FTA Card. Cell membranes and organelles are lysed and the released nucleic acids are entrapped in the fibers of the matrix. The nucleic acids remain immobilized and are preserved for transport, immediate processing or long-term room temperature storage. A tiny drop of blood / blood stain or any biological fluids may be loaded on the specified area of FTA card. Simultaneously, blood smears may be made on the glass slide in the field conditions for biochemical analysis (Fig 3).

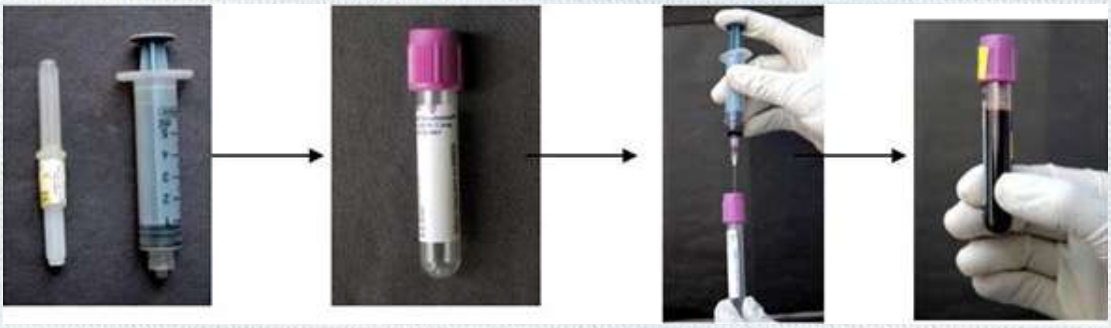


Fig. 3. Collection of blood samples in the Vaccutainer tubes

Collection of miscellaneous biological samples

Several times, fresh blood samples or tissue samples in field is not available. However, there is always ample chances to collect various other types of physical evidences e.g. hairs, blood soaked in soil or blood stains from plants, and any sort of body fluids either from the animal or from the offender. These all type of samples are need to be searched thoroughly.All types of fluids should be collected on swab, hair should be collected separately in Ziplock bag for laboratory analysis (Fig. 4).

Fig.4. Collection of miscellaneous biological samples

23. GEOGRAPHIC INFORMATION SYSTEM (GIS)

1. What is Geographic Information System (GIS)?

A Geographic Information System or popularly called as GIS is a computer aided spatial or geographic analysis software system where spatial information is stored, retrieved manipulated, analyzed and presented as maps. Different modules of GIS are used to understand and predict spatial information in a easy and intuitive forms as maps and graphs.

2. Why GIS in faunal diversity studies?

The purpose of faunal surveys is to document the distribution and diversity of fauna at different spatial scales from habitats, ecosystems, landscapes to biogeographic region. Formerly, the spatial distribution of fauna was described in text form which is imprecise and difficult to visualize. With the development of GIS tools, hand held GPS, computational distribution models, now it is possible to generate species distribution and diversity maps across spatial scales. Further, the techniques in species distribution models can predict potential species occurrence or spread of invasive aliens for effective conservation of biodiversity. Currently, GIS coupled with GPS collar and satellite telemetry is efficiently used to understand the movement pattern of endangered, migratory species or individuals which are involved in man-animal conflict. The species distribution and diversity maps generated out of field survey and GIS analysis is widely used in scientific publications, technical reports, policy documents and conservation communication. Since it is very easy and intuitive to understand and interpret the GIS outputs it has became the preferred way of communication by scientists to policy makers, politicians, general public and other stake holders.

3. GIS in taxonomic studies

Taxonomists collect large amount of primary biodiversity data and most of it is stored in irretrievable text form such as locality name, collection date, altitude, geo coordinates, sex, life stage etc this data is generally termed as label data or materials examined data. This is generally published as such without much further analysis. However, the primary biodiversity data is of immense value to ecologists and conservation biologists to understand species biology and ecology if it is brought into a database format in a GIS platform. Further, the spatial distribution data in GIS platform can be used for identifying spatial gap areas in survey, biodiversity studies, identifying key areas for biodiversity conservation and species distribution modeling.

4. Global Positioning System (GPS)

Global Positioning System (GPS) is satellite based navigation which provides geolocation and time information to a receiver. The accuracy of GPS varies with location, cloud and canopy cover. With the availability of hand held GPS and smart phones, recording of geocordinates and area covered during surveys are very accurate. Further the stored data from GPS can be directly downloaded in to a GIS platform. There are several models of GPS available in the market and manuals of particular model in use to be consulted for proper configuration and use.

4.1 Calibrating GPS: It is important to calibrate GPS before using. Make sure that following settings are done before each survey.

4.1 Battery status: GPS consumes batteries very fast, hence it is important to keep sufficient spare batteries. Lithium-Ion batteries are recommended as they last longer in field conditions.

4.2 Datum: In GIS there are several formats used to represent the spatial data in two dimension. Technically, it is a coordinate system, and a set of reference points, used to locate places on the earth or objects, it is also known as geodetic datum or geodetic system. For documenting spatial distribution of fauna, World Geodetic Dataum-84 (WGS84) format is widely used. In GIS this need to be set manually.

4.3 Date and Time: Select time as GMT + 5.30 and automatically date will be set.

4.4 Coordinate System: GIS used degree decimal coordinate system for spatial data representation. In GPS the coordinate system is to be set in decimal degree mode. By default it will be in degree minutes and need to be changed into decimal degree mode.

4.5 Understanding Decimal Degrees: Table below provides an over view of spatial scale of decimal degrees which will be useful in interpreting the GPS the data.

DECIMAL PLACES	DECIMAL DEGREES	SPATIAL SCALE	APPROXIMATE AREA AT EQUATOR
0	1	Region/Biogoegraphic Zone	111.32 X 111.32 Km
1	0.1	Landscape/Protected Area	11.32 X 11.32 Km
2	0.01	Ecosystem	1.1132 X 1.1132 Km
3	0.001	Habitat Mosaic	111.32 X 111.32 m
4	0.0001	Habitat	11.1132 X 11.132 m
5	0.00001	Microhabitat	1.11132 X 1.11132 m
6	0.000001	Individual Organism	111.32 X 111.32 mm

4.6 Altitude: Altitude reading in GPS is not very accurate hence it is recommended to carry a manual altimeter.

4.7 Accuracy: For recording data signal from minimum of three satellites are required. However due to terrain, canopy and cloud cover signal strength may be weak and accuracy compromised.

All the GPS show accuracy value and it is advised to wait to record the reading till the accuracy is 3m and below.

4.8 Recording Locality Data: Locality data of a specimen with date is most crucial data in surveys. The specimen should have a textual description of locality along with geocordinates. Following textual format for locality description is recommended.

Habitat Name (Eg. Pond, lake or stream name); village or forest beat, range, compartment etc; district and state; Latitude; Longitude; Altitude

5. Extracting data from published records:

Published papers, technical reports and taxonomic monograph has detailed locality information especially in the materials examined part. This data is usually represented in text form. This text data can be converted into a GIS readable data base by adding geocordinates to the locality. Geocordinates of localities can be found with reasonable accuracy using online resources such as Google Earth and Bhuvan.

6. Extracting data from specimen labels:

Identification labels in the specimens contain wealth of information. Once the label information including locality data is brought into a GIS platform several analysis could be carried out. In old specimen labels geocordinates may be absent. However using Google Earth and Bhuvan geocordinates of localities can be found.

7. Recording data in GPS

Once GPS is calibrated it is ready for data logging. In GPS geocordinates of place of interest can be stored using "mark waypoint" option in the GPS and retrieved directly into the GIS software. It is recommended to use track mode daily on a survey tour to measure the total area covered during a survey.

8. Recording GPS data on Smart Phones

Smart phones come with inbuilt GPS. There are several free apps which can be used to record geocordinates reliably.

9. Data formats

Locality data from surveys, museum specimens and literature need to be stored in standard format. Microsoft Excel is ideal software for storing the data. Recommended data format for GIS is provided in Table-1. The data in Excel sheet need to be exported into text (*.txt) or CSV (*.csv) based on software requirements.

9.1 Shapefile

Shapefile format is a popular geospatial data base format describing vector data such as lines, points and polygons in GIS software. Eg: roads, stream networks, ponds, rivers, lakes, political boundaries etc. The Shapefile format consists of three mandatory files with standard extension viz., *.shp, *.shx and *.dbd stored in the same directory.

9.2 Keyhole Markup Language (KML)

Keyhole Markup Language (KML) is a format used in expressing geographic information in internet based two dimensional and three dimensional browsers such as Google Earth.

10. GIS softwares

There are several GIS softwares which could be used for analysis. However, preparation of data in standard format and access to base layers (eg. Political boundaries, ecosystem boundaries etc.) is essential to start GIS analysis. It is advised to start with simple open source GIS software such as DIVA-GIS to learn the basics. The DIVA website also provide a good user manual and essential GIS layers for basic analysis. Some open source and proprietary GIS softwares are listed below. There are several online resources, manuals and video tutorials to work with GIS softwares and advanced learning.

1. DIVA-GIS
2. QGIS
3. GRASS GIS
4. ArcGIS
5. ERDAS
6. MAPINFO
7. ESRI

Useful websites

1. http://www.diva-gis.org/
2. https://www.qgis.org/en/site/
3. https://grass.osgeo.org/
4. https://www.arcgis.com/index.html
5. https://www.hexagongeospatial.com/products/power-portfolio/erdas-imagine
6. https://www.pitneybowes.com/us/location-intelligence/geographic-information systems/mapinfo-pro.html
7. https://www.esri.com/en-us/home
8. http://geodata.grid.unep.ch/
9. http://srtm.csi.cgiar.org/
10. https://asterweb.jpl.nasa.gov/gdem.asp
11. https://earthexplorer.usgs.gov/
12. https://www.google.com/intl/en_in/earth/
13. https://bhuvan.nrsc.gov.in/bhuvan_links.php
14. https://www.bing.com/maps
15. https://globil-panda.opendata.arcgis.com/

11. Species Distribution Modeling

Use of computer softwares to predict geographic distribution of species using environmental data is commonly known as Species Distribution Modeling (SDM), Environmental (or Ecological) Niche Modeling (ENM), habitat modeling or predictive habitat distribution modeling. Species Distribution Modeling is useful in assessing the potential distribution of rare and endangered species, status surveys or predicting potential areas of colonization by invasive alien species. To create SDM for a species, environmental data such as climate data (eg. rainfall and temperature), habitat data, vegetation data etc. are used. The SDM models can also used for predicting future distribution under climate change scenarios.

The SDMs can be classified into Correlative SDMs or Mechanistic SDMs. Correlative SDMs predict species distribution based on environmental conditions. Models such as Climate Envelope models, Bioclimatic Models, or resource selection function models are Correlative SDMs. On the other hand, Mechanistic SDMs used independently derived information on species physiology to develop models on species potential distribution.

A variety of algorithms are used for correlative SDMs some of the widely used ones are listed below. GIS software such as DIVA-GIS has inbuilt modeling tool such as BIOCLIM and very useful for beginners.

- BIOCLIM
- Ecological Niche Factor Analysis (ENFA)
- Mahalanobis distance
- Generalized linear model (GLM)
- Generalized additive model (GAM)
- MAXENT
- Artificial neural networks (ANN)
- Genetic algorithm for rule set production (GARP)

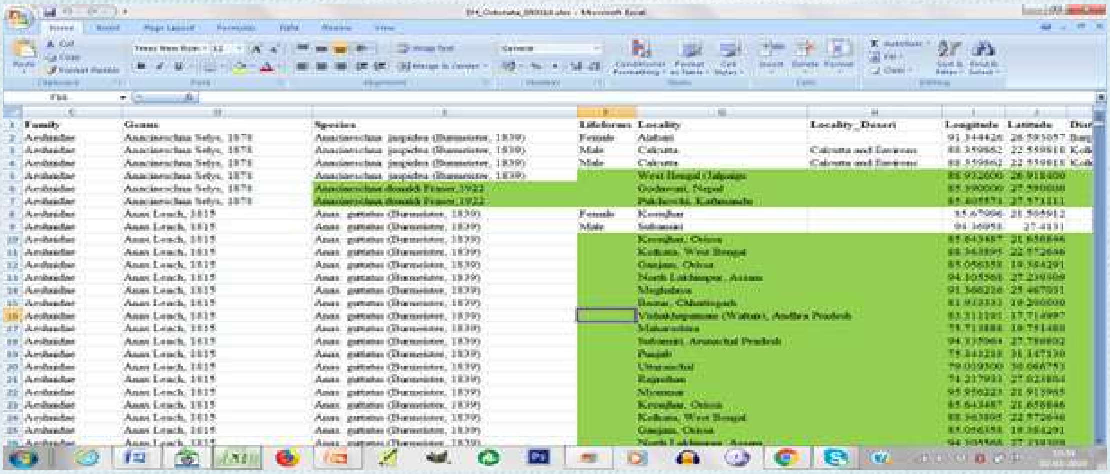
Appendix

1. Text Data

2. Data format

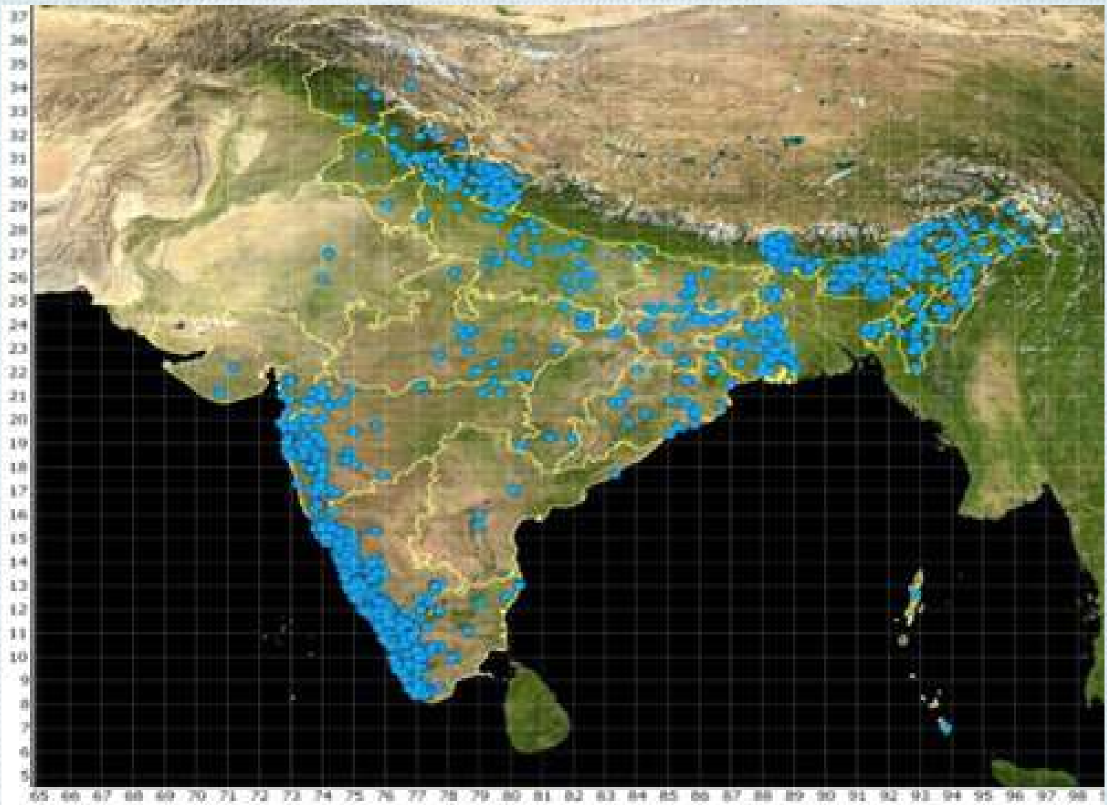
Sl. No.	Family	Species	Longitude	Latitude	Locality	District	State	Date	Collected By	Identified By

3. Screenshot of data

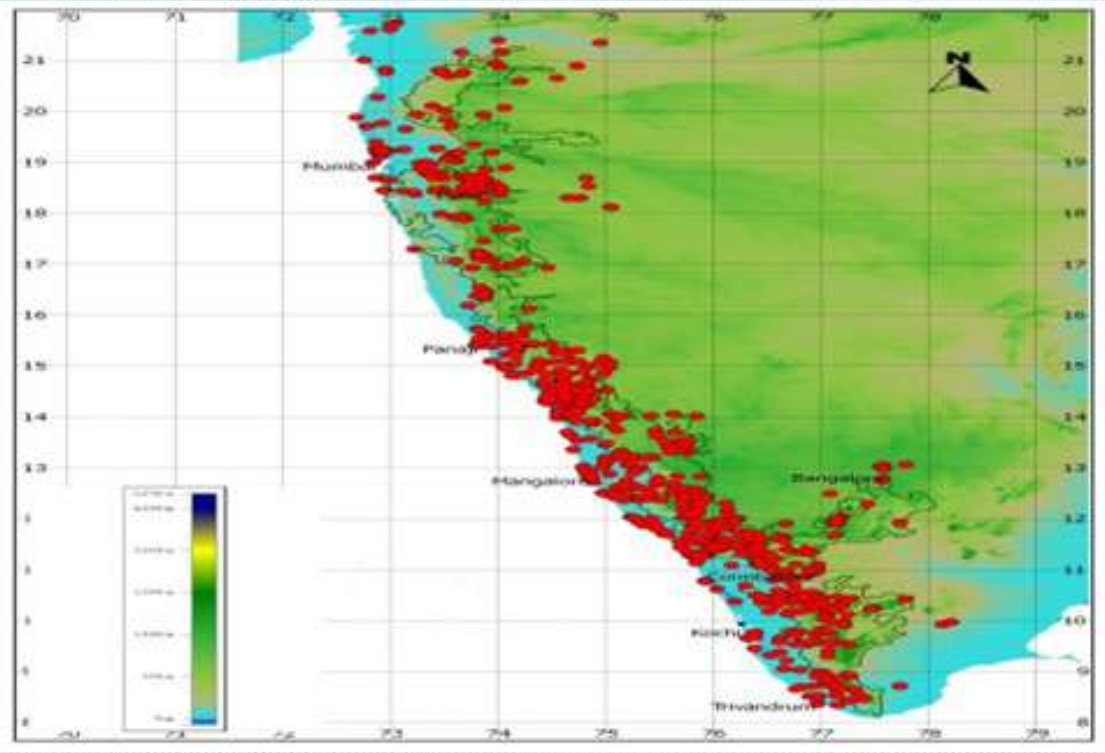


Data visualization in GIS

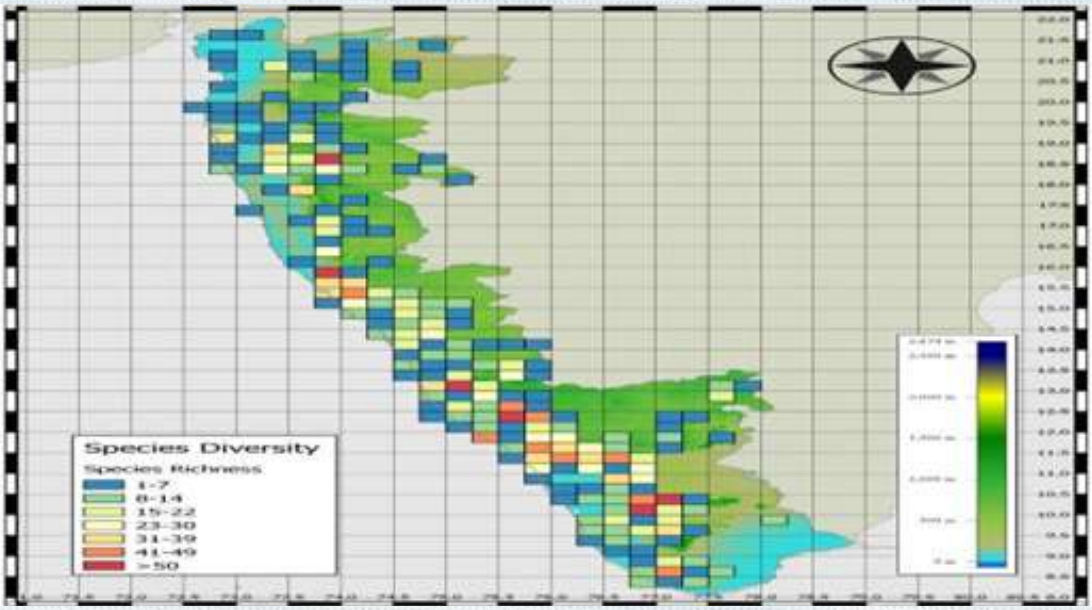
4. Data visualization in GIS

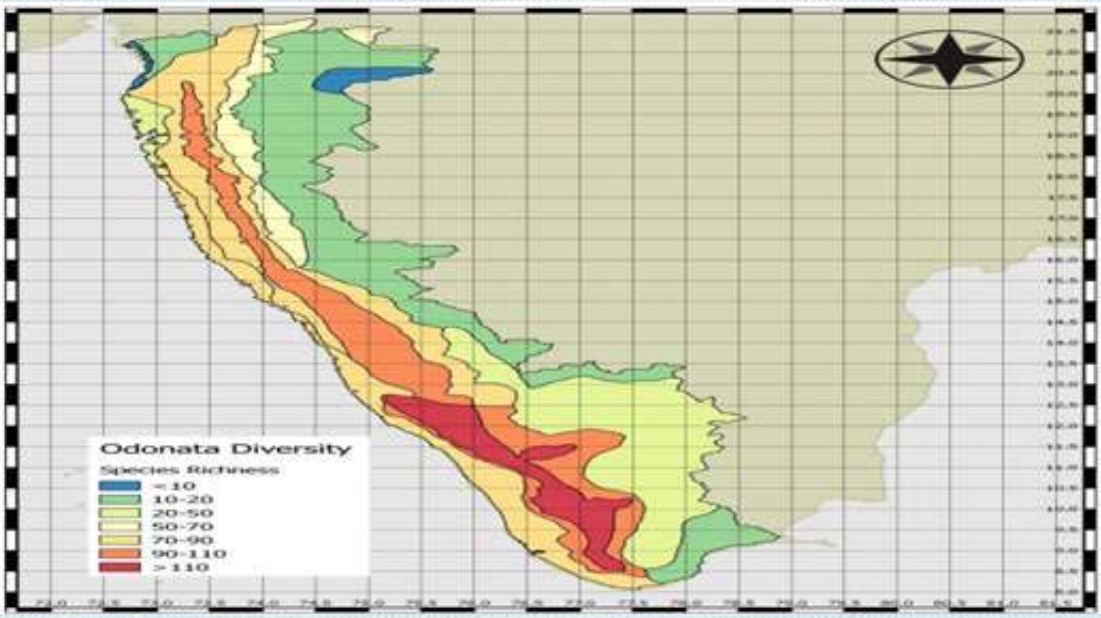


5. Species distribution map

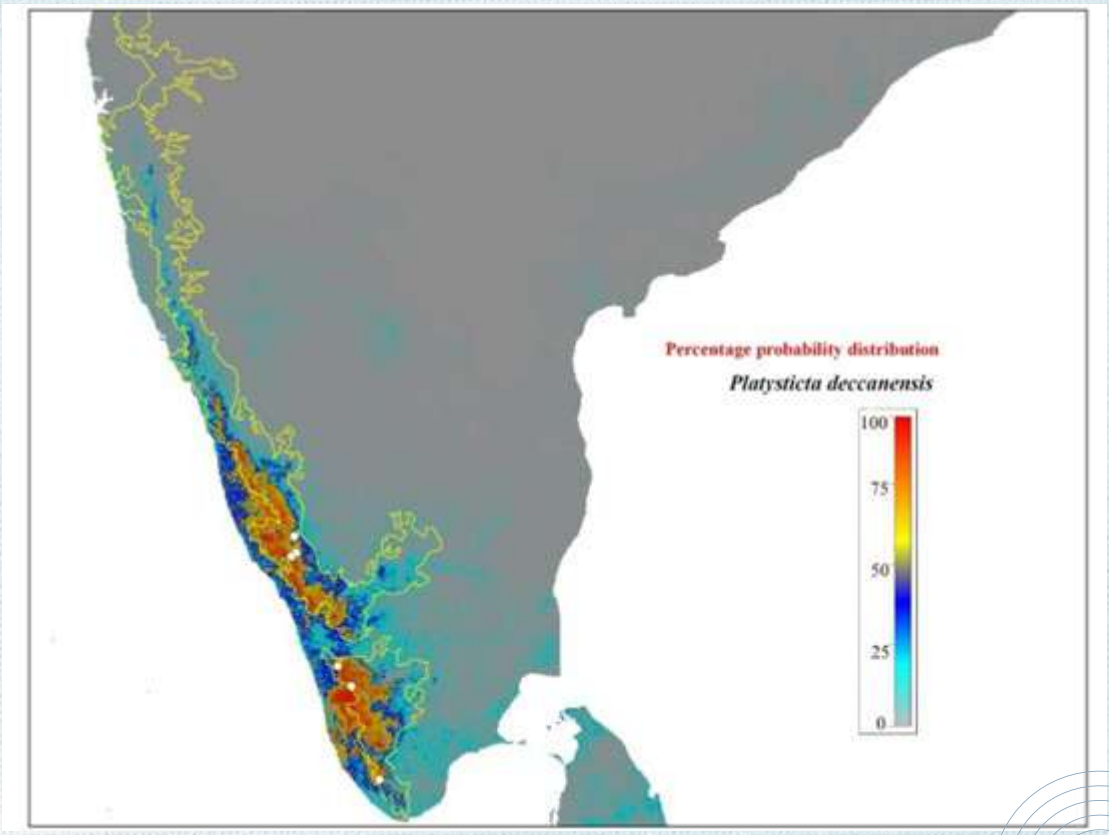


6. Diversity maps





7. Output of Ecological Niche Modeling (MAXENT)



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