

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
- 55x 500mL heat sealable sample bags
- 4x 500mL collection bottles
- External and internal stickers to be attached
- Plastic pipettes
- Bag-holding container (2L plastic bottle cut in half)
- Heat sealer

Required from Participant

- 95% Ethanol
- Ethanol waste container
- Ethanol squirt bottle
- Standard household freezer (-20°C) for sample storage
- Return shipping materials
- Plug adapter for heat sealer (if needed)
- 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 heat sealable bag (see transfer instructions below) and then rinse out the used collection bottle with ethanol 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.).

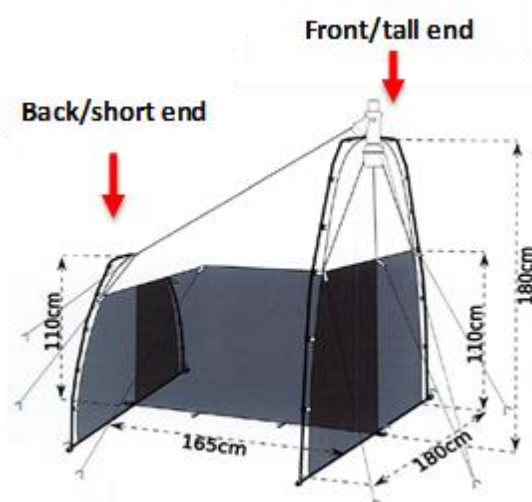
3. Prior to deployment in the field, watch a trap deployment video here:

<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:

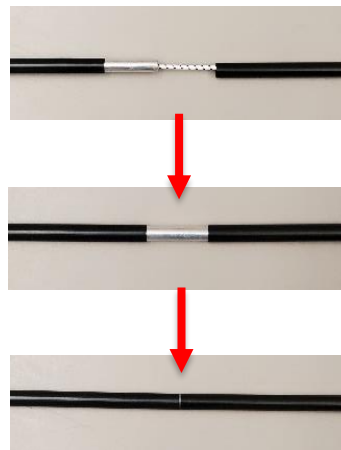
***Note: Assemble the trap on a day of the week you can consistently return to for the duration of the sampling period to collect the bottle.**



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.

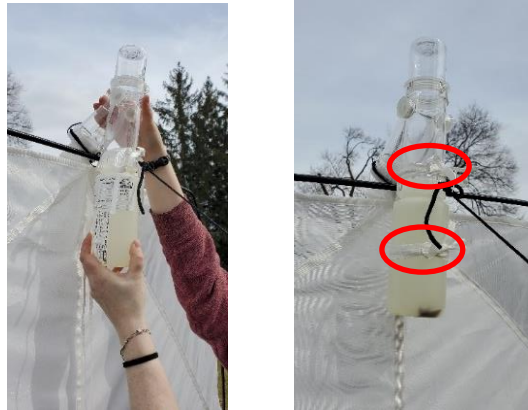


- Attach the guy lines to the trap and adjust them so that the trap erects in a proper shape and tension. Peg the guy lines into the ground using pins and stakes. Alternatively, tie the front and/or back lines to nearby trees for added support when possible.

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.



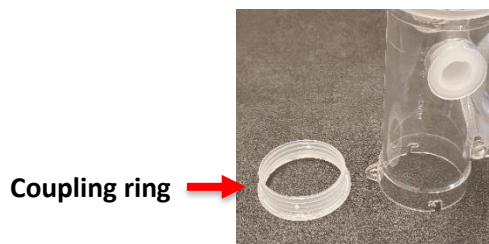
12. Deployment is complete. Follow Sampling and Monitoring Instructions below. Take an image of your Malaise site to send to CBG later.

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.)



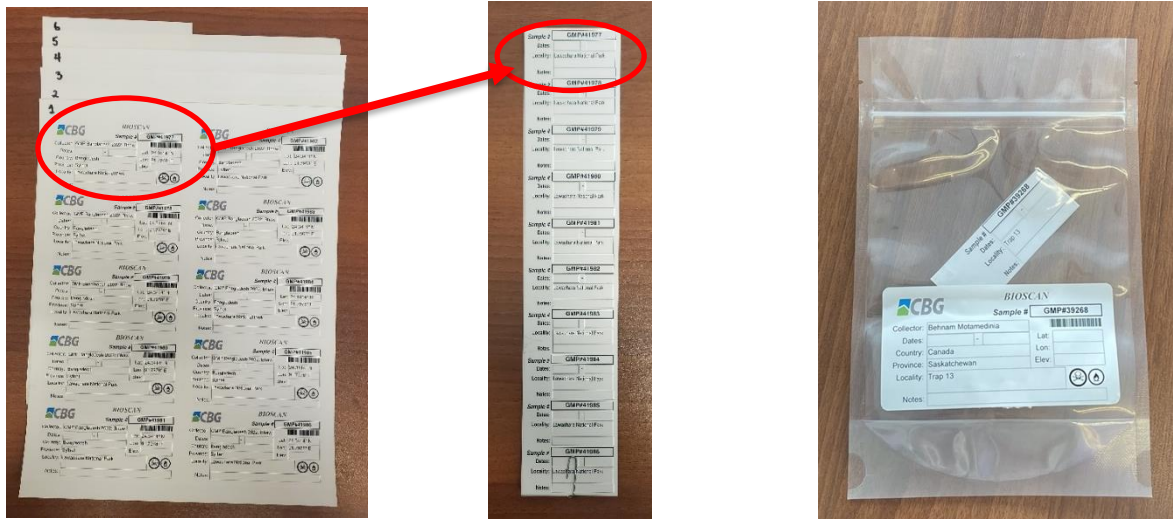
Record collection details in the Sample Log Sheet (e.g., trap down at collection, bottle dry, spider web around trap head, etc.).

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:

- After changing the collection bottle in the field, sample contents must be transferred into the bags provided for storage and shipping.

First, the sample bag must be labelled. Start with the lowest GMP# on the sticker sheets (external labels) and cut off the matching internal label from the fluid paper strips. Using PENCIL, fill out both labels with collection dates and other details in Notes (e.g., trap down, bottle dry, etc.). Place the internal label into the bag and attach the external label on the bottom of the bag.



External (sticker) labels

Internal (fluid) labels

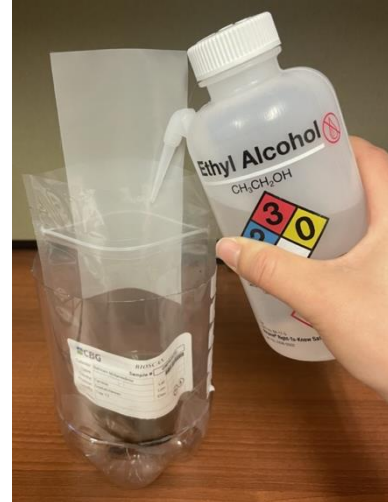
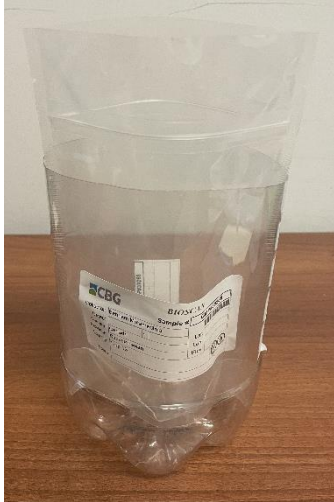
Sticker label goes on bottom of bag and MATCHING fluid label goes inside bag.

- 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 bag. The bag may be placed in a large jar or 2L plastic bottle (provided) to help hold it up during the sample transfer. 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 minimal ethanol is required in the bag and the bag may be heat sealed prior to storage (see #22 for heat sealing instructions). If the samples will be stored in a refrigerator, ensure that the full insect mass is submerged in ethanol.
18. To temporarily seal the bag, remove the air from the bag and manually close the zipper lock. **Please ensure the sample bags are stored upright and refrain from folding the tops of the bags until heat sealed to prevent leakage.**



SAMPLE STORAGE:

19. **Samples should be placed in a standard household freezer (i.e. -20°C) for storage.** 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).

Storage outside a freezer may be possible if you can provide a suitable cool, dark location such as a refrigerator. Any alternative storage arrangements MUST be discussed with and approved by a CBG contact prior to sampling.

Please ensure the sample bags are stored upright until heat sealed to prevent leakage.

SAMPLE SHIPMENT:

20. **Just before shipment, remove most of the ethanol from each bag.**

Cut a small piece of a nitex filter and attach it to a plastic pipette tip using tape. Open a bagged sample and use the filtered pipette to remove most ethanol from the sample. Take care not to remove specimens from the sample if they get attached to the filtered tip; place these back into the bag using forceps. Close the bag as before.

21. **As per International Air Transport Association (IATA) regulations, there is a 30ml (1oz) ethanol maximum per container for shipment and a maximum total of 1L ethanol per package; ensure these limits are not exceeded.**

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)

22. **Heat seal each bag.**

WARNING: Do not place the machine on blankets, sofas, clothes, or any flammable tabletop. After 30 minutes of continuous use, please let the machine rest for 3 minutes to prevent the heat sealer from burning out. Do not use wet cloth to clean the surface of the heating strip.

- I. Plug in the provided heat sealer and set the dial to 7. Place the open end of the bag over the heating element as close to the open end of the bag as possible (allowing us to reseal if needed). Make sure the bag is lined up straight.



- II. Press the lever down until the indicator light turns off, indicating the seal is complete.



- III. Raise the lever back up and move onto the next bag.

23. **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.

Place the bagged samples into a large plastic/Tupperware container or small cardboard box for added support during transport.

24. Dispatch samples to Guelph, Ontario, Canada using cardboard boxes of appropriate size and add padding material as needed.
25. Review the [Bulk Sample Shipment Checklist](#) provided and ensure all the steps are complete.