Papua New Guinea National Forest Inventory

Protocol for NFI Faunal Biodiversity Assessment

ORNITHOLOGICAL SURVEYS

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This Manual is designed for ornithological surveys conducted during the PNG National Forest Inventory (PNGNFI) throughout Papua New Guinea.

The ornithological surveys comprise a combination of four survey methods:

- Point-counts (radius r = 50m, with brackets = 10m, 15 min long)
- MacKinnon lists (10 species long)
- Automatic recordings (24 hours non-stop)
- Daily checklists

Point Counts

Point counts method has been selected as the main method for the survey as it is quantitative, repeatable method that produces estimates of bird density and species richness. It is used in many tropical countries as the standard methodology. The 15 minutes long recording of all birds seen or heard within a 50 m radius, split into 10-m wide concentric circles, represents a standard method used here. Each observation point is surveyed in two subsequent days (mornings), maximizing thus the sample size. In total, seven points is surveyed in each cluster, the maximum number manageable in one day during the period of maximum bird activity from 6.00 to 9.30 am.

The point counts survey records all bird calls within 50 m radius, with estimates of the distance from the observer in 10 m intervals. The calls that cannot be immediately identified are recorded by a recording machine, assigned a unique code and identified at a later time.

The procedure for point counts starts with the location of seven point-count stations established in the field, one central and other six regularly spaced on 300 m radius (Fig. 1); four stations coincide with the four plant plots. The consecutive three days these stations are surveyed, starting with point count from the N plot (no. 1), continuing clockwise from point 1 to 7. Start at 6.00am and finish at 9.30am. The following rules should be observed:

- 15 mins spent at each point recoding, 10 mins moving between points
- Shot-gun recorder used for unknown calls at all points (Fig. 2)
- Record all birds seen or heard, including birds flying over the point count stations.
- Separate (by a line in the protocol) observations in the first, second and third 5 mins intervals.
- Write common English birds name (rather than Latin names) as it is usually easier.
- For each recorded bird, estimate its distance from the observer in 10m brackets: 0-10, 11-20, 21-30, 31-40, and 41 to 50m.
- Fill in all information on the data sheet including date, cluster number, starting time, ending time, surveyor name (Fig. 3).
- Record the weather on the day of point counts every morning (wind, sun, rain).

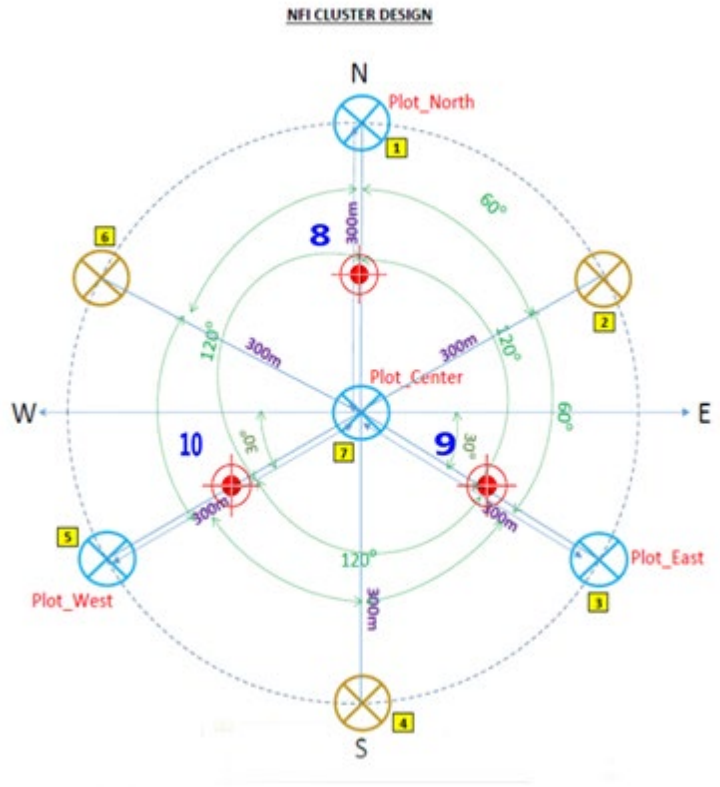

Fig. 1. The NFI cluster design with the seven locations for ornithological point counts (yellow squares no 1 – 7) and the song meter location mounted at the point no 2.

Fig. 2. The shot-gun recorder powered with two AA batteries inserted in the battery slot at the back of the recorder and one AA battery inserted in the battery slot of the microphone. One SD card (8GB) should be inserted in the card slot. Download all recordings using NFI laptops and transferred into two separate hard drives. Delete all files and re-use the same SD card at the next cluster. Repeat the same process/steps at the next cluster.

Plot ID: ..... Custer No. .....

Point-counts

Page X/Pages Y __/__

Date: __ __/__ __/__ __	Starting time:	__ __ : __ __	Ending time:	__ __ : __ __
Coordinates:		Surveyor:		

Do line after 5 or 10 minutes, or/and put number into O after 5 minutes and into □ after 10 minutes

Point	Species Name	0-10m	11-20m	21-30m	31-40m	41-50m	NOTES/REC.

Checked Date:……..Checked By:……Weather:……

Databased Date:……Databased By:……..

Fig. 3. The NFI point count data sheet

Mackinnon Lists

The Mackinnon list is a complementary method for point counts as it records birds less quantitatively but over a larger spatial area. During the random walk through the surveyed area the surveyor compiles a series of sub-lists recording the first 10 sighted or heard species in the order they were encountered. Walk throughout the surveyed areas and complete as many lists as possible. Use the Mackinnon data sheet (Fig. 4).

MacKinnon lists	
Name of surveyor:	
Period of surveys: ___/___/___ - ___/___/___ (DD/MM/YY)	
ID's of finished plots (Cluster names)	( )
( )	( )
( )	( )
( )	( )
( )	( )
( )	( )
( )	( )
( )	( )
( )	( )
( )	( )
( )	( )

Fig. 4. The NFI Mackinnon list data sheet

Song Meter

One song meter is used to make audio record of the environment for continuous 48 hours at each cluster. The protocol should follow these rules:

- Song meter is mounted at the North East (NE) point on the first day of the survey at 3.30 pm.
- Insert four D size batteries in the battery slot, they should last for 48 hours (Fig. 5).
- Insert four SD cards (8GB) into the card slot.
- Put the Power button in ON position so that the screen will display will come on.
- Go to Main Menu and scroll down until you see “24 hours” then highlight it.
- Press start program button and the meter will start recording non-stop for 48 hours.
- A yellow light on the left-hand corner must blink, that means the device is recording.
- Check the meter also on the morning of the first day during point count to make sure it is recording ok.
- Let the song meter record non-stop for 48 hours and take it down at 3.30pm on the third day.
- Fill in the data sheet (Fig. 6) when mounting and removing the song meter each time.
- All recordings from the Song Meter and Shot-gun recorders must be downloaded using NFI laptops on site. All files to be copied onto two separate external hard drives and stored safely in the field.
- Delete all files from the previous cluster and re-use the same SD cards in the next cluster.
- Please repeat the same steps when you get to a new cluster.

Fig. 5. The song meter recording device.

SONGMETER CODE: ___ ___ ___ ___ Name of surveyor responsible for songmeter ....

Exposed on plot ID:		Date:		Time	
Collected from plot:		Date:		Time	
Data back-up file name:		Location of back-up:			
Notes on potential disturbance:					
Exposed on plot ID:		Date:		Time	
Collected from plot:		Date:		Time	
Data back-up file name:		Name of back-up			
Notes on potential disturbance:					
Exposed on plot ID:		Date:		Time	
Collected from plot:		Date:		Time	
Data back-up file name:		Name of back-up			
Notes on potential disturbance:					
Exposed on plot ID:		Date:		Time	
Collected from plot:		Date:		Time	
Data back-up file name:		Name of back-up			
Notes on potential disturbance:					
Exposed on plot ID:		Date:		Time	
Collected from plot:		Date:		Time	
Data back-up file name:		Name of back-up			
Notes on potential disturbance:					
Exposed on plot ID:		Date:		Time	
Collected from plot:		Date:		Time	
Data back-up file name:		Name of back-up			

Fig. 6. The Song Meter data sheet

Daily Checklists

- Every day in the afternoon, a checklist of all observed bird species will be constructed, so the surveyors will have chance to record even the bird species which were encountered during the night.
- This activity will contribute to complete checklist of the birds for the whole cluster and will keep the surveyors updated about the presence of some birds. Collaboration with botanists or other team-members reporting some bird species encountered is possible and welcomed.
- Surveyors will put a tick in in the box for each species on each date (Fig. 7).

Plot ID: .... Custer No. .... **Checklists** Coordinates: ....

Day 1 date:	Day 3 date:	Surveyor name:				
Day 2 date:	Day 4 date:					
English name	Scientific name	1	2	3	4	Note
Cassowaries						
Northern Cassowary	Casuarius unappendiculatus					
Brushturkeys + scrubfowls						
Brown-collared Brush-turkey	Talegalla jobiensis					
* New Guinea Scrubfowl	Megapodius decollatus					
King Quail	Excalfactoria chinensis					
* Brown Quail	Coturnix ypsilonophora					
Waterbirds						
Pacific Black Duck	Anas superciliosa					
Little Pied Cormorant	Phalacrocorax melanoleucos					
Forest Bittern	Zonerodius heliosylus					
Rufous Night-heron	Nycticorax caledonicus					
Intermediate Egret	Mesophoyx intermedia					

Fig. 7. The daily checklist data sheet. Example for 4 days, each observed species must be ticked for each of the 4 days when observed.

Fig. 8. The use of binocular when identifying bird species for point counts or Mackinnon lists.

Data Safety and Management:

- All completed datasheets **must** be kept in A4 envelopes and labelled with the cluster number. The datasheets must be stored securely in a water-proof bag in the camp during the field work and placed in a specific box after return from the field.
- All completed sheets must be photographed as a back-up in case they are lost or damaged.
- Individual surveyors will be responsible for immediate data-entry to Microsoft Excel, database and make back-up copies within Binatang Research Centre (BRC).
- All recordings (song meter and shot-gun recorders) **must** be downloaded from the recording machines after each cluster using NFI laptops and then transferred to two
- External Hard Drives and kept safely. All teams to have two External Hard Drives each.
- Photographs of all completed data sheets **must** be taken in the field before moving to the next cluster.

Time flow of the survey:

First day (arrival day):

Place a song-meter in the field – as soon after arrival at the cluster as possible. Preferably 3 hours before dusk of the first day. Locate the position of all seven point-counts in the field. It is important that the surveyor familiarizes himself with the terrain and position of the sampling points around the plant plot cluster and will establish and label with flagging tape the positions of all seven ornithological points. It might take around four hours to establish all points. While surveyors are moving through the study site locating points they might carry out first MacKinnon lists.

Second day and third day:

Identical programme for both days. From 6:00 – 9:30am complete all seven point counts, then start MacKinnon lists from 9:30 till about 10.00 or 11.00 am, when the bird activity decreases. Complete more MacKinnon lists, if possible, from 2.00 - 3.00pm till dusk.

Fourth day:

If available, repeat again the same protocol as in the 2 previous days.

Equipment List for Ornithology

Item	Quantity
Song meter recorder	1 per cluster
Shot-gun recorder	1 per cluster
Directional Microphone	1 per cluster
SD Cards (8GB)	5 per cluster
GPS (Global Positioning System)	1 per cluster
Compass	1 per cluster
External Hard Drive	2 per cluster
“AA” Battery	1 box per cluster
“D” size Battery	1 box per cluster
Binocular	1 book per cluster
Bird book	1 book per cluster
Point count data Sheets	14 sheets per cluster
Mackinnon list data sheets	14 sheets per cluster
Checklist data sheets	1 per cluster
Flagging Tape	2 per cluster
Bush Knife	1 per cluster
Head Torch	2 per cluster
Action Packer	2 per cluster
Head Set	1 per cluster
Card Reader	1 per cluster
Camera	1 per cluster
Note Book	1 per cluster
Wrist watch	1 per cluster
Duct tape	2 per cluster
"AAA" battery	1 box per cluster
Biro/pen	3 per cluster (Red, Blue, Black)
Permanent Markers	1 per cluster (Blue/Black
Pencil	1 box per cluster
Eraser/rubber	1 per cluster
Clipboard	1 per cluster
Hiking shoes	6 pairs
A4 Envelopes	5 per cluster

Papua New Guinea National Forest Inventory

Protocol for NFI Faunal Biodiversity Assessment

GEOMETRID MOTH (Lepidoptera: Geometridae) SURVEYS

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1. Introduction

This protocol will be used during the Multipurpose National Forest Inventory (NFI) Zoological survey and contains step-by-step instructions for the Geometridae Moths Light Trap sampling aiming to provide standardized quantitative samples of moth communities.

2. Methods

2.1 Equipment

Geometridae Light Trap Equipment Master Checklist for 2 Light Trap Teams in 1 Cluster
IMPORTANT: Make sure all equipment for sampling are available and ready before actual sampling in each cluster

Generator/ Light Equipment
2 x Honda Genset
10L Unleaded Petrol
1 x 500mL Engine oil
2 x Light bulb (+ 1 spare)
2 x Light bulb Cord (+ 1 spare)
5 x Whitesheet (2 for use + 3 spare)
4 x bushknife
1 x Knife File
2 x Small tarpaulin
20m Thin Buckline
20 x Clothes pegs
4 x Raincoats
6 x Umbrellas
3 x Extension Cord (2 for use + 1 spare)

Collecting equipment
10 x soft forceps
4 x hard forceps
10 x killing jars
5 x syringe (2 for use + 1 spare)
1 x box Surgical gloves
250mL Ethyl acetate
6 x Toilet tissue packs
6 x lunch box containers (30cm x 20cm x 15)
2 x Safety eye-glasses
6 x Headtorches (2 good torches for staff, 2 Chinese ones for assistants + 2 Chinese spares)
1 x box Head torch batteries (AA and AAA)
4 x Gumboot (2 for staff [sizes for each staff], 2 for assistants [size 10]
8 x Socks
4 x Hand gloves

Data Recording equipment
20 x Geometridae Field Datasheet
2 x Waterproof Field notebook
Pre-printed Field labels
2 x Permanent Micron pen
10 x Pencils
10 x Eraser
5 x Clipboard
2 x Scissors

Sorting, Drying & Storage equipment
6 x mail master box
400 Entomological pins no 2 (4 x packets with 100 pins each)
1 x Mounting pins box (size 2 and 3)
Hard and soft forceps (quantity already listed under **Collecting equipment**)
2 x Magnifying glass
100 cm2 of Styrofoam
2 x Pagi's Geometridae Field Booklet
1 x roll of Wax paper
800 Glassine Envelopes (400 small & 400 medium)
2 x 2L container Silica Gel
2 x box Zip-lock bags (medium and large)
1 x medium Patrol Box drier
1 x Coleman Lamp
5 x Mantles
5L Kerosene
4 x Large Action Packers for equipment storage
1 x Medium Action Packer for specimen storage

Electronic equipment
2 x 8 GB Flash drive
1 x Ipad/Laptop
2 x 8GB SD cards
2 x Multiple SD card reader
1 x Camera
1 x medium sized Action Packer for equipment storage

2.2 Site selection

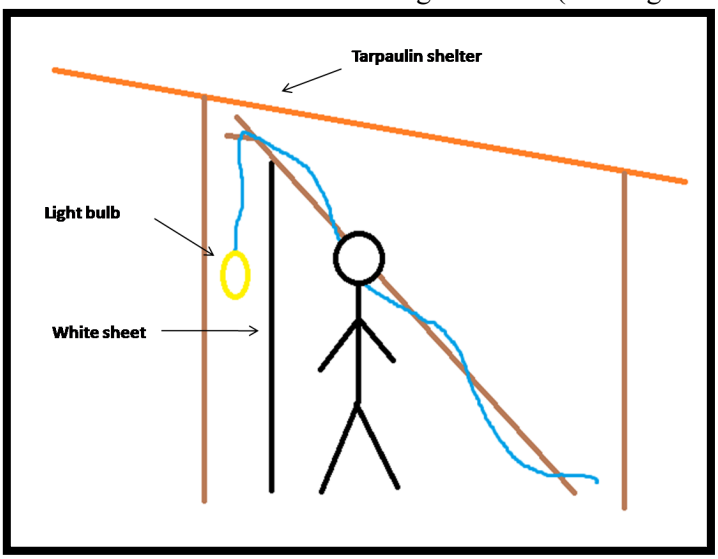
During the first day select two suitable spots for light trapping within the cluster area. These spots can be anywhere in the cluster but should be at least 100 meters apart so light from one light trap is not visible from the other. Good spots for light trapping include:

- On top of a ridge or small hill with a clearing overlooking the forest
- In a clearing or at the edge of the forest, e. g. tree fall gap or an open patch of grass
- On the bank of a river or creek
- DO NOT select an area where forest is too thick and enclosed and where light cannot travel far
- Be careful not to select an area that can be flooded during rain

Important to remember is that we want the light to be visible as far as possible. It may be necessary to clear a bit of undergrowth at the spot if the area is too enclosed. Make sure that with clearing undergrowth or cutting down trees you are not in or near a botany plot. The side of the white sheet with the light bulb must be always on the exposed side that faces an open area.

2.3 Light trap setup

- Prepare the area during the day before light trap starts. Place two strong sticks in the ground 4 meters apart. You can use two nearby growing trees or saplings that are strong enough. Build the frame for a small shelter for the tarpaulin to cover the light sheet and bulb in case of rain during sampling. Make sure the shelter will cover the light well so wind does not blow rain onto the light bulb, but not too close to the bulb as not to block the light from in (see diagram below)



- Tie the rope between the sticks, tight enough so the white sheet will not pull it down. Height of the rope is determined by how far you can reach up to collect the moths.
- Place the white sheet on the rope and use pegs to secure it in place. Make sure there are no big creases or wrinkles on the white sheet that will create shadow. Secure the sheet on the ground with ropes or some heavy sticks so it does not blow around if there is a wind. Important: the white sheet must reach to the ground and fold at least 10 cm on the ground.
- Place the tarpaulin over the shelter frame and adjust and secure with ropes. The tarpaulin must protect the light from rain but not shade it too much. You can also use umbrella to help to
- Put another stick over the white sheet towards the side where you want the light and hang up the light (or you can use one of the sticks extending out from the shelter frame). Do not hang the light too close to the white sheet or it will burn the sheet. Once you turn on the light you can adjust the bulb higher or lower or outwards from the sheet to get the best position where all the sheet is evenly lit and there are no big dark areas on it. You can also adjust the sheet if there are plenty of creases or folds on it.
- After you have completed all your setup and are satisfied, check through all your equipment, check genset fuel and oil and test it.
- At 6.00 pm turn on the genset and get ready to start collection. **Important: Do not be late, start at 6.00 pm!**
- Note: Setup your light trap and shelter frame during the day and towards the evening you can bring your genset, white sheet, bulb and collecting equipment.

2.4 Moth collection

SAMPLE SIZE:
- Normal sample size is 2 light traps (A and B) operating for 2 nights (night 1 and 2), creating thus four samples: A1, A2, B1, B2
- Each sample includes all geometrids coming to light from 6.00 pm to 10.00 pm
- In case of exceptionally large sample the collection on a particular night can be limited to only a part of white sheet and stop at about 300 geometrid individuals collected; however we still have to continue sampling on the next nigh so we get all 4 samples from the cluster
- In case of exceptionally small samples, below 200 geometrid individuals total from all 4 samples, the sampling should be extended to the third night by both light traps (if possible), creating thus samples A3 and B3

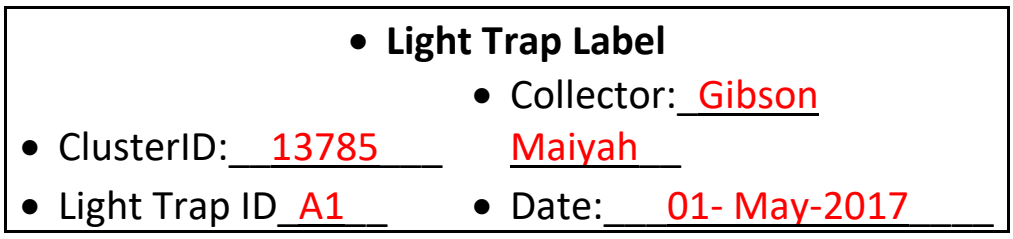
When moths start to arrive start collecting all geometrid moths into the killing jars. If you not sure about whether a moth if a geometrid, collect it as well. Use killing jars to collect moths from the white sheet and insect net to collect those flying around. Make sure your killing jars have been provided with fresh ethyl acetate, but not too much so the paper inside does not get wet or else the moths will become wet and lose color. During the night, when you see that the ethyl acetate is getting weak (i.e moths take long time to die) use a syringe to refill.

The dead moths from a killing jar are removed and placed on a layer of toilet tissue in a lunch box. During the night, create additional layers of toilet tissue and then finally cover the top layer with toilet tissue, place a label in the box and close, The toilet tissue must be tight enough so that the moths in the toilet tissue layers do not move.

Notes on good collecting techniques:

- Make sure your killing jar has enough ethyl acetate. Once you notice it getting weak refill it.
- When collecting, be mindful of disturbing other Geometrid moths. Be quick but gentle with the killing jar against the white sheet.
- Always move and look around the white sheet - there may be some geometrid moth sitting on the ground near the white sheet, or in the folded white sheet on the ground
- Do not collect too many moths into one killing jar as they will start to disturb the others and lose their colors and patterns. Once the bottom of your killing jar is covered with moths let it stand for all the moths to die and continue with another empty killing jar.
- Remove any large beetles or moths that keep flying into the white sheet - they may disturb the moths you want to collect.
- Check for moths systematically, start at one end of the white sheet and search up and down and towards the other end.
- Take turns checking both sides of the white sheet.
- Check also the tarpaulin roof of your shelter, the sticks and nearby vegetation. Sometimes very interesting moths will come and sit for hours without you noticing them. Shake out the tarpaulin and bushes occasionally to get the moths flying.
- Once the moths in a killing jar are dead, pour out the specimens onto the layer of toilet tissue in the lunch box. Use soft forceps to carefully pull out any moths sticking to the cardboard inside the jar.

- Use soft forceps to carefully arrange all the moths so that their wings are spread out. This is very important because it will make it easier for mounting and identification the next day.
- Arrange all the specimens in this way until the layer is covered with moths. Then cover the moths with another layer of tissue and continue.
- IMPORTANT: If you notice some moths still alive and moving around, use your forceps to place them back into the killing jars and later put them back into the lunchbox. If there are lots of moths still alive and moving, crumple some tissue and pump some ethyl acetate into it and place them in the lunch box near the corners and close the lid. Remember: putting too much ethyl acetate can drip onto the specimens and make them wet and damage them.
- Use plastic lunch boxes with tightly closing lid for layering moths, always close the lid so that the moths do not dry out. Layer moths between toilet tissue so that they do not touch. IMPORTANT: Make sure the box is packed with enough toilet tissue so that the moths are not moving inside the box – this would damage their wings.
- At the end of collection, wait for the last collected moths to die and then place them into the lunchbox and make a final layer of tissue to cover all the specimens.
- Fill out the Light Trap Label and place into the container and close the lid firmly. Light trap label details should have: Cluster ID, Light Trap ID (A1, B1, A2, B2 – trap A and B sampled on day 1 or day 2, so for instance A1 is a sample from trap A on day 1), Collector name, Date.



At certain sites there may be an overwhelming number of moths coming to the light trap. In situation like this:

- **Select ½ of the whitesheet** and collect Geometrid moths only from that area.
- If still too many specimens, collect until you have **300 individuals of Geometrids collected during that particular night** and then you can stop collecting (but you still go on the day 2 for another collection).
- **This is the only exception where you can stop collecting before 10pm**.

Golden Rule for collecting: If you are in doubt, just collect the specimen so you can confirm later during sorting in camp or back in Nagada.

2.5 Closing the sampling

- Make sure all your equipment and specimens have been packed.
- Slowly remove the white sheet and shake off the moths and fold it neatly.
- Disconnect the light bulb power cord from the genset and turn the genset off (see Genset Protocol)
- Let the light cool while you finish packing.
- If it is raining or there is some moisture on the shelter tarpaulin, wait until you remove and pack the light bulb before removing the tarpaulin. You can also use umbrella to protect the bulb from the rain.

- When the light bulb has cooled down enough to handle, use a cloth or tissue or its own box to cover the bulb and carefully unscrew it and pack it safely.
- IMPORTANT: Never touch the light bulb with your bare hands before it has cooled down. It can burn your hands but also dirt and grease from your hand can damage the bulb so it will not last long in the field. Always use a dry cloth, tissue or cardboard box to handle it.
- After packing away light bulbs and other equipment, remove the tarpaulin and after making sure all your equipment and specimens have been packed you can head to camp.
- IMPORTANT: Make sure your specimens are safely and well packed and there is no chance of the box opening and specimen pouring out or moving around too much. The person carrying the specimen must be mindful of this when moving back to camp.
- At camp stow away your equipment. Make sure datasheets are safe and not mixed with wet things. Make sure chemicals are not leaking, etc.
- Check your specimen container and make sure they are ok. If there is need (if some moths are still alive, put some more toilet tissue with ethyl acetate and place in the container overnight and store your specimen container safely for work the next day

2.6 Sorting of moths

Equipment:
Hard and soft forceps
Storage cartoon boxes
Entomological pins no 2
Geometridae Field ID Guide
Newspaper (to make envelopes)
Glassine Envelopes
Silica Gel
Zip-lock bags
Permanent Markers
Labels

The next morning, begin sorting the collection. Start early in the morning when the moths are still fresh, easy to spread. Start sorting the first sample (A1). Sort the specimens into separate species (following the advice below). Then number the species from 001, 002, 003, ... and count the total number of specimens for each species and record in data sheet for A1. Finally pin one good specimen from each species, plus put 2 specimens from the same species (if available) into glassine envelopes. Record the total number of specimens from each species in data sheet.

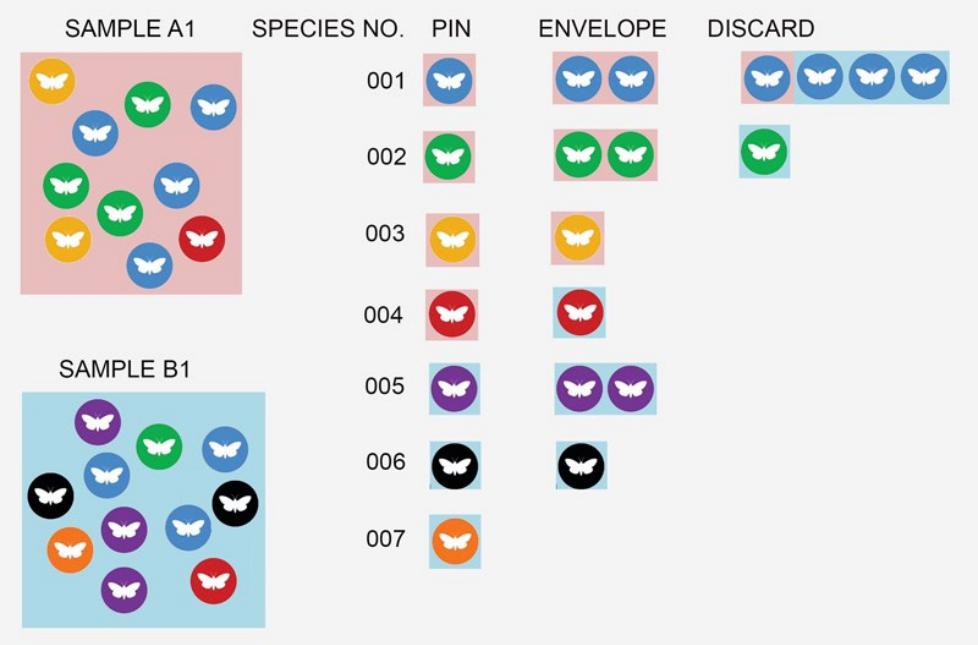
After A1 sample is finished, continue sorting B1 sample (from the second light trap). Use the species from the reference collection created from A1 sample and continue adding new species to it from B1. When sorting is finished, count the total number of specimens for each species and record in data sheet for B1. Pin one specimens from all new species, and put 2 specimens into glassine envelopes. If any species from the previous sample does not have 2 specimens in glassine envelopes already, add them if they are available.

After the second sampling night, continue with sorting A2 and then B2 sample. Continue adding species to a single reference collection and numbering system created for the entire cluster. Start new reference collection, starting from 001 species, for each cluster.

Important: We will create one reference collection for each cluster. At the same time data each of the four samples (A1, B1, A2, B2) must be recorded separately.

Sorting example: Sample A1 had 10 individuals sorted into 4 species. First specimen from each species is pinned, next 2 specimens put into envelope, others discarded.

Next sample is B1 from the same cluster, with 11 individuals from 6 species, including 3 species already found in A1 and 3 new species. Use the reference collection already created for A1 sample for repeated species (no. 001, 002, 004); for the repeated species where we do not have 2 individual in the envelope, add missing specimens from this sample (here species 004). New species continue numbering from the previous sample: the three new species thus become 005, 006 and 007.



Continue in the same way also for the samples A2 and B2, building the same reference collection with continued numbering of new species (no. 008, 009, etc.).

Start new reference collection, starting from 001 species, for each cluster.

Advice on sorting moths

Prepare a dry, clean surface (you can spread old newspaper, paper, cardboard on a bench or table) and pour out all the moths from one light trap onto the surface. If there is a lot of moths, you can work on one layer at a time to make it easier.

Sort and tally from each night and trap separately, 4 samples per cluster in total. If two people are working on sorting you can sort samples from Light Trap A and B simultaneously but make sure to keep them separate.

Start sorting and separating into different morphotypes. Start with the easier ones and then go onto the difficult ones. When there is a lot of material you can start with rough sorting into big groups (small green species, large brown species, etc.), then continue to split these groups into species.

Try your best to group together very similar moths. If you have any doubt or if you see that one morphotype looks very similar but is not exactly the same as the others in the group, separate it into a group of its own. **It is always better to split one species by mistake into two incorrect species then to make the opposite mistake by combining two true species into one mixed species!**

You can use the Geometridae Field ID Guide to sort the moths. But you must be careful and keep in mind that many of the species can be new. So it is very important to look and check each moth carefully. Use the magnifying glass to help you see some of the features clearly. These are some tips on sorting. Look very carefully at each individual moth and examine all these features:

- Wing colours and patterns - Some patterns are obvious and others are hard to see clearly. Look very carefully and use patterns to sort.
- Wing shape / size – Often similar-looking moths can be separated by the shape and size of the wings.
- Underneath wing patterns and colour - this is as important as looking at wing patterns and colour on the top side. Always check the underneath to confirm if species are similar.
- Antenna shape/length/color- antenna types in Geometrids are either simple (slender and straight) in females or bi-pectinate (feathery) in males. You can use antenna shape, length, color to separate species in combination with the other above features.

NOTE: All the features mentioned above are used in combination to help you sort and group moths.

Sorting is very important in this moth collection and so you must really look very carefully when separating species. If you are not careful and group together different-looking species, then our data will be wrong and you can end up removing species that should have been separated and tallied correctly.

Golden Rule for sorting: If you are in doubt, separate the species. It will be easier to check and group them together later if some of them are the same.

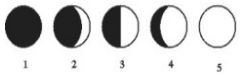
2.7 Completing data sheets

This is an example of the field datasheet:

NFI Geometridae Field Datasheet

Cluster ID:_13785____
Province:__Madang___
Date:__01/May/2017___
GPS:_____

Light trap ID:_A1___
Collectors:_Shen_
__
Moonlight:__1__
_



Weather notes:___Light rain from 8-9pm_____
_____
Habitat notes:___On small ridge near North Plot_____

Morphospecies code	No. of Individuals	Remarks
001	12	
002	5	
003	15	similar to 001 but small in size
004	1	not really sure if Geom, check later in Nagada
005		
006		
007		
008		

- **Cluster ID**: This is the unique identification number that PNGFA is using for each cluster
- **Province**: Province where cluster is located in
- **Date**: Date of light trap collection (Use first 3 letters of the month, not number, e.g. 12 Apr 2022)
- **GPS**: GPS coordinate of light trap location
- **Light Trap ID**: Since there will be 4 Light traps samples within a cluster, this is the code used to identify each light trap night. We have traps A and B operating on nights 1 and 2, so the sample codes will be A1 and B1 from the first night and A2 and B2 from the second night. If we have low sample size and collect on the third night, sample codes will be A3 and B3.
- **Collectors**: name of the collector (s)
- **Moonlight**: Since moonlight affects light trap samples, it's important to record this. Use numbers 1 (no moon) – 2 (less than half moon) – more than half moon - 4 (full moon). In the cloudy night when overcast, indicate that in a note (clouds, moon not visible).
- **Weather notes**: brief description of weather during sampling
- **Habitat notes**: brief description of light trap habitat and surroundings

Note: The information above will filled in during the actual light trap sampling so remember to take a datasheet copy with you to the light trap. The following information must be completed in the camp next morning:

- **Morphospecies Code**: code you give to each morphospecies, composed from the letter G (for Geometridae) followed by cluster ID number (5 numbers long), hyphen and morphospecies number starting from 001.

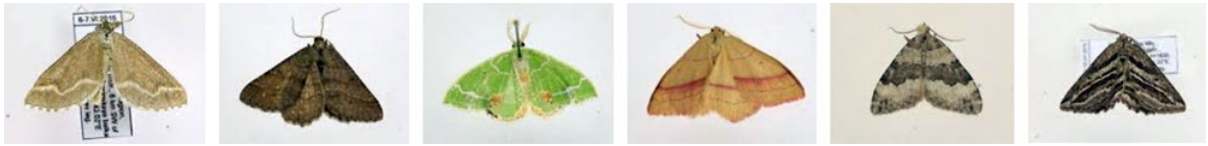
Example: G13785-001 (where 13785 is ID of the cluster, 001 the morphospecies number)

- **No. of Individuals**: number of individuals from each morphospecies
- **Remarks**: any sort of comments/remarks regarding the species that will be helpful during later work on the sample in Nagada

Tallying After finishing the first sample (A1), continue in numbering new morphospecies in the next sample (B1) following the highest number from the previous sample, but record the numbers of individuals separately for each sample.

2.8 Processing specimens

- Each morphospecies in the cluster should have 1 individual pinned and 2 individuals in glassine envelopes (if possible, some morphospecies will have fewer than 3 individuals).
- Pinned specimens: use pins no 2. Pin the moth through thorax, pin going straight down through the body. The moth body should be positioned at about two thirds of the height of the pin – the length of the pin above the body must be just enough to hold the pin in 2 fingers without touching the moth. Do not leave the body too low on the pin. The pinned moths must have wings open and if possible also part of hind wings visible. **Important**: each specimen has to have a label pinned underneath. Examples:



- Specimens in glassine envelope: Put only **1 specimen in each envelope**. Depending on the specimen size, use the appropriate envelope size. Select specimens in good condition. Remember to put the moths into the glassine envelopes with wings folded as in the example below.

- Important: there must be label with the specimen in each envelope.

Specimen Label

Papua New Guinea	Papua New Guinea
Nat. Forest Survey	Nat. Forest Survey
Species code	G13785-001
Date	28-Jun-17
Sample	A1 A2 B1 B2
Colector	B. Gewa

Papua New Guinea: Nat. Forest Survey
Species code (example shows species 001 from cluster 13785).
Date
Sample (A1, A2, B1, or B2: circle the correct one depending on the trap and the night of collection)
Collector name:
Some parts of this label will be pre-printed.

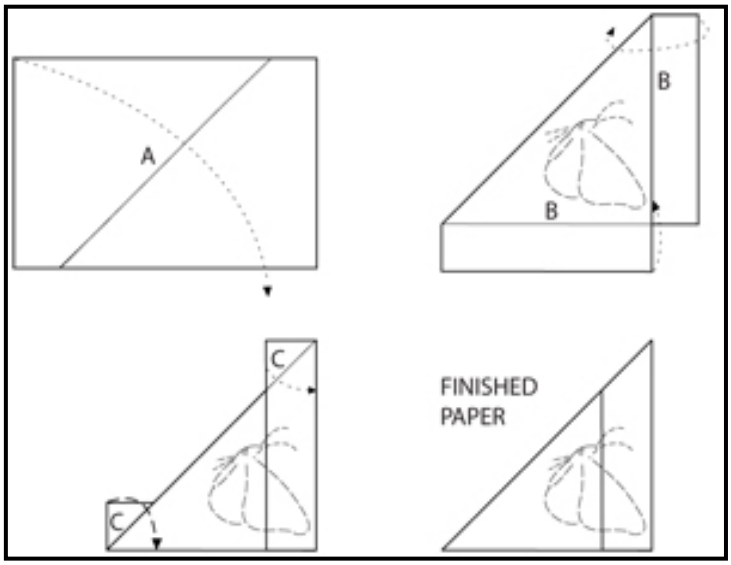
Making 'Triangles' to preserve moths

If you run out of glassine envelopes in the field or if there is a big specimen which cannot fit inside the envelope, you can use newspaper to make a triangle to store the moths. Follow diagram with steps:

A. Depending on the size of your specimen, cut out a rectangle of suitable size and fold in half as shown below.

B. Place your specimen and label inside and fold the paper to cover it.

C. Fold over the tips (C) firmly to hold the triangle so it doesn't open up. On the finished triangle you can write specimen details like in step 3 above.



Drying moth specimens

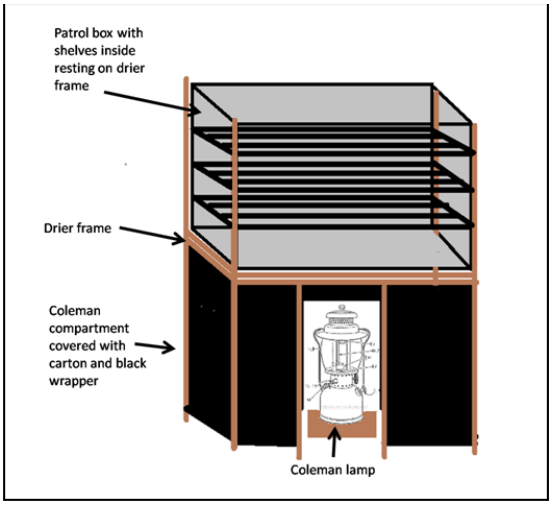
Drying is a very important process of moth preservation in the field. Properly dried specimens will not get mouldy, and will be in good condition for identification back in Nagada including taking DNA samples from them so it is very important that all moth specimens are dried well in the field before storing and transportation. We should dry both pinned specimens and specimens in envelopes. There are 3 methods for drying specimens: [i] Coleman Lamp Drier, [ii] Light Trap Bulb (only when Coleman does not work), and [iii] Silicagel.

Coleman Lamp Drier Metal Patrol Box fitted with a wooden shelves in a small frame (see the figure below) placed above a Coleman lamp. There should be about 20 cm space between top of Coleman lamp and the patrol box so that lamp is not in direct contact with the patrol box. After putting the lamp inside and specimen shelves above it leave the patrol box lid slightly open.

Drying Moth Specimens with Light Bulb It is possible to use light bulbs (not LED lights) instead of the Coleman lamp. It is possible to use the same light bulb used for light trapping but only if you have a spare one. When drying the specimens with Coleman lamp or light bulb:

- always monitor the dryer and make sure the heat is not too high to damage specimens.
- after at least 5 hours of drying, turn the enveloped on the other side
- make sure the drying box is protected from ants – use insect glue to protect its stand from them.
- when you add new fresh specimens from 2nd night light trapping, put them to the bottom trays and move the old specimens to the top.
- after specimens have been dried remove and store them in silicagel.

- be careful, dry specimens are easy to damage.



Drying and Storing Specimen in Silicagel

- Prepare a storage container. It must be airtight and labelled as 'NFI Geom Sample Storage'. This container must be kept safe and dry at all times and handled carefully during transportation. The lid has to be always kept closed to preserve silicagel.
- Put all glassine envelopes from each cluster into a large **Snaplock** bag (use Snaplock, not Ziplock) and fill out 'Storage Label' for each bag with Micron pen. Write this same information on the bag with permanent marker. The label will look like this below, and include Cluster ID. Keep the back closed at all time.
- Fill two large newspaper envelopes with fresh dried silicagel in each snaplock with the glassine envelopes. The silicagel has to be fresh. Pack the silicagel and moth envelopes in the box so that they do not freely move around – fill the space in the box with toilet tissue or newspaper as needed.
- Change silicagel for fresh one every 2 weeks in the field. Dry the envelopes again immediately after arriving to Nagada.
- When you place your hand into a container with silicagel, you must feel cold, this is how you know that silicagel is still fresh and working. Some silicagel also changes colour from blue (good) to red (bad). To make silicagel fresh again, heat it in a pot over fire.

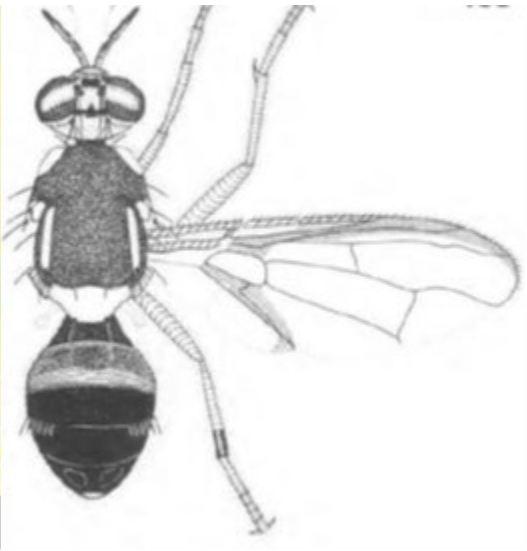
Data and Specimen Safety and Quality Control

- Keep pinned specimens in a different place (action packer) than envelope specimens so in case there is some disaster at least one collection survives.
- Pay attention to the safety of specimens. Keep checking the silicagel.

- All data sheets must be photographed and the photos from the camera card downloaded to two back-up hard drives.
- All data sheets must be organized in a folder at a safe location.

Protocol for NFI Faunal Biodiversity Assessment

FRUIT FLY (Diptera: Tephritidae) SURVEYS



Redley Opasa and Ruma Umari

The New Guinea Binatang Research Center

BINATANG RESEARCH CENTER

Fruit fly sampling guidelines

Fruit fly study protocol is part the insect protocol for the PNG National Forest Inventory (NFI) by the PNG Forest Authority. The protocols are designed so that they can be implemented for a cluster during 48hours of field work per site, with one lead researcher and two assistants.

Material list for one cluster

- Steiner traps x 10 + 2 spare
- Cue lure x 10 + 2 spare
- ME lure x 10 + 2 spare
- Zingerone lure x 10 + 2 spare
- Insect sticky glue x 1 small container
- Flagging tapes x 2 1 each of different colors (pink & yellow)
- 5mL vials x 40
- 2.5L ethanol (70%), pure medical grade
- Wash bottle x 1
- Hand glove
- Ziplock bags (Large storage) x 2 box
- Ziplock bags (snack) x 2 box
- Vial labels (hand written or typed)
- Cluster map (Laminated)
- Cluster information recording sheet
- Clipboard with clear folder- water proof
- Action packer (storage patrol box).

NFI Cluster Fruit fly sampling protocol

1.1 For a survey of each cluster use 10 Steiner traps, each baited with a combination of three lures: Cue lure, Methyl eugenol (ME) lure and Zingerone lure, also infused with insecticide.

1.2 The traps will be hung at about 1.5 meter high above the forest floor or ground. The 10 traps labelled 1-10 will be stationed at 10 plots identified within the cluster

1.3 Insect sticky glue will be put on the ropes/branch where the trap is hung on to prevent ants from coming into the trap.

1.4 Traps can be opened at any time of the day; the start hour must be recorded and the traps closed after 48 hours (=2 days) of sampling.

1.5 Three vials filled with ethanol (70% concentration, dilute from 96%, use pure medical grade ethanol only) should be labelled for each trap and packed in a small ziplock bag so there are 10 ziplock bags each containing three labelled vials prepared for collection.

1.6 Each trap has its sample code composed with letter F (for fruitflies) followed by the cluster number, hyphen and trap number from T01 to T10. Example: F64296-T04 is a code for fruitfly trap 04 in the cluster 64296.

1.7 Collection of trapped fruit flies from 10 traps will be made 48 hours after the start of the sampling. The samples will be carefully collected into respective labelled vials filled with ethanol for preservation. Use as many vials as possible depending on the size of the sample. Each must have a label. The vials must be placed back into its respective ziplock and all placed inside a small packer for safe keeping.

1.8 The specimens to be sent back to BRC for sorting and identification.

1.9 Basic information of the cluster location, climate, forest type and any other comments to be recorded on the site information sheet provided. Write the number of insect vials filled from each trap in the protocol.

IMPORTANT: Lures must be kept in air tight plastic bags the whole time, except when put into the traps. Once you close the trap, put the lures back to plastic bag and close.

IMPORTANT: Handle the lures with gloves and do not contaminate outside of the trap, the ropes and anything around the trap – this contamination will attract fruitflies away from the trap.

Data and specimens safety

Always look after the specimens well. Data sheets must be photographed and the photos downloaded from the camera card to back up hard drives. Data sheets must be kept in a folder at safe location.

Location of 10 Fruit fly traps in the cluster:

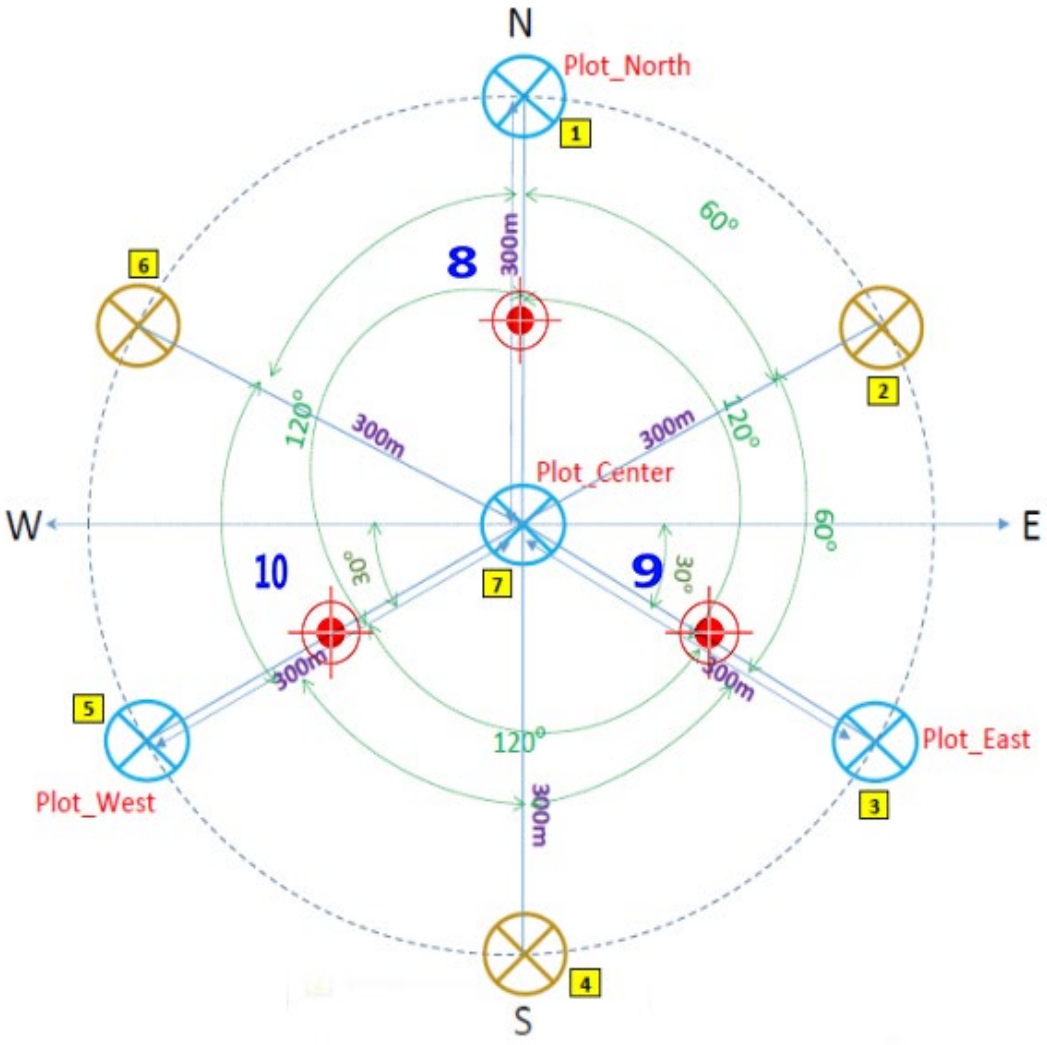
The fruit fly traps must be at least 100 m apart. The traps are located

- at each of the 4 plant plots (North, trap no 1, East, trap no 3, West, trap no 5, Center, trap no 7)

- at mid-points between Center and the other three traps, 150 m from the Center (Center-North trap no 8, Center-East trap no 9, Center-West trap no 10)

- at mid-point between West-North (trap no 6), Nort-East (trap no 2) and East-West (trap no 4), always 300 m from the Center.

NFI CLUSTER DESIGN



NFI Cluster Site Information Sheet

Fruit fly sampling record

Name of recorder: ……
Cluster ID: …… Date of sampling: ……..
Name of village nearby: …… District: ……Province: .....

General weather at time of sampling:

Forest type: Circle the appropriate description and record any history.
(Primary undisturbed forest, Primary disturbed forest, Primary part secondary)

Other comments/notes:

No. of vials with fruitflies for each trap:

T01..... vials T02..... vials T03..... vials T04..... vials T05..... vials

T06..... vials T07..... vials T08..... vials T09..... vials T10..... vials

.....

NFI Cluster Site Information Sheet

Fruit fly sampling record

Name of recorder: ……
Cluster ID: …… Date of sampling: ……..
Name of village nearby: …… District: ……Province: .....

General weather at time of sampling:

Forest type: Circle the appropriate description and record any history.
(Primary undisturbed forest, Primary disturbed forest, Primary part secondary)

Other comments/notes:

T01..... vials T02..... vials T03..... vials T04..... vials T05..... vials

T06..... vials T07..... vials T08..... vials T09..... vials T10..... vials

.....

NFI FRUIT FLY Vial labels

CL…… T01 Fruitfly NFI Site:…….. Date: …….. Collectors:……..	CL…… T02 Fruitfly NFI Site:…….. Date: …….. Collectors:……..	CL…… T03 Fruitfly NFI Site:…….. Date: …….. Collectors:……..
CL…… T04 Fruitfly NFI proj. Site: Date: …… Collectors:	CL…… T05 Fruitfly NFI proj. Site:…….. Date: …….. Collectors:……..	CL…… T06 Fruitfly NFI Site:…….. Date: …….. Collectors:……..
CL…… T07 Fruitfly NFI Site: Date: …… Collectors:	CL…… T08 Fruitfly NFI Site:…….. Date: …….. Collectors:……..	CL…… T09 Fruitfly NFI Site:…….. Date: …….. Collectors:……..
CL…… T10 Fruitfly NFI Site:…….. Date: …….. Collectors:……..		

Example of filled vial label:

CL64259-T09 Fruitfly NFI S 1200 S, 145'2400 E Date: 12-13/FEB/2016 Collectors: Sputnik. J, Muno. I	CL64259-T10 Fruitfly NFI S 1200 S, 145'2400 E Date: 12-13/FEB/2016 Collectors: Speedy. Y, Brunette. I

Protocol for NFI Faunal Biodiversity Assessment

ANT (Hymenoptera: Formicidae) SURVEYS

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BINATANG RESEARCH CENTER

Introduction

Ant survey involves two methods: TUNA BAITS and HAND COLLECTION. Tuna bait method will be done first, followed by hand collection. Hand collection could be done on the first day if there is time left after tuna baits, or on the second day. **Both methods should be done during dry weather (at least partly sunny if possible).** When the weather is good, do not delay ant collection and try to do it on day 1. In cae of rain, wait for better weather. If rains continue for the whole field work at the cluster, do the ant collection even in bad weather on the last day.

Tuna bait sampling

1. Two transects are set up for the work, with 10 baits at each transect (see Figure below). In total, there are 20 baits, with one sample from each.
2. Prepare vials, fill them with undiluted (96%) ethanol and cut labels in the camp so that you do not have to spent time on that in the field. Use 2ml vials where possible, keep 5ml vials for larger samples with bigger ant species. Vial should be filled by ants only up to its half (not overfilled), with ethanol above them. Try to use one vial for each sample – if necessary you can use 2 vials but in that case they both have to have a label (keep spare labels for this).
3. Tuna bait is made from Dianna tuna **in oil** (important – not in brine or anything else) mixed with cordial. Take one large tin (425g) can of Dianna tuna and mix 5 spoons of tuna with 1 spoon of cordial in a jar. One can of tuna is enough for 20 traps.
4. Start the first transect in the middle of the Central Plot. Clean a patch of ground by removing litter, place 10 x 10 cm gauze on the cleaned ground, then put one spoon of tuna bait on it. This will be bait A01 (Ant trap 01). Facing north direction, take 30 long paces and place second bait (A02), continue another 30 long paces in the same direction, until you put 10 baits along the 300m transect, reaching the North Plot. Record time of bait exposition after setting each bait, starting from A01. Place a flagging tape with bait number written near each bait. This will make it easy to find the bait when you come to check it.
5. After 1 hour of exposition, check the baits starting from A01 and record time of collection. At each bait collect all recruited ants and those roaming around baits. In cases there is a large number of ants on baits, **collect 5-10 individuals per species and record estimated abundance for each species** (on the scale 0 / 1-10 / 11-50 / 51-100 /over 100 in the data sheet)**.** Use soft forceps and aspirator for ant collection. Make sure to turn the gauze with bait and collect smaller ants underneath and those on the gauze. Place all ants from the bait into one 2ml or 5ml vial (depending on size & number of ants) in absolute (96% undiluted) pure (medical grade) insect ethanol.
6. In the protocol in **column "notes"** describe how each ant species looks like (color, size) so that its abundance in the data sheet can be later matched with specimens in the vial. Indicate forest type on data sheet. Different ant species can be recognised by their size, color and movements and behavior (**important**: note that the same

species do not bite / fight with other species so friendly ants belong most likely to the same species, fighting ants to different species).

7. Its important that after collection at each bait (A01,A02, …A20) place completed label into the vial and close lid tight. Each bait must be sampled into a different vial. **The label must include:**
   - Sampling Method: BAITING
   - Cluster Code (e.g. CL64259)
   - Trap Code (e.g., 01-A01): the first number (01 or 02) indicates the transect, followed by trap number from 01 to 20
   - Date of collection (e.g., 02 Jun 2017; use three letters for months, NOT numbers).
8. Leave the flagging tags marking the baits in place so that you can return for hand collection.

<u>Hand collection</u>

1. Hand collection can be done in the same day fllowing baits (approx. 2 hours after bait is removed), or on the next day if necessary. Always try to do it on good weather. Hand collection has to be done around the A03, A06, and A09 baits in transect 1 and A13, A16 and A19 baits in transect 2 (see the figure below).
2. Ants will be collected by 2 persons within 2 x 2 m plots from the ground to the height of 2 m above ground level.
3. Start at Tuna bait A03, use a tape measure to mark 2 m x 2m square. Mark the corners with sticks, and also mark 2 m heght with flagging tape. Search for ants within the marked area for 15 mins in 2 persons.
4. Search on soil, understory vegetation, tree trunks, leaf litter and nesting sites-cavities, dead wood,under stones and use aspirator to collect ants. If a nest or a large number of ants of one species is discovered at a spot collect only 5-10 ant workers per species (not all ants). Do not collect brood and pupae, only workers.
5. Scrape the litter and other material from the groung and put it on 1 m^2 white cloth for better search for ants.
6. Collect all ants from all species into prefilled 2 mls or 5 mls vial using aspirator and forceps from the 2x2 m plot. For nests or vary common species, collect 5-10 representatives per species.
7. In the protocol, write your estimate of all ants in total you collected from the whole square to the vial (0/1-10/11-50/51-100/over 100) in the data sheet). *Do not record and describe the species individually as in baits, as it can be many species found in the circle, and they will be sorted later in the lab.*
8. Each vials must have label with the following information:
   - Sampling Method: HAND-COLLECT
   - Cluster Code (e.g. CL64259)

- Trap Code (e.g., 01-A01): the first number (01 or 02) indicates the transect, followed by trap number: you will only use 01-A03, 01-A06, 01-A09, 02-A13, 02-A16 and 02-A19 trap codes
- Date of collection (e.g., 02 Jun 2017; use three letters for months, NOT numbers).

Data and specimens safety

All vials have to have labels written by ethanol-proof Micro pen inside of the vials (it is not allowed to tie two or more vials with a rubber band together with one label only). Vials from the baits sampled have to be kept in a zip lock bag marked with the method (baits) and cluster number using permanent marker; likewise vials from hand collection have to be in another zip lock. All protocols and data sheets have to be photographed, then safely filed. The photographs must be backed up on a hardrive.

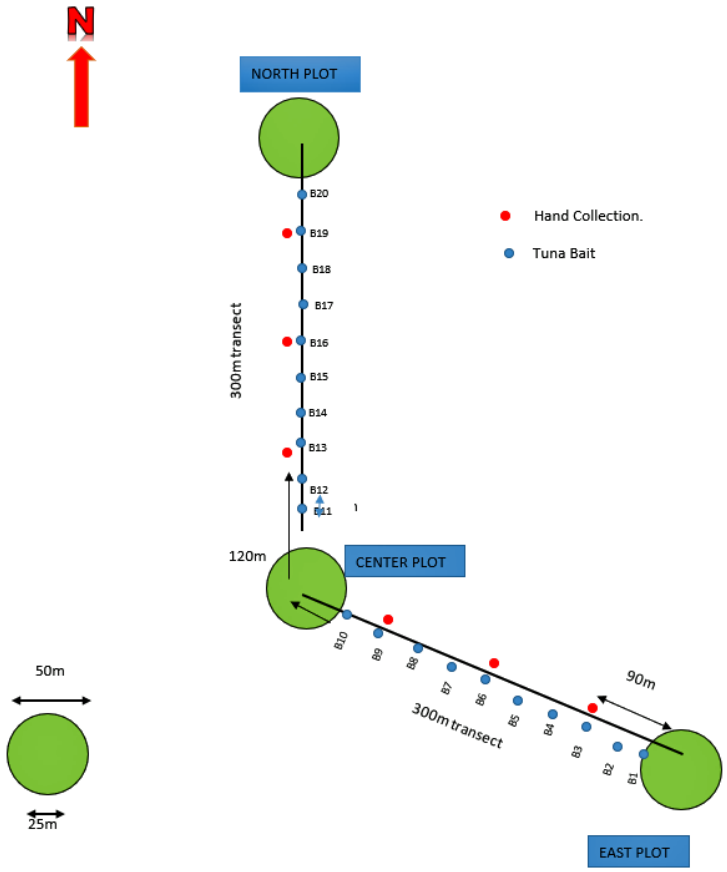

Figure: Tuna bait and Hand collection transect. Walk for 30m and set bait. For Hand collection, walk 60m and collect ants at every odd bait number