

SAMPLING KIT

Package Contents

Paperwork (digital documents also attached in the CCDB outshipment email from shipping@ccdb.ca):

- Standard Operating Procedures (PDF)
- Bulk Sample Shipment Checklist, Biological Material Transfer Policies, and Customs Declaration Form (PDF)
- Sample Log Sheets (Excel)

Equipment:

- 1x Malaise trap kit
- 1x Decanting lid (i.e. spare coupling ring)
- 2x Nitex filters
- Shipping stickers
- 52x pre-labelled 250ml sample bottles
- 4x 500ml collection bottles
- Rubber bands
- Funnel

Required from Participant

- 95% Ethanol
- Ethanol waste container
- Ethanol squirt bottle
- Standard household freezer (-20°C), preferred, or refrigerator for sample storage
- Return shipping materials
- 2x 6-8ft posts (optional)



SAMPLING PROTOCOL

It is critical that we employ standardized operating procedures for collecting Malaise trap bulk samples for DNA analysis at the Centre for Biodiversity Genomics (CBG). Our coordinated efforts will ensure optimal specimen preservation for sequence analysis and high-quality data recording, allowing for the comparison of sites at a global scale.

PREPARATION PRIOR TO DEPLOYMENT:

1. **Fill one 500ml collection bottle with 95% ethanol** at the time of deployment. Do not substitute with other kinds of alcohol or other preservatives.

Four collection bottles are included in your kit so that they can be swapped out in between bottle changes every week. Ensure that the entire sample is transferred to a 250ml bottle (see transfer instructions below) and then rinse out the used collection bottle with water and let dry, before re-using it for the next collection.



2. **Site Selection** - Deploy the trap at a site which is subject to minimal disturbance and ideally in a habitat's climax vegetation (i.e. placement in a national park or other protected area is preferred). When possible, position the trap at a right angle to an insect flight line, in areas with low undergrowth; forest edges or clearings and elevated sites are recommended.

Consider possibilities of wildlife disturbance and/or human vandalism – try to avoid either scenario as much as possible; the trap may be relocated if consistent issues persist after deployment. Ensure that all proper specimen collecting permissions are obtained (i.e. from local authorities, property owners, etc.).

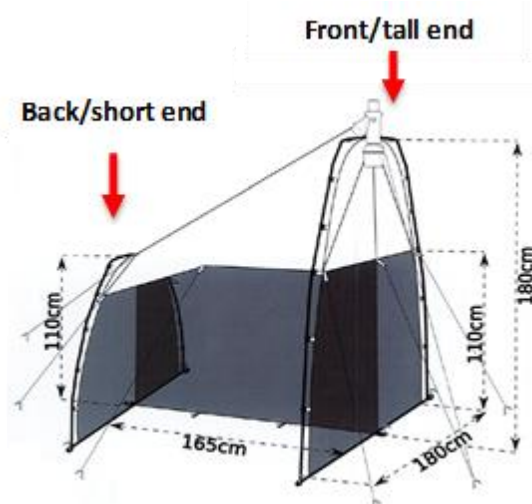
3. Prior to deployment in the field, watch a trap deployment video here:

<https://www.youtube.com/watch?v=sU9rW71f5ZA&t=9s>

While this video features an older trap design, there are useful tips that are applicable to the new design.

MALAISE TRAP ASSEMBLY AT SITE:

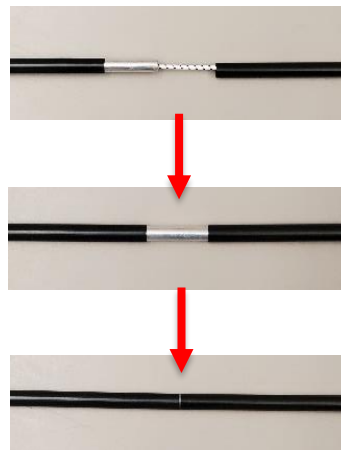
***Note: Assemble the trap on a day of the week you can consistently return to for the duration of the sampling period to collect the bottle.**



4. Bring the carrying bag with all components and a prepared 500ml collection bottle to selected site. Additional materials may include two 6 to 8ft posts for added trap support. Trap deployment is ideally performed by two people.
5. Unfold and lay out the fabric trap body on the ground with the white side facing up.



6. Assemble the short and long shock-corded poles. Make sure the narrow end of each pole is securely seated in the neighboring pole section.



7. Push the spring pins at the corners of front of the trap into the ends of the long pole. Clip the hooks sewn onto the trap around the pole to form a mesh panel. Repeat with the back panel of the trap with the short pole.



- With two people, stand up both mesh panels temporarily from the top of the poles to erect the trap. The inverted Y-shaped collection head should sit over top of the long pole. Please double check that the moth excluder at the trap head has been removed; in case it is still on, please take it out by pushing and twisting it through the trap head.



- Adjust the base of the trap so that the trap body is fully stretched and use the alloy metal pins and plastic X-stakes to stake the trap to the ground. Ensure the 2 spots within the middle panel are also staked down for a total of 8 pins/stakes.

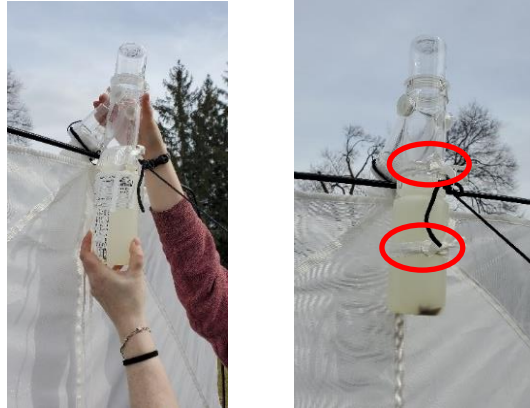


- Attach the guy lines to the trap and adjust them so that the trap erects in a proper shape and tension. Peg the guy lines into the ground using pins and stakes. Alternatively, tie the front and/or back lines to nearby trees for added support when possible.

There are 3 attachments at the front end, 3 at the back end, and 1 at each of the sides (for a total of 8 lines). For the sides of the trap, follow the line on the white panel to get proper trap tension. This serves to funnel flying insects up to the trap head.



11. Attach the prepared collection bottle to the trap head and fasten ribbons around trap head and bottle for added support. Ensure the collection head stands up straight.



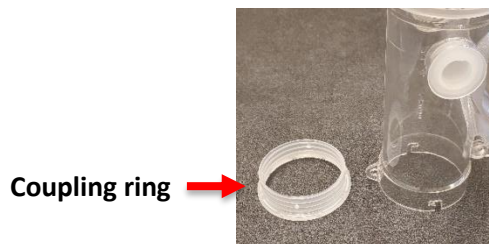
12. Deployment is complete. Follow Sampling and Monitoring Instructions below. Take an image of your Malaise site to send to CBG later.

Optional: Attach the trap body to an 8-foot post at the front end and a 6-foot post at the back end for additional stability; use zip-ties or rope to attach the poles to the posts. These are very helpful against high winds and other weather events, especially for traps placed in exposed areas.



SAMPLE COLLECTION AND MONITORING:

13. **Remove the catch WEEKLY**, on the same day each week. Remove the collection bottle from the trap head and replace with a new one. Take care when removing the bottle that the coupling ring stays on the trap head; re-attach it if it comes off with the bottle. (The decanting lid to be used in draining ethanol is a spare coupling ring.)



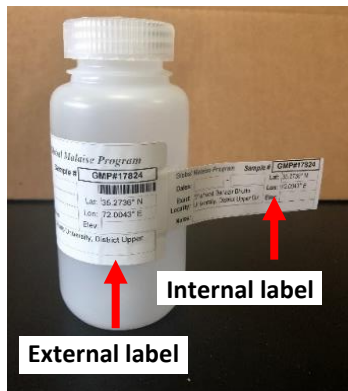
Record collection details in the Sample Log Sheet (examples: trap down at collection, bottle dry, spider web around trap head, etc.).

Visit the trap frequently to monitor for and repair damage, and to avoid sample overflow. In particular, check the trap after strong winds or heavy precipitation. In the event of damage, malfunction or other concerns contact ccdbcol@uoguelph.ca for maintenance suggestions and replacement parts.

SAMPLE TRANSFER:

- 14. After changing the 500ml collection bottle in the field, sample contents must be transferred into the 250ml sample bottles provided for storage and shipping. As much as possible, use the bottles in sequential order based on the number on their lids.

First, fill out the attached external and internal labels with collection details in PENCIL. Include collection details in Notes. Then, tear off the internal label and place it inside the bottle.



- 15. Using the decanting lid and nitex filter provided, drain off a little more than half (~200ml) of the ethanol from the collection bottle into a waste ethanol container. Leave enough ethanol that the specimens are still covered.

Watch a decanting video here: https://www.youtube.com/watch?v=KNpXTKZLO_Q



- 16. Swirl the sample gently to suspend the insects and then pour the contents into the labelled sample bottle. Ensure any specimens remaining on the bottle or nitex filter are rinsed off with ethanol and added to the bag. Thoroughly clean the filter before using again.



17. If the samples will be placed in a freezer, they may be pre-emptively prepared for shipping. This means that they will require very minimal amounts of ethanol. If the sample will be stored in a refrigerator, ensure that the full insect mass is submerged into ethanol.

SAMPLE STORAGE:

18. Ideally, place the samples in a standard household freezer (i.e. -20°C) for storage. If placed in a freezer, minimal ethanol is required in the bottle.

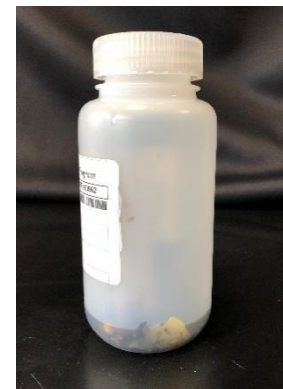
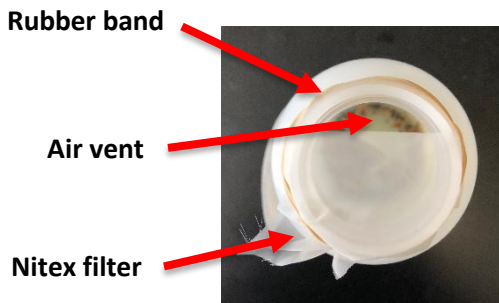
If a freezer is unavailable, store the samples in a refrigerator or cool, dark location. Add fresh ethanol to the sample as needed to ensure that the entire insect mass is fully submerged in ethanol before storage OUTSIDE of a freezer.

This is critical to preserve the DNA in the samples; improper storage will result in DNA degradation rendering samples unusable for DNA sequencing (e.g. under constant light, heat or variable temperatures).

SAMPLE SHIPMENT:

19. **Just before shipment, remove most of the ethanol from each bottle.**

Instead of the decanting lid, use a rubber band to hold the filter around the bottle opening; make sure there is still a small air vent after placement. Thoroughly rinse the filter between samples.



20. **There is a 30ml (1oz) ethanol maximum per container for shipment and a maximum total of 1L ethanol per package as per International Air Transport Association (IATA) regulations.**

If shipping 26 samples (half a year), each sample can have up to 30ml (1oz) of ethanol.

If shipping 52 samples (full year), please REMOVE AS MUCH ETHANOL AS POSSIBLE to stay under IATA limit (52x30ml = 1560ml = 1.56L which is over the 1L per package limit)

21. **The samples must be double bagged for shipment:** put absorbent material (e.g. paper towels) between the two bags and seal both tightly by taping generously or using a heat sealer.
22. Dispatch samples to Guelph, Ontario, Canada using cardboard boxes of appropriate size and add padding material as needed.
23. Review the [Bulk Sample Shipment Checklist](#) provided and ensure all the steps are complete.