





Direct pinning method Source: NMK - Entomology

Lesson Objectives

- The participants shall identify and describe sampling techniques of arthropods.
- 2. The participants shall describe the preservation methods of arthropods.
- 3. The participants shall appreciate curation of arthropod collection.
- 4. The participants shall appreciate the process of museum specimen preparation

Learning resources

- 1. Text
- 2. Video
- 3. Photo



Introduction

Collection and preservation of arthropods is important in biodiversity studies. It enables researchers, scientists and enthusiasts to study, identify species and understand their ecology.

The methods used to collect arthropods are dictated by the ultimate goal of the samples collected. For example, sampling may be conducted to measure biodiversity to help identify appropriate areas to be included in reserves.

Sampling aquatic species may help to detect changes in water quality. Pest species may be sampled to assess their presence, absence or abundance in order to determine whether control measures are necessary.



A basic kit for collecting arthropods

A basic kit of items for collecting arthropods consists of the following:

- Field notebook and pencil
- Forceps
- Vials with alcohol or other preservative
- Killing bottles of various sizes
- Aspirators
- Small paint brush



Entomological kit Source: NMK - Entomology



Specimen relaxing jar Source: NMK - Entomology

- Hand lens
- Tissue paper/cotton
- Plastic bags or zip locks
- Hoe
- Machete
- GPS machine



Killing jar Source: NMK - Entomology



Methods of collecting Arthropods

A range of methods are available for collecting arthropods. Some methods are suitable for collecting arthropod groups that appear in different habitats. The method of collection used depends on the targeted group and whether live or dead specimens are required.

Collection methods can either be active or passive. Active methods involve the use of nets, forceps and aspirators to collect arthropods. Passive methods involve use of traps left in the field to collect the specimens which are then harvested later.

- **Beating Sheets:** Beating sheets are white or light-coloured canvas cloths stretched with two pieces of wood held below the vegetation. They are useful in collecting sessile or wingless groups of arthropods such as some beetles, bugs, stick insects, caterpillars and spiders.
 - Arthropods are knocked from the vegetation by tapping it with a stick or gently shaking the branches. Arthropods fall from the vegetation and land on the cloth. They can then be examined or collected.
- Aspirator: A basic aspirator design includes a jar and a tight-fitting cork with two flexible tubes. An aspirator, also known as a pooter, is used to collect small insects by sucking them into the jar. It is a useful method when collecting small specimens that need to be kept alive.
 - One tube goes into the mouth to apply suction, and the other goes over the insect so that it is sucked into the jar. A small piece of mesh covers one end of the first tube to prevent specimens from being swallowed.



Hand collecting: This method is used to collect sedentary or slow-moving arthropods. Forceps are mainly used to pick the specimens up. It is useful where no sampling method is applicable.

Specimens are obtained from various parts of a plant: stems, flowers, leaves, roots and fruits. Other arthropods are obtained from crevices or rough-bark trees, under stones, logs and in leaf litter.

- Collecting Nets: Collecting nets are made of metal screening or heavy scrim with a canvas band affixed to the metal rim. They come in three basic forms: aerial, sweep and aquatic nets.
 - Aerial nets are used for collecting flying insects like butterflies, bees, wasps, grasshoppers, dragonflies and flies. Both the net and the handle are relatively lightweight.



Aerial net Source: iStock



- Sweep nets are used to collect arthropods found on grass and vegetation. It is swung through the vegetation to dislodge the specimens from the plants into the bag of the net. The net and the handle are stronger as compared to the aerial net.
- Aquatic nets are used to collect arthropods from water. They have heavy duty net bags and handles
 with square to triangular thick wire hoops. The specimens are collected by dragging the net through
 the base of a pond or stream and then depositing the accumulated material into a large white pan to
 sort through.
- Berlese or Tullgren funnel and Winkler bag: A <u>Berlese funnel</u> also known as a Tullgren funnel consists of a funnel with a wire mesh insert that supports the leaf litter or humus above a container with alcohol or another preservative. It is an extraction tool used to separate and extract small arthropods from leaf litter or humus.

Species collected by this method are photophobic, preferring moist conditions. A light bulb is positioned above the sample leaf litter or humus. The light and drying effect of the hot bulb pushes the specimens downwards to the collecting container.

• <u>Winkler bag</u> uses the same principle as the Berlese funnel but is made of canvas and allows the sample to dry under natural conditions. It is used when there is no source of power.



- **Light Traps:** A simple light trap consists of a suspended white sheet with a light bulb hung in front of it. A variety of light traps with different light designs are used. The ultraviolet (UV) light is highly effective in attracting insects. Different insects are attracted to different light wavelengths and intensity.
 - This method is used to collect nocturnal arthropods. They use natural light from the moon and stars for navigation. Artificial light confuses them and makes them hover around the light. It is the best method for collecting moths.
- **Malaise trap:** Malaise trap is a tent-like structure made of net material with a high point in the roof where the insects collect and from where they are funnelled into a container.
 - The top of the malaise trap forms a large funnel-shaped opening that guides insects into the trap. Flying insects get into the trap, then follow the upward slope of the structure and are funnelled into a collecting container.
 - This trap is used to collect small to medium-sized insects that fly close to the ground or within vegetation. Captured specimens are collected at regular intervals, often daily or weekly.
- **Pitfall Traps:** A pitfall trap is a simple tool used to catch small arthropods that spend most of their time on the ground. It consists of a container buried so that its top is level with the surface of the ground.
 - The container is filled with water mixed with odourless detergent which acts as the killing agent. The mixture of water and detergent is filled up to three quarters of the container.
 - The specimens collected are harvested daily.



- Butterfly Baited traps: Butterfly baited trap is a cylindrical gauze, closed at the top with a landing platform at the base. A gap is left between the gauze and the platform to allow entry point for butterflies. Bait made of fermented fruits is put at the base of the trap. The trap is hoisted on a tree branch with a rope. Butterfly Baited traps are specifically designed to capture butterflies which feed on fermented fruits, sap exudates from tree barks, animal waste and rotting meat. These butterflies tend to be fast fliers in high canopy and belong to Charaxes and other nymphalids. The trap is monitored regularly.
- Pan traps: A pan trap is made of a bowl with shallow sides filled with water, mixed with odourless detergent.
 The traps are painted with different colours mainly yellow, blue and white mimicking flowers of different
 plants. Some pollinators confuse the traps with natural flowers hence they are efficient when sampling for
 pollinators.

To use the trap, place the bowl on the ground and partially fill with water mixed with the detergent. The liquid detergent is used to break the surface tension of the water, so the insects will fall through. To collect the arthropods captured, the water is poured through a fine aquarium mesh net. It is then rinsed with water into a jar of 70% ethanol.

Pan traps need to be checked at least once a day.



Pheromone traps: A pheromone trap uses pheromones, a chemical substance produced by insects for communication, to attract insects. Sexual pheromones are used in traps to attract members of the opposite sex.

The pheromone traps use lures loaded with a synthetic form of the pheromone scent for the target insect. They are placed on a sticky liner that slides into a triangular Delta trap.

The traps are species specific and can be used to monitor the presence of certain species in the ecosystem.



Pheromone trap Source: iStock



Killing methods of arthropods

After the live specimens are collected, different killing methods are applied. These methods include:

Use of liquids: 70% ethanol is used to kill non-scaly insects. Larvae and other stages of soft bodied arthropods are killed in near boiling water.

Freezing: This is done by putting specimens in a freezer for 48 hours. It is suitable for reared moths and butterflies.

Pinching: Larger butterflies are stunned or killed by pinching the thorax between the thumb and forefinger.

Killing bottle: This device is used by entomologists to kill captured insects quickly and with minimum damage. The bottle should be wide-mouthed and glassy. Absorbent material is placed at the bottom of the bottle and a few drops of ethyl-acetate added. Collected specimens are put inside and left to die.

Field preservation of specimen

There are different ways of preserving specimen in the field:

- 1. Storing in alcohol, for example spiders and ants.
- 2. Placing on paper envelopes, for example butterflies and moths.
- 3. Direct pinning into boxes. This method requires space, consumes time and poses a challenge during transportation of the specimens.
- 4. Stuffing abdominal and thoracic cavity with cotton wool, for example grasshoppers and mantids.



Preservation Methods

Arthropods are permanently preserved either dry, in liquid or on microscope slides. The method of preservation depends on the nature of the specimen.

Dry preservation

It is mounted in a way to facilitate study and permanent storage. Specimens are pinned immediately after killing; the dried specimens are relaxed or softened. Preservation of dry specimens must be done in a dry air oven at 37 degrees celsius (°C).





Dry preservation of museum specimens Source: NMK - Entomology



Slide specimen

A specimen slide is a small flat rectangular piece of glass on which specimens can be mounted for microscopic study. Thrips, aphids, parasitic wasps, scale insects, lice, fleas, mites and insect parts are mounted in microscope slides for study under a compound microscope. Each arthropod group requires a specialised mounting technique.



Drawers showing slide preservation method of arthropods Source: NMK - Entomology



Slide preservation of arthropods Source: NMK - Entomology



Wet Preservation

This is where immature stages of arthropods and all soft bodied insects are preserved in alcohol. This method is preferred as they become distorted when they dry.

Most arachnids, spiders and scorpions are preserved by this method. They are preserved permanently in 70-95% ethanol.





Wet preservation of specimens Source: NMK - Entomology



Relaxing specimens for pinning and mounting

Before long term preservation, dry preserved specimens need to be pinned. Specimens should be pinned immediately after killing, while still soft. Dry specimens to be mounted are first relaxed to make them flexible for pinning.

Specimen relaxation is done in a relaxing jar, an airtight plastic jar, with moist sand or cotton at the base. Phenol crystals can be added to prevent fungal growth. Dry specimens are placed inside the jar and left for at least one day.

Field preservation of specimen

There are different ways of preserving specimen in the field:

- Storing in alcohol, for example spiders and ants.
- Placing on paper envelopes, for example butterflies and moths.
- Direct pinning into boxes. This method requires space, consumes time and poses a challenge during transportation of the specimens.
- Stuffing abdominal and thoracic cavity with cotton wool, for example grasshoppers and mantids.



Methods of pinning and mounting of arthropod specimens

Direct pinning

The specimen is pinned directly in the thorax using an entomological pin. The pins are made of stainless steel and come in different sizes. Most insects are pinned to the right of the centre of mesothorax.

A pinning block is used to adjust the specimen on the pin to the appropriate height. The antennae and the legs are spread close to the body using bracing pins. Pinned specimens are dried at 37°C in an oven for about 3 days.

Setting of specimens is done on a setting board and the wings held in position with setting tapes. Butterflies, moths, dragonflies, lacewings and antlions are set by having both their wings spread. Grasshoppers, cockroaches, stick insects and mantids are set with one pair of wings spread.



Direct pinning method Source: NMK - Entomology



Drawer showing direct pinning of insects Source: NMK - Entomology



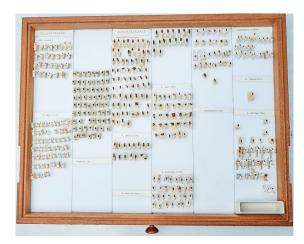
Card point pinning

Small specimens are mounted on small card points about 12mm long and 3mm wide. The specimen is glued at the tip of the card using clear drying glue.

The specimen is spread carefully for every body part to be visible. The card is pinned with an entomological pin and adjusted to appropriate height using a pinning block.



Card point pinning Source: NMK - Entomology



Drawer showing card point pinning Source: NMK - Entomology



Double pinning

This is done for very small specimens. Stainless steel micro-pins measuring 10-15mm long are used, without heads. Under a microscope, the specimen is pinned using a micro-pin at the thorax.

The pinned specimen is pinned at one end of a polyporus strip. The other end of the polyporus strip is pinned with an entomological pin and levelled using a pinning block.



Double pinning insects Source: NMK - Entomology



Drawer showing double pinning Source: NMK - Entomology



Basic slide preparation procedure

The basic procedure for preparing a microscope slide are:

- Clearing and cleaning: This is done to destroy soft internal tissues leaving the hard cutaneous part and cutaneous membranes intact. 10% potassium hydroxide is commonly used in this process called maceration.
- Rinsing: This is done to neutralise the action of potassium hydroxide. Water or ethanol mixed with a few drops of acetic acid is used.
- 3. Dehydration: Used if the mountant is not water soluble. It is done in series of increasing ethanol concentrations: 30%, 50%, 90% and 96% for 10-15 minutes in each series.
- Clearing: The material is cleared in clove oil or xylene if the mountant is not water soluble and in chloralphenol if the mountant is water-based.
- Mounting: Commonly used mounting media are Canada balsam, Euparal, Berlese fluid, Hoyer's medium or Polyvinyl alcohol. If a water based mounting medium is used, the coverslip must be sealed with colourless nail varnish or Canada balsam to prevent excessive dehydration and discoloration.



Labelling of Specimens

All specimens collected for any research purpose should be well labelled. Specimens without labels are of no scientific value. All specimens collected from the field should contain the following information:

- Locality information: This should include the country, province and the local name of that area. GPS coordinates should also be taken. Altitude information is important for some groups.
- Date of collection: The date of collection should be written as 12.v.2023. The month should always be written in Roman numerals.
- Name of collector(s).
- Host or habitat records.
- 5. Specimen accession number.



Labelling of museum specimens Source: NMK - Entomology



Curation of Arthropod Collections

Arthropod collections should be stored in a safe place and monitored regularly for any damage. Curation of arthropod collection entails:

Arranging the collection: Identified specimens of the same species should be kept together in the same drawer, row or unit tray. A label showing the name of the species is put in the drawer below each of the arranged species.

Controlling pest infestation: A number of insect pests infest and destroy preserved dry specimens like booklice, museum beetles and certain moths. All drawers and specimens should be inspected for any signs of insect infestation. If an object is found, it should be isolated immediately and put into quarantine. Insect repellents like mothballs and camphor are placed in the drawers.

Protection of specimens from light: Strong light causes colour of the specimens to fade. Specimens should be stored in tight fitting drawers in cabinets to reduce light exposure.

Topping up of alcohol in wet collection: Regular monitoring of the wet collection is conducted to ensure the right levels of ethanol are maintained. Topping up of ethanol should be done when the levels go down due to evaporation.