





Micro-Poll Pollinator Sampling Protocol

1.0. Overview

Plant and pollinator surveys will be conducted in replicate fixed plots in and around each of the 10 study villages (Figure 1.1) every two weeks during the active pollinator season. The active season begins in April when plants start to flower, crops are planted, and pollinators first become active. The season ends in October/November when most plants stop flowering and pollinators disappear for the winter.

This data will allow us to:

- a) Identify important insect pollinators of crop plants.
- b) Identify wild plant species and habitats around the village which support these pollinators.
- c) Record seasonal changes in the plant and pollinator community through the year.
- d) Construct replicate plant-pollinator networks to analyse community structure and model the effects of climate change.

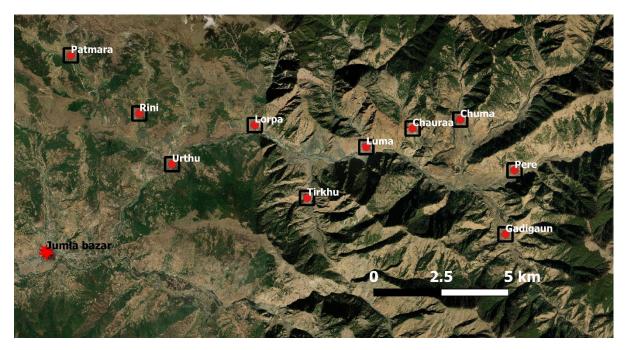


Figure 1.1: Location of study villages (red circles) and surrounding survey areas (black squares) in Patarasi Rural Municipality, Jumla District.

If anything is unclear at any point, contact Tom Timberlake Email: <u>thomas_timberlake@bristol.ac.uk</u> or WhatsApp: +44 7858 804607

Table of Contents

1.0. Overview	1
2.0. Establishing the survey plots:	4
2.1. Overview	4
2.2. Plot selection criteria	5
2.2.1. Choosing plots in semi-natural habitat	5
2.2.2. Choosing plots in crop land	5
2.2.3. Choosing plots in the village	5
2.3. Establishing the fixed plot	6
2.3.1. Establishing a fixed plot in semi-natural habitat or cropland	6
2.3.2. Establishing a fixed plot in the village	8
2.4 Plot replacement protocols	9
2.5 Recording details about the plot	9
3.0. Recording the pollinators	10
3.1. Appropriate conditions for surveying pollinators	10
3.2. Pollinator survey equipment	10
3.3. Preparing to start the survey	10
3.4. Moving around the survey plot	11
3.5. Catching pollinators	12
3.6. Transferring pollinators to killing tubes	13
3.6.1. Transferring non-butterfly insects	13
3.6.2. Transferring butterflies and moths	14
3.7. Recording missed or escaped pollinators	14
3.8. Recording details about each pollinator	15
3.9. Survey rules and avoiding bias	16
3.10. Emptying the killing tubes	16
3.11. Justifying the killing of pollinators	17
3.11.1. Why we need to kill pollinators	17
3.11.2. Effects on pollinator populations	17
4.0. Floral abundance surveys	
4.1. Floral abundance survey methods	
4.1.1. Placing quadrats in cropland and semi-natural habitat	
4.1.2. Placing quadrats in village gardens	19
4.1.3. Recording flowers in each quadrat	20

	22
4.1.4. Recording plants flowering outside the quadrats	
4.1.5. Identifying plants	
4.1.6. Recording and photographing new plant species	
4.2. Survey forms for floral abundance	24
5.0. Pollinator specimen preservation	25
5.1. Preserving medium-large insects	25
5.2. Preserving very small insects	
5.3. Preserving butterflies and moths	27
5.4. Transporting and managing the specimens	
6.0. Timescale and timeline	
6.1. Timings of individual plot surveys	
6.2. Pollinator survey timeline	
7.0. Training of field staff	
7.1. Training procedures	
7.2. Training topics	
7.3. Logistics to organise for course	
8.0. Management of field team	Error! Bookmark not defined.
8.1. Field team meetings	Error! Bookmark not defined.
8.2. Monitoring and evaluation	Error! Bookmark not defined.
8.3. Data management strategy	Error! Bookmark not defined.
9.0. General recommendations from survey experience	
9.1. Recommendations from Tom	
10.0. Health and safety considerations	
Appendices	
Appendix 1: Preparing insect killing jars	
Appendix 2: Preparing body bags for insects	
Appendix 3: Making triangular envelopes for butterflies	
Appendix 4: Making a quadrat	
Appendix 5: Full equipment list	
Appendix 6: Identifying pollinator groups	

2.0. Establishing the survey plots:

2.1. Overview

- In each of the 10 study villages, a 600m x 600m square will be drawn around the central point of each village using satellite imagery (see Figure 2.1). This square will constitute our entire sampling area and will include:
 - 1. the village households and gardens,
 - 2. the surrounding crops and
 - 3. semi-natural vegetation such as grassland, forest and scrub.
- Habitats will be crudely mapped using GIS software but will be confirmed and mapped out more precisely using participatory rural mapping by the data collectors.
- Within this study square, three fixed plots of 60x60 metres will be established in each of the three habitat types (village, crops and semi-natural vegetation) giving 9 plots in total (see Figure 2.1).
- The location of plots will be chosen by selecting random points within each habitat of interest on the village maps.
- The randomly chosen points in each village will be visited by data collectors to check their suitability and establish the plot in the nearest suitable area to this point, following a set of rules/criteria (see Section 2.2 below).
- Each plots will be visited by Sujan/Dambar to validate the work. Once confirmed, the four corners of each plot will be marked on the ground with coloured wooden pegs or poles so that data collectors can locate the same plot each visit.

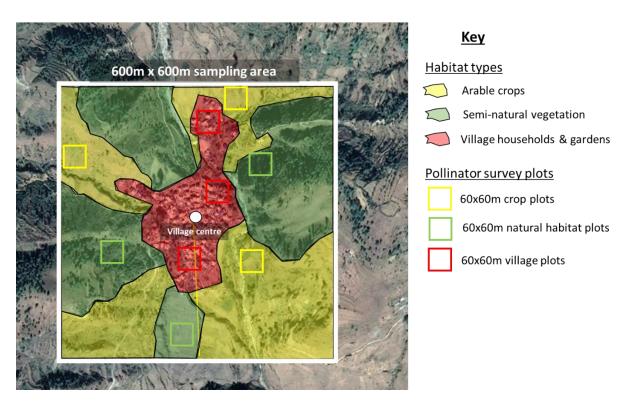


Figure 2.1: Example of a study village and surrounding 600m x 600m pollinator survey area, with habitats shown in different colours. Three replicate fixed plots of 60x60m will be placed in each habitat following a set of criteria detailed below.

2.2. Plot selection criteria

Once the randomly chosen point has been identified on the ground, you should establish the plot in the nearest place to this point which meets the criteria outlined below. The criteria are slightly different for each habitat.

2.2.1. Choosing plots in semi-natural habitat

- Is the area around this point safe to walk around in? I.e., no steep cliffs or dangerous ground
- Will you be able to get permission to survey in this area?
- Can this point be reached from the village without danger to the data collector?
- Is there enough space around this point to establish a plot of 60x60 metres (or equivalent area but a different shape) which is primarily in semi-natural habitat?

If the answer to all these questions is YES, then you can proceed to set up the plot using the methods detailed in section 2.3.1

If the answer to any of these questions is no, then look around you and try to find the nearest area to this point where all of these conditions are met. DO NOT search for areas which seem to be better for plants or pollinators, the choice of location must ONLY be based on the practical criteria above.

2.2.2. Choosing plots in crop land

- Does the area around this point include patches of flowering, pollinator-dependent crops such as pumpkins, beans, carrots, squash, buckwheat, apples, peaches, mustard, soybeans, karela or various other fruits and vegetables? It MUST NOT be totally dominated by non-flowering crops such as rice, wheat, maize, sorghum, millet and barley.
- Is the area around this point safe to walk around in? I.e., no steep cliffs or dangerous ground
- Will you be able to get permission to survey in this area?
- Can this point be reached from the village without danger to the data collector?
- Is there enough space around this point to establish a plot of 60x60 metres (or equivalent area but a different shape) which is primarily in cropland?

If the answer to all these questions is YES, then you can proceed to set up the plot using the methods detailed in section 2.3.1

If the answer to any of these questions is no, then look around you and try to find the nearest area to this point where all of these conditions are met. DO NOT search for areas which seem to be better for pollinators, the choice of location must just be based on the practical criteria above and the crops that are being grown.

2.2.3. Choosing plots in the village

• Does the area around this point include patches of fruit or vegetable garden? It is fine for the surveying area to include houses, roads and bare ground but it MUST NOT be totally dominated by these areas. At least three quarters (75%) of the area should be comprised of gardens and other vegetation.

- Do the gardens in this area include some flowering, pollinator-dependent crops such as pumpkins, beans, carrots, squash, buckwheat, apples, peaches, mustard, soybeans, karela or various other fruits and vegetables?
- Will you be able to get permission to survey in these gardens?
- Is there enough space around this point to establish a plot of 60x60 metres (or equivalent area but a different shape) which is primarily in the village? You will probably need to link together multiple houses and gardens to form a plot that is big enough.

If the answer to all these questions is YES, then you can proceed to set up the plot using the methods detailed in Section 2.3.2

If the answer to any of these questions is no, then look around you and try to find the nearest area to this point where all of these conditions are met.

2.3. Establishing the fixed plot

2.3.1. Establishing a fixed plot in semi-natural habitat or cropland

Semi-natural habitat and cropland is normally fairly open and easy to move around in (unlike village gardens), so once you have found a suitable location for your surveying, the fixed plot can be established using the systematic rules listed below:

- Stand on the randomly chosen point shown on your map, or the new point you have chosen to meet the criteria shown in Section 2.2. This will be the approximate centre point of your plot and you can temporarily mark this with a rock or a small stick.
- Spin a pen/pencil on the ground to choose a random direction to walk from this central point.
- If this direction is not practical or safe to walk in, or it immediately passes into a different habitat, spin the pen again until you find a direction that is safe and suitable.
- Walk 40 large paces in this direction and then stop and mark this point with a bamboo pole. If you reach the edge of a different habitat before 40 paces (i.e., crops or village) then stop at this point. Otherwise continue for the full 40 paces. This will be the first corner of your sampling area (Figure 2.2).
- Now turn and face the central point where you came from. Turn 45 degrees (approximately) to the right of this and measure out 60 metres along this line using a measuring tape.
- At 60 metres place another bamboo pole to mark your second corner
- Now turn 90 degrees to the left and measure out another 60 metres along this line to reach your third corner. Mark this with a bamboo pole as well. Repeat this for the fourth pole too.
- Turn 90 degrees to the left once more and you should be able to see your first pole in a clear line in front of you. It should be approximately 60 metres away.
- It is NOT A PROBLEM if the plot passes into a bit of another habitat. In fact, it is important that we include some habitat edges in our surveys. However, a minimum of three quarters (75%) of the plot MUST be within the correct habitat.
- Finally, place an additional two poles halfway (30 m) along opposite sides of the plot. If one edge of the plot is marked by a fence/wall/hedge/field edge, make this one of the sides with the halfway marker. If none of the plot edges have a boundary feature, it does not matter which sides these are placed but they must be opposite each other (Figure 2.2). These will be used to mark the start and end of the floral abundance surveys.

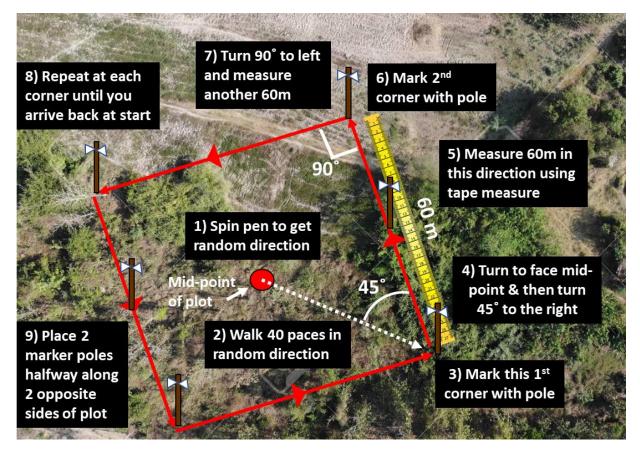


Figure 2.2. Diagram showing how to mark out a 60m x 60m fixed plot in open habitat such as cropland or semi-natural habitat

Being practical

- It will NOT always be possible to establish the plot following the systematic rules above. For example, a fence/river/wall may run through the plot, making it difficult for data collectors to keep crossing back and forth when they are doing their survey.
- In these cases, it is FULLY ACCEPTABLE to shift the location of the plot so that it ends at the fence/river/wall and this forms one edge of the plot. This edge must still be included in your pollinator surveys though.
- Don't worry about the plot being exactly 60x60m in area just as long as it is approximately this size.

Changing plot shape

- It is also acceptable to change the shape of the plot so that it fits within the area of habitat that is accessible. The total plot area <u>must</u> remain the same though and it is important that you calculate the new width and length correctly.
- For example, a normal square plot is 60x60 metres = 3600m². However, if there is a strip of accessible habitat which is only 30 metres wide and therefore your plot needs to be 30 metres in width, you can divide 3600m² by 30m to give 120m. Therefore, 120m will be the length of your new plot because 120 x 30 = 3600m²
- The plots do not need to be EXACTLY the right size and shape it is too difficult to measure out precise areas in the field. They just need to be approximately 3600 m².

2.3.2. Establishing a fixed plot in the village

Establishing a plot in the village is more difficult because the area is normally divided up into lots of small gardens separated by walls or houses. Therefore, it is not possible to establish plots using the rigid systematic methods shown above. Instead, it is important to be flexible and practical but follow these guidelines:

- Find a cluster of neighbouring gardens which can be joined together to form a plot of roughly 60x60m (3600m²) (Figure 2.3).
- As explained above, the shape of the plot can change, but not the overall area.
- Use garden walls or fences as the outer boundaries of the plot if you need to.
- Tie some coloured ribbon or a place a bamboo pole on the outer corners of the plot so that data collectors can remember where the plot ends.
- The plot will now be composed of multiple small gardens with houses or walls dividing up the area. This will make it difficult to move freely around the area in the plant and pollinator surveys, so each sub-section of the plot may need to be surveyed separately, dividing up the total sampling time between the different sections.

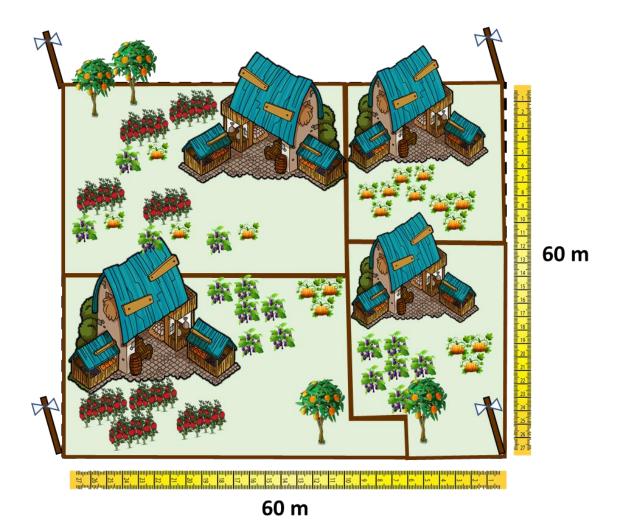


Figure 2.3. Diagram showing how groups of neighbouring houses and gardens can be joined up to form one full 60m x 60m survey plot.

2.4 Plot replacement protocols

Consider replacing a survey plot if:

- It becomes very difficult or dangerous to access (e.g., you no longer have permission)
- The area is destroyed in some way so that it is no longer the same habitat it started as (e.g., an area of grassland is ploughed for crops or a forest is cut down). Note that it does not matter if an agricultural plot is ploughed, weeded or planted – this will naturally happen throughout the year)

If you do decide to replace the plot, choose a replacement that is:

- Within the same habitat type (village, crop or semi-natural) as the existing plot
- Meets the plot criteria shown in Section 2.2
- Still within the 600mx600m survey area
- The same total area as the original plot (3600m²)

2.5 Recording details about the plot

Once the plot has been established and all four corners marked, you should record the following details in the provided ODK form or paper form:

- Give the plot a unique code. This should be the first four letters of the village name (e.g. <u>LORP</u> for Lorpa or <u>GADI</u> for Gadigaun, followed by an Underscore (_) and then the first letter of the habitat type (<u>V</u> for village; <u>C</u> for crop and <u>N</u> for natural), another underscore (_) and then number 1, 2 or 3 as there will be three plots in each habitat. For example, the second village plot in Rini would be: "RINI_V_2"
- Take a GPS waypoint at each of the four corners so that we know their location even if the marker poles are removed.
- Give the local name of this area.
- Briefly describe the type of habitat (e.g., apple orchard with surrounding hedges OR mix of pine forest and grassland OR village gardens and waste ground etc.)
- Record which crops are present (or are likely to be planted) in this plot.
- Record the names and phone numbers of anyone who owns the land and may need to be asked for permission or contacted at any point.
- Take lots of photographs of the plot so that we can see the vegetation type, the outline of fields, the topography etc.

<u>Note</u>, that this information only needs to be recorded <u>once</u> by Sujan or Dambar at the time of plot establishment.

3.0. Recording the pollinators

Pollinators will be surveyed for 30 minutes in each plot once every two weeks from April – November. These surveys should take place BEFORE the floral abundance surveys to avoid disturbing any insects in the site. Keep a track of the date on which you survey each plot so that you know when you next need to survey it (2 weeks later).

3.1. Appropriate conditions for surveying pollinators

Pollinator surveys should only be conducted:

- Between the hours of 10am and 6pm
- When the sun has already reached that part of the valley (if the plot is situated on a westfacing slope which only gets sun later in the day, survey this plot in the afternoon)
- When it is not raining
- When wind is light or absent (branches swaying is fine but not whole trees)
- When there is limited disturbance in the plot (i.e., harvesting/planting/ploughing should not be occurring at the time of the survey)

[All of this information will be asked at the start of the ODK form to check suitability]

3.2. Pollinator survey equipment

The following items should be carried by each data collector when they go to survey pollinators and plants (Figure 3.1):

- Shoulder bag for carrying all equipment
- Insect sweep net for catching pollinators
- Tablet or smartphone with ODK form loaded (ensure fully charged before leaving)
- Plant and pollinator atlas (printed and bound)
- Paper survey forms in case of phone failure
- 50 small killing tubes loaded with ethyl acetate (see Appendix 1 for preparing these)
- 2 large killing jars loaded with ethyl acetate (prepared in the same way as the tubes)
- Stopwatch for measuring the duration of the pollinator survey
- Cut squares of paper (10x10cm) for making body bags (see Appendix 2)
- Pre-prepared paper envelopes for storing butterflies (see Appendix 3)
- Hat to reduce glare on screen
- Spare battery pack for charging phone in field
- 1m² quadrat for floral abundance surveys (see Appendix 4 for how to make a quadrat)

See Appendix 5 for a full list of pollinator survey equipment required.

3.3. Preparing to start the survey

- Walk to the survey plot and stand on the edge
- Check that the conditions are right (see Section 3.1) and the area is safe to survey
- Check with landowners that it is okay to survey
- Prepare all equipment (see Section 3.2)
- Think about everything you are going to do and read the instruction summary once more
- Load the ODK pollinator survey tool on your phone/tablet
- Start your timer and begin the 30 minute survey



Figure 3.1: Equipment to prepare and take on each plant and pollinator survey

3.4. Moving around the survey plot

- Start on a corner and begin to walk randomly around the plot, moving from flower to flower (Figure 3.2).
- Inspect each patch of flowers very thoroughly and catch any insect you see visiting a flower (see Section 3.5)
- As soon as you catch the insect, stop the timer while you process the specimen and record its details. Only restart the timer once you are ready to start searching again.
- If there are multiple insects at this flower patch, continue catching them for a maximum of <u>5</u> <u>minutes</u> on your timer and then continue so you don't get stuck in one place.
- If you see no insects at a flower patch, continue to the next patch immediately.
- If you have covered the rest of the area and have not found any other insects, you can return to patches that had lots of insects before.
- The aim is to <u>maximise</u> the number of insects you collect, but also to try and cover the <u>whole</u> <u>area</u> of the survey site thoroughly within the 30-minute period.
- Once you have completed the 30 minute survey, stop looking for insects
- Look through all of your records from the plot and check that you have completed all information that is required

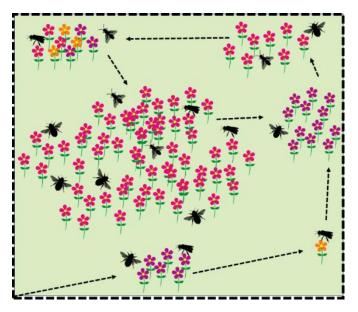


Figure 3.2. Diagram showing how data collectors should move around the 60x60m survey plot when searching for pollinators

3.5. Catching pollinators

- Whenever you encounter an insect visiting a flower, catch it by sweeping the net at the flower so that the insect enters the net. Sometimes you will need to hit the flower as well (see Figure 3.3a)
- For very small insects or low flowers, it is sometimes better to place the net over the flower and then tap the flower so the insect flies up into the net (Figure 3.3b)
- Once the insect is in the net you must swipe the net from side to side so that the insect moves down to the end of the net where it is trapped by flicking the net over the frame (Figure 3.3c)

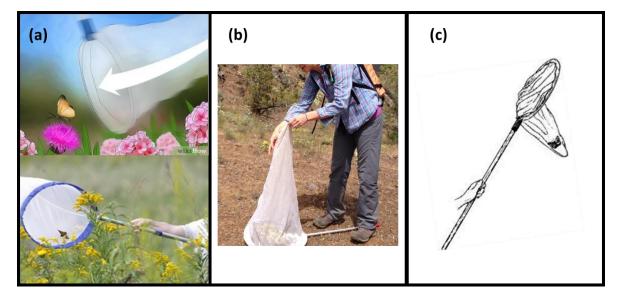


Figure 3.3. Images showing how insects should be captured from flowers.

The first thing to work out once you have caught an insect is broadly what type of insect it is because this will determine what you do with it.

- If it is a <u>non-butterfly insect</u> (e.g., a bee, fly, wasp, beetle etc.), you will transfer it straight to a small killing tube following the methods in Section 3.6.1 and then record its details following the methods in section 3.8.
- If it is a <u>butterfly or moth</u>, you will pinch it in the net, put it in a prepared paper envelope and then transfer it to a killing jar following the methods in Section 3.6.2 and then record its details following the methods in section 3.8.
- If it is a <u>bumblebee</u>, you should <u>NOT kill it</u> in the first month of surveying (April) because it is likely to be a queen, but you should still record all of its details following the methods in section 3.8. After April, you can catch and kill bumblebees as normal. See below for more details.

Queen bumblebees generally emerge from hibernation in March and April and are very important as they will be the ones to lay lots of eggs and raise offspring, so killing them will kill a whole colony and is therefore <u>not ethical</u>. They are sometimes difficult to tell apart from worker bumblebees, so to be safe, we will NOT KILL any bumblebees in March and April. It is still very important that we record these bumblebees though, so if you see a bumblebee visiting a flower in March or April, you must still catch them to confirm they are a bumblebee and then give them a unique specimen code and record all of its details in the normal way (see Section 3.8). But after recording the details, instead of transferring it to a killing tube, you will release it.

After April (May onwards), queens normally stay in their nests so it is only workers that we see flying around and it is therefore fine to start capturing bumblebees and collecting them in the same way as the other insects.

3.6. Transferring pollinators to killing tubes

The protocols for transferring insects to killing tubes is slightly different for butterflies and moths than for other insects like bees, wasps, flies etc. so these will be discussed separately.

3.6.1. Transferring non-butterfly insects

This approach should be used for all insects apart from moths and butterflies:

- Once the insect is at the bottom of the net, hold the net bag near the bottom so that the insect is trapped at the end.
- Get a killing tube and open the lid (Figure 3.4b)
- Move the tube down the net towards your other hand and push it through the bunched up net so that its opening is available to the insect to enter (Figure 3.4c)
- Keep moving the tube down towards bottom of the net so the insect is forced to enter and then quickly replace the lid while still in the net (Figure 3.4d)
- Write a small paper label with the insect's unique code and put in the tube.
- Only put one insect in each tube

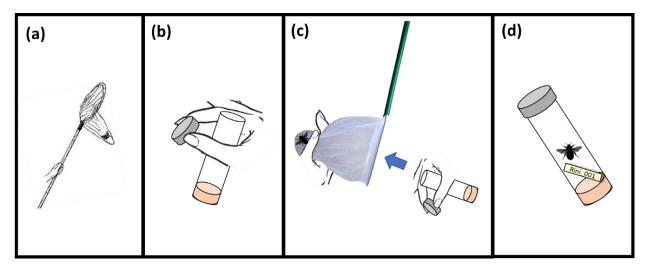


Figure 3.4. Diagram showing how to transfer insects from the net into a killing tube

3.6.2. Transferring butterflies and moths

Butterflies and moths are normally too large to fit into the small killing tubes and can damage their wings in the tube, making identification more difficult. Therefore, follow these alternative methods:

- a) Once the butterfly is at the bottom of the net, hold the net bag near the bottom so that the insect is trapped at the end (Figure 3.5a).
- b) Hold the butterfly by its thorax (chest) while it is still in the net bag and give this part of its body a gentle pinch to stun it and stop it moving. It is important that you squeeze the chest, not any other part of its body (Figure 3.5b).
- c) Take a pre-prepared triangular paper envelope (see Appendix 3 for instructions on making these) and write the unique specimen code on the <u>back of this</u>, using a <u>pencil (Figure 3.5c)</u>.
- d) Now that the butterfly is immobilised, open the net and transfer the butterfly gently into the labelled triangular paper envelop with its wings held <u>closed</u> above it (Figure 3.5d).
- e) Fold over the top flap of the envelope tightly so that it remains firmly shut (Figure 3.5e).
- f) Place the envelope into the large killing jar and leave for 2-3 hours to complete the process.

3.7. Recording missed or escaped pollinators

Sometimes you will see an insect visiting a flower and try to catch it, but you are unsuccessful, and it escapes. Or you manage to capture it but it escapes while you are transferring it to a tube. This is not a problem, but it is important that you <u>still record these insects</u>.

For each of these escaped insects, you will record it in the normal way by giving it a unique code, classifying what pollinator group it was, recording the plant it was found on and the habitat you found it in (following Section 3.8). However, you will note in the ODK or paper form that it was a missed capture.

It is important that these pollinators are recorded even if they are not captured because they will still be used in our data analysis.

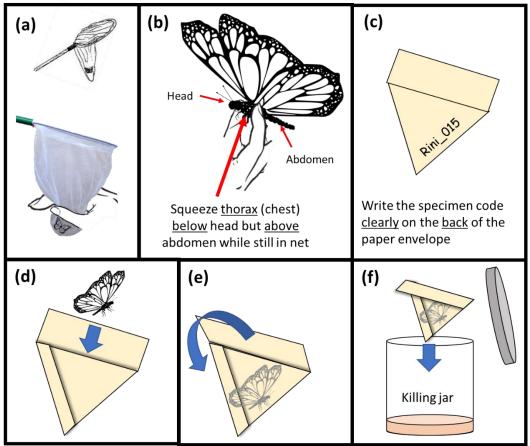


Figure 3.5. Diagram showing how to stun butterflies or moths in the net and transfer them safely to a paper envelope and killing jar. See Appendix 3 for instructions on how to prepare a triangular paper envelope for storing butterflies.

3.8. Recording details about each pollinator

As soon as each insect has been captured and transferred to a killing jar, record the following details about it in the ODK form or traditional paper form (if ODK not available).

- A unique code linked to that specimen. This will be the name of the site (e.g., Village A) followed by number a unique number (e.g., 001). Each specimen captured in that village should be given a unique sequential number i.e., the first one will be 001, the second 002 and so on, throughout the whole year. (very important)
- The species of plant that the insect was captured on (very important)
- Type of insect (HONEYBEE, SOLITARY BEE, BUMBLEBEE, BUTTERFLY, MOTH, FLY, BEETLE, WASP or UNKNOWN) see Appendix 6 for an identification guide to these groups.
- The type of habitat where it was captured (for example: forest, bushes, river edge, rice field)

After processing each specimen, <u>write down the unique code</u> you just gave it on a piece of paper or a notebook so that you can remember what the next code number needs to be when you catch another pollinator in the next plot, or even the next day.

When using the ODK form, plants will be selected by scanning the appropriate QR code in the plant atlas and the pollinator group and habitat will be selected from a drop-down list. See Section 4.1.5 for more details on how to record plant species using the plant atlas.

If using a traditional survey form, it will look something like the one below (Figure 3.6).

Village: Rini Plot code: R3 Date: 25/05/2021						
<u>Jnique specimen</u> code	<u>Pollinator typ</u>	e <u>Plant captured on</u>	Habitat where captured			
Rini_001	Bumblebee	Pumpkin	Vegetable field			
Rini_002	Bumblebee	Tomato	Vegetable field			
Rini_003	Honeybee	Pumpkin	Vegetable field			
Rini_004	Fly	Anthriscus sylvestris	Edge of field			
Rini_005	Wasp	Prinsepia utilis	Hedgerow			
Rini_006	Honeybee	Pumpkin	Vegetable field			
Rini_007	Beetle	Carrot	Vegetable field			

Figure 3.6. Example of a traditional pollinator survey form

3.9. Survey rules and avoiding bias

Rules to follow when surveying pollinators:

- Only catch insects INSIDE the 60x60m plot area. Ignore any outside this area.
- Only catch insects that you see visiting a flower the information is useless without a link to the flower.
- If an insect moves from one flower to another, just record the <u>last flower</u> it visited on your survey form.
- If you catch two insects on the same flower, they must each still have a <u>unique code</u>.
- Record and label insects <u>as soon</u> as they are in the killing tube (straight away) do not leave it until the end of the survey as you will forget the details.

Avoiding bias when surveying pollinators:

Some insects are easier to see and catch than others and this can create bias in our results. To avoid this, you must:

- Look carefully at each flower and catch <u>all</u> insects visiting the flower, not just the big ones but also small ones like flies.
- Focus on searching for flowers rather than searching for insects you should not wait until you see an insect flying or moving and then follow it. Instead, look for them on flowers.

3.10. Emptying the killing tubes

- If you have enough killing tubes left for the next plot or you are going to pin the specimens immediately, you can leave the insects in their individual tubes.
- But if you need to empty the tubes and use them again before pinning, do the following before the next survey:
 - 1. Check the insect is completely sedated or dead
 - 2. Make a body bag by wrapping a small piece of paper around your finger and folding up one end (see Appendix 2 for instructions on how to make these).
 - 3. Label the bag with the unique specimen code
 - 4. Tip the dead/sedated insect into this bag and fold up the end

- 5. Repeat with all insects and place all of the body bags into one single large killing jar
- 6. Leave in this jar for a minimum of 3 hours before they are ready to pin

3.11. Justifying the killing of pollinators

We will need to kill all of the pollinators we catch (with the exception of bumblebees in March/April) and it is important to explain to data collectors, beekeepers and the local community why we need do this.

We also need to explain that the numbers we catch will NOT have any meaningful effect on the populations of these pollinators.

3.11.1. Why we need to kill pollinators

- It is almost impossible to identify most pollinators while they are alive they need to be closely inspected, often under a microscope by a trained taxonomist.
- Even honeybees can look very much like other wild pollinators (Figure 3.7) and it is important that we can identify them later.
- Without knowing the identity of the pollinators we catch, we cannot understand how to protect them or make specific management recommendations to boost their pollination
- Pollinator collections are extremely valuable for research many museums and universities keep collections so that they can learn the different species and have a record of the pollinators of their country.
- Nepal has a limited collection of pollinating insects our collection will provide an important contribution to the national archives, strengthening research capacity.

3.11.2. Effects on pollinator populations

- We will only be catching pollinators in a small 60x60m area for <u>30 minutes every 2 weeks</u>.
- In this time, we are very unlikely to catch more than 10 or 15 individuals of any particular species.
- Honeybee hives often have over 10, 000 bees in them, so taking 10 or 15 every two weeks will have absolutely <u>no impact on the colony</u>.
- The number of bees we are killing is extremely low compared to mortality from disease and predators such as birds.
- A recent peer-reviewed paper demonstrated that sampling pollinators every two weeks and killing them to identify the specimens has <u>absolutely no effect</u> on their overall populations or provision of ecosystem services (Gezon *et al.* 2015)

Gezon, Z. J., et al. (2015). "The effect of repeated, lethal sampling on wild bee abundance and diversity." *Methods in Ecology and Evolution* **6**(9): 1044-1054.



Figure 3.7. Example of the similarity between a solitary bee (left) and a honeybee (right).

4.0. Floral abundance surveys

After completing the 30-minute pollinator survey in each plot, you must then conduct a floral abundance survey in the same plot so that we can estimate the number of flowers of each plant species in the plot at that time.

Why is this important?

- Different plants flower at different times and we want to find out when each plant is flowering this will allow us to model the effects of climate change which shifts flowering times.
- If we know how many flowers there are of each species, we can work out which plants are important to pollinators because they are actually preferred by them, not just because they are more abundant. We therefore need to record flowers independently of pollinators but in the same plots.

4.1. Floral abundance survey methods

Floral abundance is surveyed by placing 15 quadrats of $1m^2$ at regular intervals throughout the 60x60m plot. Quadrats are rigid square frames used for measuring a fixed area in a systematic way (see Appendix 4 for instructions on making your own quadrat).

Because of the differences in how easy it is to move around the 60x60m sampling area, different rules will need to be used for placing quadrats in villages than for placing them in cropland and seminatural habitat.

4.1.1. Placing quadrats in cropland and semi-natural habitat

- Start from the corner of your 60x60m plot and pace out 10 large strides along the edge of the plot towards the marker post at the other corner. Do not pace towards the middle marker post, you should instead be walking along the <u>other</u> plot edge (Figure 4.1).
- When you reach exactly 10 strides, place the quadrat immediately to the <u>right</u> of your foot
- Do NOT aim to include extra flowers the placement must be systematic, not biased
- Record all of the species in flower in this 1m² area and give each one an abundance score (Section 4.2).
- Pace out another 10 strides along the same line and this time place the quadrat immediately to the <u>left</u> of your foot. Alternate the placement between right and left each time.
- When you have completed 5 quadrats along this line, walk to the <u>middle</u> marker post and repeat the same process heading in the direction of the opposite middle marker post (i.e. passing directly through the middle of the plot).
- After 5 quadrats along this line, repeat the process once more along the far side of the plot to give a total of 15 quadrats (Figure 4.1).
- If any of your quadrats fall on bare ground with no vegetation or flowers, that is fine, just record everything as zero and continue on to your next quadrat. These zero values are still very important to record.

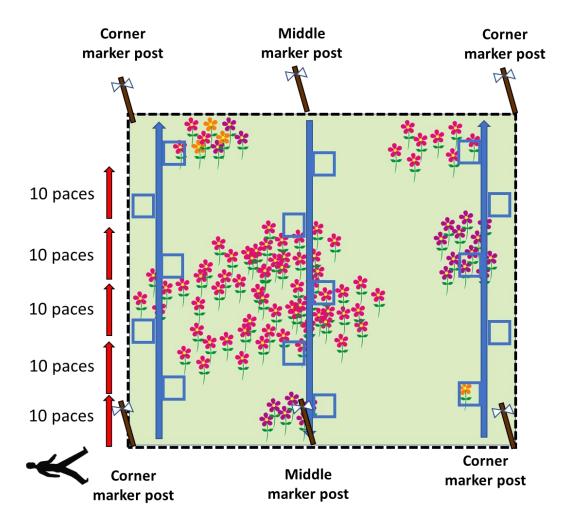


Figure 4.1. Diagram showing how quadrats should be placed in the 60x60m survey plot

4.1.2. Placing quadrats in village gardens

Village gardens will be more difficult to sample because they aren't a single open space. Some fenced crop fields might be like this too.

In these cases, we need an alternative method for placing quadrats systematically across the area WITHOUT BIAS (i.e., following set rules).

Methods:

- Start at the corner of the first garden within the area of your 60x60m plot area.
- Walk 5 large paces towards the opposite corner of the garden so that you are going straight across the middle of the garden (see Figure 4.2).
- After exactly 5 paces, place a quadrat immediately to the <u>right</u> of your foot.
- Take another 5 paces and place a quadrat immediately to the <u>left</u> of your foot.
- Keep repeating these 5 paces, alternating sides each time until you reach the far corner of the garden.
- If you reach the edge of a house in the middle of a garden, skip this section of the walk and continue on the other side of the house.

- Once you have completed one field or garden in this manner, move onto the next one and repeat the same procedure.
- Repeat this procedure with all of the gardens in your 60x60m survey area.
- If there areas <u>between</u> gardens within the survey plot (like patches of weeds or paths etc.), you should continue your 5 metre quadrats <u>in between</u> one garden and the next, following a straight line from one gate or wall to the other
- If any of your quadrats fall on bare ground with no vegetation or flowers, that is fine, just record everything as zero and continue on to your next quadrat. These zero values are still very important to record.
- You may end up with more than 15 quadrats following this protocol this is NOT A PROBLEM.

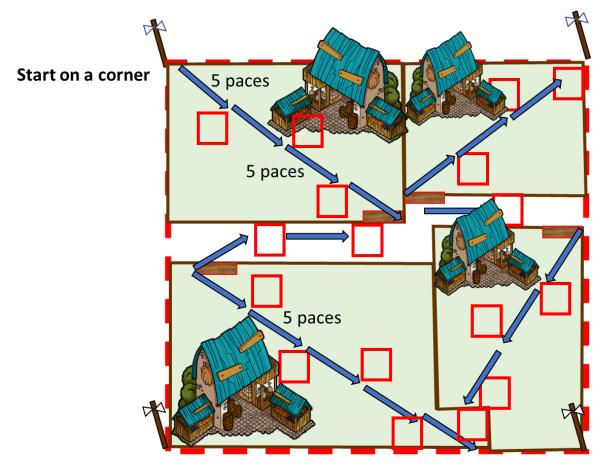


Figure 4.2. Diagram showing how quadrats should be placed within village garden plots.

4.1.3. Recording flowers in each quadrat

For each plant species that is flowering in a quadrat, you must:

- Identify the plant species using the plant atlas and scan its QR code in the ODK form. If you cannot identify the species or find it in your plant atlas, you will be asked to give a description of it and take a photograph (see Sections 4.1.5 and 4.1.6)
- Next, you will give the plant species an abundance score for its flowers using the following scale:

0 = no flowers present

1 = rare (<3 flowers present)</pre>

2 = scattered (3-10 flowers present)

3 = common (10-20 flowers)

<mark>4= dominant (<20 flowers)</mark>

(These should be very rough and rapid estimates)

- If there is more than one species of flower present in your quadrat, you should identify and record all of them in the same way.
- Once you have recorded all of the different species of flower in your quadrat, move onto the next quadrat.
- If there are no plants flowering in your quadrat (e.g. grass or bare ground), you will record this as a zero value and move on – zero values are still important, so you MUST record them.

Difficult quadrats

- If the quadrat is placed on a bush or underneath a tree, include all of the flowers directly above and below it in your abundance estimates, as shown in Figure 4.3.
- Some plants group their flowers together into tight clusters so when estimating the number of flowers for these species, just consider one cluster as an individual flower (Figure 4.4).
- The general rule is that we consider a cluster of flowers as one single unit if insects can easily move between flowers within the cluster without flying.

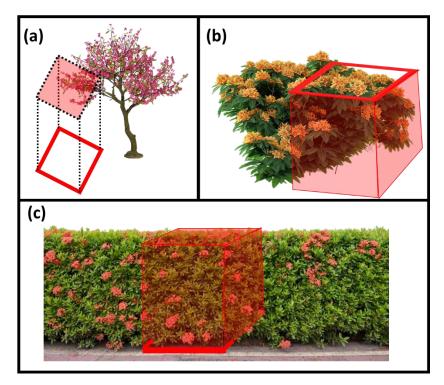


Figure 4.3. Diagram showing how quadrats should be placed over bits of vertical vegetation such as bushes, trees and hedges. For any trees overhanging the quadrat (a), imagine a vertical extension of the quadrat directly above where it is placed on the ground and include all flowers within this vertical column. If the quadrat lands on a bush (b), place it directly over the bush and count all flowers beneath the quadrat. For tall hedges or bushes where it is not possible to place the quadrat on top (c), place the quadrat underneath the hedge and count all flowers directly above it.

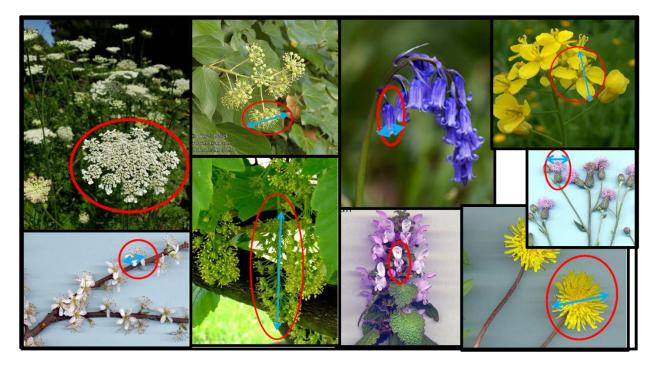


Figure 4.4. Some examples of what to consider as an individual flower unit when estimating flower abundance. The red rings show what should be considered as a single flower. In general, consider a cluster of flowers as a single flower when you think an insect could move between them without having to fly.

4.1.4. Recording plants flowering outside the quadrats

There will be some plant species which are flowering within the 60x60m plot area but do not appear in any of the 15 quadrats. Data collectors should record these in the following way:

- After all 15 quadrats have been completed, spend a few minutes walking around the remaining area of the 60x60m plot looking for flowering plants that have not been recorded
- List each of these plants in the designated place on the survey form or scan them in the ODK form
- For each plant, give them an abundance score based upon the number of flowers seen around the whole survey plot. Use the scoring system below:

A = Rare – just a few individual flowers scattered throughout the plot

B = Scattered – appears fairly regularly around the plot but in small numbers or very isolated patches which is why it didn't appear in the quadrats

 ${\bf C}$ = Common – appears regularly throughout the plot and was only missed from the quadrats by chance

4.1.5. Identifying plants

A plant atlas will be provided to each data collector to help them with the identification of the plants they find. The atlas will have a picture and brief description of all common plants likely to be found in this area and will list the local name and scientific name to minimise confusion. Each plant species will also have a QR code which can be scanned by data collectors to log the correct plant species in their ODK form (e.g. Figure 4.5a).

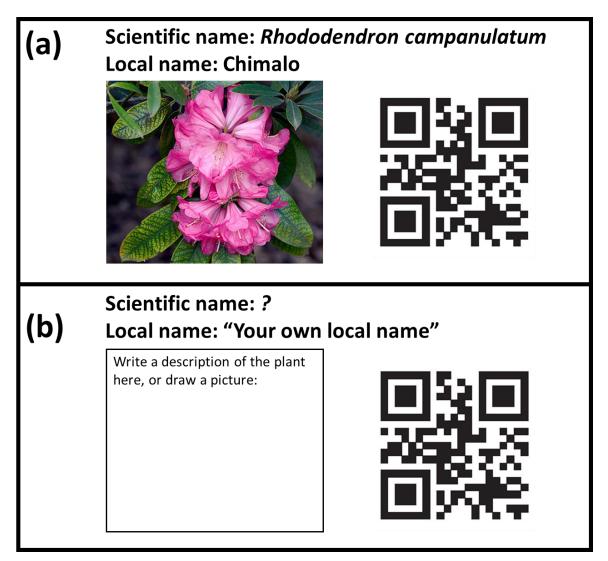


Figure 4.5. An example of what the plant atlas will look like. Each plant species will have a clear picture with scientific and local name included. There will also be a QR code to scan. For new species (e.g. b), there will be spaces to write your own description so that you can use this QR code in future

4.1.6. Recording and photographing new plant species

If there are any plants that the data collectors do not recognise or cannot find in their plant atlas, they should give the plant its own unique description (e.g., purple bell flower with grey spiky leaves) and take a photograph in the ODK form so that it can be later identified. They should then keep using this same unique description whenever they see this plant. There will be a section of the plant atlas where they can record these new plant species next to a QR code so that they can scan this when they encounter this species in future (e.g. Figure 4.5b). When taking photographs of a new plant species, it is important to photograph it from multiple angles and to photograph different parts of the plant (e.g. leaves, flowers, whole plant) as this will help us to identify it. The ODK form will remind you how to take good photographs, but it helps to remember these basic rules:

- Make sure the plant is still (i.e. not moving around with wind)
- Avoid getting lots of other plants in the same picture. To avoid this you can put a piece of paper behind the plant so that it is totally clear.
- Do not bring the camera too close to the flower/leaf. It is better to keep the camera 30 cm away and zoom in on the flower
- Make sure the plant is in-focus and not blurred in the picture
- Avoid taking the picture in very bright harsh sunlight. If it is easier, you can pick a stem with leaves and flower from the plant and move it to a place with better light where the picture will be more clear
- If any of the pictures aren't clear just re-take them before moving on

4.2. Survey forms for floral abundance

An ODK tool will be created to allow data collectors to log the information from each quadrat by scanning the QR code for a plant and then entering its abundance score from a drop-down list. There will also be an option to take photographs of a plant if they cannot identify it.

In the event that the ODK form fails, data collectors can use the traditional survey sheet. An example is provided below in Figure 4.6.

	F	lot	code	e: R3	5	D	ate:	25/	05/	2021	L				
						Flow	er ab	undar	nce sc	ore ir	n eact	n quac	Irat		
Image	4	1	l = rare	e (<3 flo	owers p	resent)		2 = s	cattere	d (3-10	D flowe	rs pres	ent)	
Code		-	-	3 = cor	nmon (1	10-20 f	lowers)		4= d	lominar	nt (<20	flowers	s)	1
	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15
	3	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	2	0	1
	2	0	0	0	0	0	0	1	0	0	4	0	0	0	0
	0	0	0	0	0	0	0	0	2	0	0	0	0	1	3
	4	0	0	4	0	0	0	2	0	0	0	0	0	0	0
C4352	1	0	0	0	0	0	0	0	0	0	1	0	2	0	0
C4353	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Abunc	dance	score	(A,B o	r C)	Notes	:									
	Α			1											
	<u>Code</u> (4352 (4353)	Q1 3 2 0 4 C4352 1 C4353 2	Code Q1 Q2 Q1 Q2 0 3 0 2 0 2 0 0 0 4 0 0 0 C4352 1 0 0 C4353 2 0 0	Code Q1 Q2 Q3 3 0 0 0 3 0 0 0 2 0 0 0 0 0 0 0 4 0 0 0 C4352 1 0 0 Abundance score (A,B or A) 0 0	Code 3 = col Q1 Q2 Q3 Q4 3 0 0 1 3 0 0 1 2 0 0 0 0 0 0 0 4 0 0 4 C4352 1 0 0 Abundance score (A,B or C) 0 0	$\begin{tabular}{ c c c c c } \hline I = rare (<3 flowers $$$ $$ $$ $$ $$ $$ $$ $$ $$ $$ $$ $$ $$	$\begin{tabular}{ c c c c c c } \hline \hline I &= rare (-3 flowers present) &= common (10-20 flowers) &=$	$\begin{tabular}{ c c c c c } \hline \hline I &= rare (<3 flowers present) & 3 &= common (10-20 flowers) & 0 &= 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & $	Image Code 1 = rare (*3 flowers present) 3 = common (10-20 flowers) Q1 Q2 Q3 Q4 Q5 Q6 Q7 Q8 3 0 0 1 0 0 0 0 3 0 0 1 0 0 0 0 2 0 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 1 4 0 0 4 0 0 0 0 0 2 0 0 0 0 0 0 0 0 4 0 0 0 0 0 0 0 0 2 0	Image 1 = rare (<3 flowers present) 2 = s Code 3 = common (10-20 flowers) Q1 Q2 Q3 Q4 Q5 Q6 Q7 Q8 Q9 3 0 0 1 0 0 0 0 0 0 3 0 0 1 0	Image Code 1 = rare (<3 flowers present) 2 = scattere 0 1 Q2 Q3 Q4 Q5 Q6 Q7 Q8 Q9 Q10 3 0 0 1 0	Image Code 1 = rare (<3 flowers present) 2 = scattered (3-10)))))) 2 = scattered (3-10 ($\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$	Code 3 = common (10-20 flowers) 4= dominant (<20 flowers) Q1 Q2 Q3 Q4 Q5 Q6 Q7 Q8 Q9 Q10 Q11 Q12 Q13 3 0 0 1 0	$\begin{tabular}{ c c c c c c c c c c c c c c c c c c c$

Figure 4.6. Example of a paper survey form for recording floral abundance in each plot.

5.0. Pollinator specimen preservation

Insects will often start to rot if they are left for too long without preservation. Therefore, at the end of each day, it is important to carefully preserve all of the specimens.

We do this by pinning the insects in a specimen box while they are still fresh (and not dried out)

This allows them to be safely stored and viewed by taxonomists and other researchers who can later identify them.

There are different ways of doing this depending on the size and type of the insect.

5.1. Preserving medium-large insects

Preservation of medium-large insects (not butterflies) should be done through the following steps:

- 1. Write a VERY small but clearly readable label on a very small piece of card. This should be the unique specimen code that you gave to this insect.
- 2. Remove the insect from its killing tube or labelled 'body bag' and place a pin very carefully through the middle of its chest (thorax) from above, so that it comes out between its legs (see Figure 5.1).
- 3. Press the pin through the card label and into the foam of the specimen box (Figure 5.1) so that each insect is clearly displayed with its specimen code underneath.
- 4. Pin the insects very close to each other so that there is enough space for all of them and try to pin them in order of their sample codes (Figure 5.2).
- 5. Place small bags of silica gel and preservative chemical in the box to keep the specimens dry and prevent pests from entering.
- 6. Keep the box closed in a safe, cool place

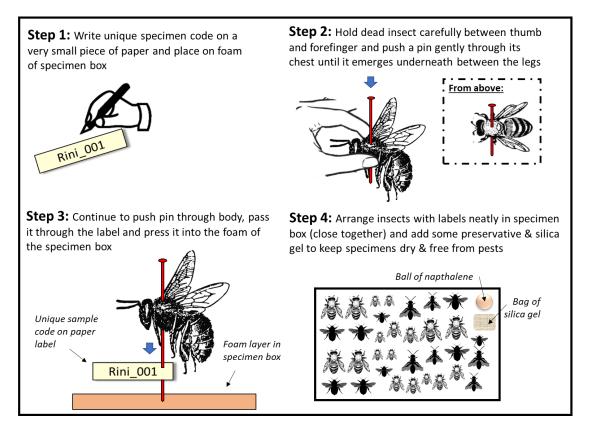


Figure 5.1. Diagram showing how to pin and preserve medium-large insect specimens



Figure 5.2. Example of an insect specimen box with labels underneath each insect

5.2. Preserving very small insects

Some insects such as small flies, wasps and bees are too small to put a pin through without destroying the specimen. In these cases, specimens should be preserved through the following steps:

- 1. Write a VERY small but clearly readable label <u>at one side</u> of a very small piece of card and place on the foam of the specimen box. This should be the unique specimen code that you gave to this insect (Figure 5.3)
- 2. Put a tiny drop of clear or white PVA glue on the end of the label
- 3. Use a pair of tweezers to transfer the tiny insect from its killing jar to place it gently on the drop of glue, facing sideways so that you can clearly see the whole insect
- 4. Pass a pin through the far side of the card and fix into place in the specimen box. The label and insect should both be clearly visible

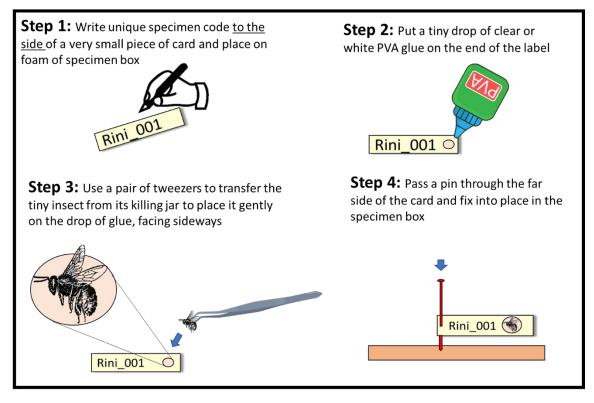


Figure 5.3. Diagram showing how to pin and preserve small insect specimens

5.3. Preserving butterflies and moths

You do <u>not</u> need to pin and mount butterflies and moths because they are already safe in their paper envelopes. Instead, you just need to do the following things:

- After 2-3 hours in the large killing jar, remove the triangular paper envelopes containing butterflies and transfer them to a secure airtight plastic box containing silica gel and naphthalene.
- Stack them neatly together in the box to save space and ensure they do not move around too much (Figure 5.4).
- You can store them in this box for weeks or even months until they are ready to be processed and identified.
- However, the envelopes <u>MUST</u> remain tightly sealed with labels clearly written on the outside. The silica gel and naphthalene must also be replaced from time to time to keep the box dry and free from pests.



Figure 5.4. Stacks of triangular paper envelopes containing butterflies being stored in a secure box

5.4. Transporting and managing the specimens

These collections are very precious and fragile so at every team meeting (monthly?) they should be carefully transported to the field office where they can be sorted and transferred to a larger box for long-term storage in a safe, cool, dry environment. Eventually, all specimens will be transported to Kathmandu where they can be identified by trained taxonomists.

The following rules should be followed when storing and transporting specimens:

- Only members of the Micro-Poll team (or trusted contacts if totally necessary) should be given responsibility for transporting specimens. They should <u>never</u> be left unattended.
- Specimen boxes should NOT be placed on the back of a motorbike or loose in the back of a car. They should be carried by hand or strapped securely to your back wherever possible.
- If it is ever necessary to transport them by car, each box should be carefully wrapped in foam and placed on a seat under the supervision of a team member.
- When transporting specimens outside of the district, carry survey permission letters with you at all times.
- Boxes are expensive so each one must be completely filled with no wasted space between specimens.
- The samples should be kept completely dry and free of pests at all times and every specimen MUST remain attached to its unique sample code – this is what links it back to the survey record.

6.0. Timescale and timeline

6.1. Timings of individual plot surveys

- Once practiced, each plot should take a total of <u>2 hours</u> to complete (including the pollinator survey and flower recording)
- Allowing for travel and set-up, it should be possible to do at least <u>2 plots per day.</u>
- There are <u>9 plots</u> in each village, so it should take maximum <u>5 days</u> to complete a full cycle.
- Allowing time for processing samples and bad weather, we suggest one sampling cycle every <u>2 weeks.</u>
- This should also allow some time to assist the dietary recall data collectors.

6.2. Pollinator survey timeline

Step 1: Produce crude maps of each village using satellite imagery – Tom [Jan-Feb]

Step 2: Procure & prepare equipment, survey tools & training materials – Tom & HERD [Feb-March]

- <u>Step 3:</u> Recruit pollinator survey data collectors HERD [Feb-March]
- <u>Step 4:</u> Pollinator training & rural mapping with data collectors HERD, Naomi, Daya? [late March]
- Step 5: Select plot locations within desired habitats from community maps Tom [late March]
- Step 5: Visit field sites with data collectors to confirm & mark plots Sujan/Dambar [early April]
- Step 6: Main phase of pollinator surveying (monitor & adapt as needed) [April Nov: every 2 weeks]

Step 7: Pollinator specimens identified by taxonomists [Nov-Feb]

7.0. Training of field staff

Once we have recruited all data collectors, we will host a 5-10 day pollinator training workshop in a convenient location in Jumla. **See separate training document for more details.**

7.1. Training procedures

We will use a variety of training approaches and materials such as:

- Written materials e.g., booklets with simple field protocols with diagrams
- Verbal lectures with slides & diagrams
- Practical demonstrations where data collectors can practice the techniques.
- Quizzes to test the understanding of data collectors.
- Visual observation to ensure protocols have been correctly understood.

7.2. Training topics

We will cover the following topics in the training course:

- <u>Project overview</u> so that data collectors understand the purpose of this research.
- <u>Choosing survey plots</u> explain how and why we are choosing these plots
- <u>Pollinator identification</u> how to recognise different pollinator groups
- Plant identification how to identify plants and use the plant atlas
- <u>Pollinator survey</u> how to move around the plot and look for pollinators without bias
- <u>Pinning & preserving</u> how to pin and label insects in specimen boxes & store securely
- <u>Flower survey</u> how to survey and measure abundance of flowers in your survey area
- <u>Using ODK tools</u> how to effectively use the ODK pollinator tool and manage data securely
- Public engagement how to explain this research and its importance to the community
- Health and safety how to minimise risks and administer basic first aid in the field
- <u>Putting everything into practice</u> a practical assessment session

7.3. Logistics to organise for course

- Accommodation and food for all data collectors
- Venue to host the training course with indoor and outdoor areas
- Printed training materials
- Pollinator survey equipment to practice with
- Staff travel to and from course location

9.0. General recommendations from survey experience

9.1. Recommendations from Tom

- <u>Be flexible!</u> Everything about this project is new and challenging so we must be prepared to alter our methods if they do not seem to be working correctly or if we think of a better way of doing things. Methods must change consistently across ALL sites though and this should be discussed with the whole team first.
- <u>Invite feedback</u> from data collectors they will have the best idea of how the methods are working on the ground and they may have good ideas. However, it should be clear that they cannot change the methods without first checking with the project manager.
- <u>Minimise the decision making</u> that data collectors need to do this is often where confusion and error occur. Having clear, simple rules to follow will minimise the decisions they need to make.
- <u>Don't assume a data collector has understood</u> something until you have actually seen them do it correctly for themselves. Watching and evaluating data collectors will be key.
- Following the correct protocols is more important than getting lots of data. It is common for field assistants to bend the rules in order to catch more pollinators or count more flowers, but this creates great bias. It must be clear to them that recording a plot with zero (or very few) flowers or pollinators is still a very important result they must not feel like they always have to find something.
- <u>Data collectors must feel comfortable</u> asking questions or seeking clarification. Many data errors occur because assistants don't want to disturb the manager, so they don't ask for clarification and continue making mistakes.
- Make sure data collectors really <u>understand the logic</u> of why we are doing all this work and why it is important to follow protocols and conduct replicate plots. It is easy for data collectors to become demotivated if they don't see the value of the work or understand why we are doing it.

10.0. Health and safety considerations

There are minimal health and safety considerations associated with this fieldwork but these few risks are outlined below, along with their mitigation plan. A first aid kit will be kept in a secure but accessible location in each village for use by the two data collectors (dietary recall and pollination).

Risk	Mitigation plan
Ethyl acetate – risk while storing, transporting or handling ethyl acetate which is a highly flammable liquid and toxic when ingested or inhaled.	 <u>Storage:</u> Ethyl acetate should be stored in a tightly sealed screw-top glass bottle in a cool, dry place away from any flammable sources, out of sunlight and the reach of children. It should be clearly marked as FLAMMABLE and POISON. <u>Transportation:</u> keep bottles upright, securely held and away from any flammable sources to minimise risk of spillage. <u>Handling:</u> Only handle ethyl acetate outside, or in well-ventilated conditions.

	Wear gloves and safety glasses when transferring between bottles. Use a pipette to transfer small amounts of liquid, rather than pouring.
Glass killing tubes – pose an injury hazard if they break whilst in use.	 When removing and replacing caps on the glass tubes, do NOT squeeze the sides of the tube too hard or push down on the cap too hard. Be gentle and patient. If a tube shows signs of cracking, wrap it in paper and discard it immediately - do not continue using it.
Insect stings – there is a small risk of data collectors being stung by bees or wasps while catching and handling the specimens.	 Only catch insects by net, not by hand and follow correct catching protocol to minimise risk. When in net, transfer insect to tube
	without touching it directly and close the lid, keeping your fingers on the upper side of it.
	 Only remove insect from tube when fully sedated or dead and do not attempt to handle or pin any insect until it has spent over 2 hours in a killing tube and is clearly dead.
	 In the unlikely event of being stung, remove the sting by scratching the skin with your nail until it comes out. Wash with cold water and soap and keep cool. If pain continues, apply antihistamine cream (from 1st aid kit) onto sting
Hazardous ground – risk of falling, slipping,	Plots will be chosen to avoid particularly
twisting ankles etc on steep or hazardous terrain	 hazardous areas. Data collectors should choose their routes to and from plots to avoid hazardous areas and keep to paths or flat areas wherever possible.
River crossing	• Plots will be chosen to avoid river crossings wherever possible. If a river crossing is necessary, only cross by a secure bridge.
Weather – risks associated with particularly cold, snowy and foggy conditions, or with extreme heat and exposure to sun.	
COVID-19 – risk of contracting COVID-19 during fieldwork, or transmitting it to others	
Toxic plants and animals - danger from rubbing or ingesting toxic plants or animals	 Avoid touching any unknown plants or animals in the field. Wash your hands with cleansing gel and
	water before eating or touching your face

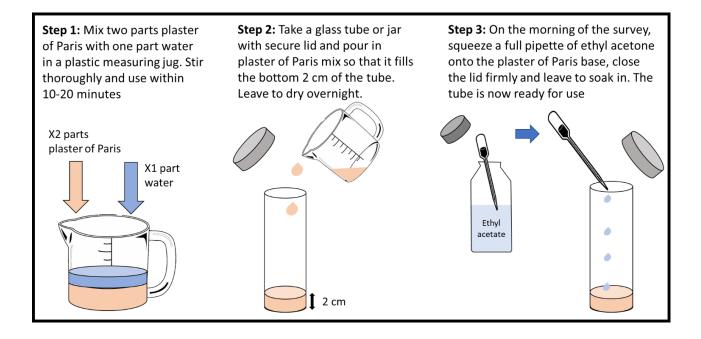
Appendices

Appendix 1: Preparing insect killing jars

A killing jar is a device used by entomologists to kill captured insects quickly and with minimum damage to their bodies. The jar must be glass and completely sealable with an absorbent substance (e.g., plaster of Paris) within it to hold the killing agent (ethyl acetate).

The figure below shows how the killing tubes should be prepared and dosed with ethyl acetate. At the beginning of the study, plaster of Paris should be poured into all tubes to hold the ethyl acetate which will be dropped into the killing tubes each morning.

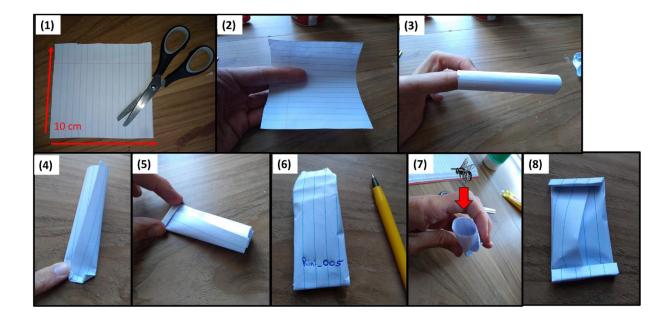
Ethyl acetate evaporates into the air over time so a killing jar will stop working after approximately 1 day. Therefore, killing jars need to be re-dosed with ethyl acetate each morning before the surveying starts (see Step 3).



Appendix 2: Preparing body bags for insects

Body bags are small paper bags used to hold insect specimens in a killing jar until they are ready to be pinned. They are made by following these simple steps:

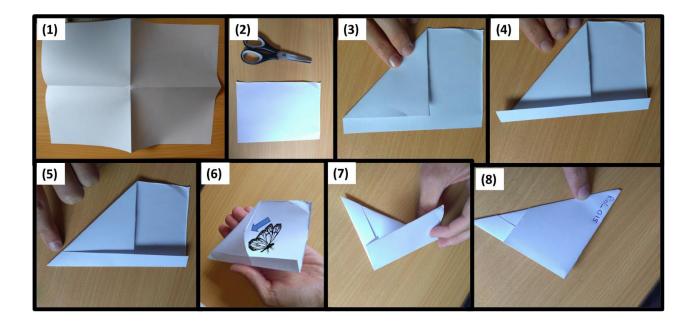
- Cut a piece of plain paper to approximately 10cm x 10cm (See figure below)
- Roll it around your thumb or big finger to make a tube
- Make 4 small tight folds up one edge of the tube so that it is tightly closed at one end
- Write the unique specimen code in pencil above the fold make sure it is clear
- Now insert the insect into the paper tube either by tipping it from the glass tube or transferring it with a pair of tweezers
- Make four small tight folds on the other side of the tube to seal the insect in
- Be careful not to crush the insect in the middle part of the tube
- Adjust the sizing as necessary, depending on the size of the insect
- Place the sealed up body bag into the large killing jar to complete the killing process and await pinning



Appendix 3: Making triangular envelopes for butterflies

The best way to store butterflies and moths is to put them in a hand-made paper envelope in the shape of a triangle. These can be prepared before the survey (following steps 1-5 below) and then sealed up and labelled (steps 6-8) after you have caught the butterfly.

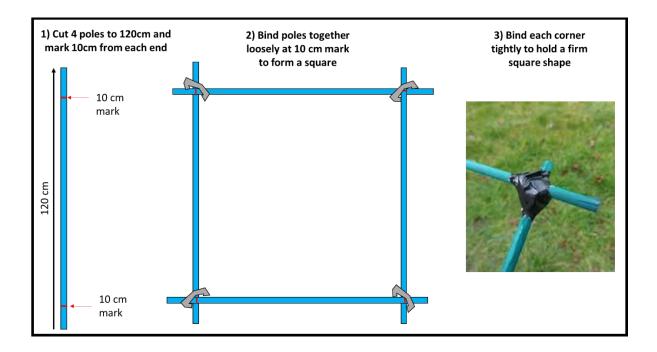
- 1. Fold a piece of A4 paper in half and then in half again to make four equal sections.
- 2. Cut along these folds with scissors to produce four smaller paper rectangles (each one of these rectangles will make a separate envelope)
- 3. Fold one corner of the paper inwards, leaving a gap of about 1 cm at the end
- 4. Now fold over this 1 cm strip and tightly press the edges to stop it coming open
- 5. Fold over the little corner so that it seals up the bottom. Your envelope is now ready for a butterfly to be inserted.
- 6. Once you have caught and stunned a butterfly by pinching it in the net, you can slide it into the triangular envelope with the wings folded closed above it
- 7. You now fold over the large top flap very tightly
- 8. Finally, you should tuck in the corner to complete the sealed triangle and write the unique specimen code very clearly on the outside of the envelope without crushing the insect inside



Appendix 4: Making a quadrat

A quadrat is a square, rigid frame used to measure out a fixed area in a systematic way. It can be made out of plastic, wood, bamboo, metal etc, just as long as it is rigid, lightweight and strong. Quadrats can be made very simply by hand following these methods:

- 1. Cut 4 lengths of thin bamboo pole, plastic tubing or similar material to 120 cm length (1.2 m)
- 2. Mark a point exactly 10 cm in from the each end of the poles so that there is exactly 100cm in-between each mark
- 3. Place two poles together at right angles so that they are touching at their 10cm mark (see Figure below)
- 4. Place a small piece of duct tape over the joint to hold it loosely in place
- 5. Repeat this for all four poles so that they are joined into a 100cm x 100cm square with ends overlapping
- 6. Now bind each joint very tightly with either duct tape or rubber (e.g., from a bicycle inner tube) so that the four corners remain firmly in place
- 7. Check that each length of the square is 100 cm in length
- 8. Make sure the bindings remain firm over time and replace the tape or rubber if they start to loosen



Appendix 5: Full equipment list

Specialist Items	Qty
Insect collection nets (sweep nets)	20
Glass tubes with sealed lids for killing insects	600
Ethyl acetate (1 litre bottle)	20
Plaster of Paris powder (kilogram bag)	5
Silica gel 1KG	10
Napthalene 1KG	5
Insect mounting pins (1 packet = 100 pins)	50
Insect mounting boxes (18*12*3)	50
Mounting tweezers	15
Measuring tape (50 or 100m length)	3
Smartphones/tablets	10
Battery packs for charging smartphones	10
Non-specialist items	Qty
Shoulder bags for carrying field equipment	15
Clipboards	25
50/100m measuring tape	2
Field pencils	100
Pencil sharpeners	20
Wooden posts/sticks with coloured ribbon or paint	360
Paper for making body bags and butterfly envelopes	10
Paper for printing survey forms	10
Card for making specimen labels	10
Scissors	15
Plastic pipette/ink-dropper	30
Liquid PVA glue	10
Plastazoate sheets	10
Stop watches	15
Set of ziplock bags	20
1.2 metre Bamboo/plastic/wooden poles for making quadrats	50
Duct tape for binding quadrats	3
Hats for shielding smartphone screens	10

Appendix 6: Identifying pollinator groups

