A List of Do's and Don'ts for Lindgren Funnel Trapping

The ease with which a trapped insect sample can be processed and the quality of the results obtained is strongly influenced by events that occur long before the sample lands under an identifier's microscope. The proper collection of data, the handling of the sample during its removal from the trap, the storage of the sample and its handling during shipment all play into the efficiency of the sorting and identification process.

The staff members of the Identification Services arm of the Carnegie Museum's Biodiversity Services Facility (BSF) have had the luxury of examining thousands of samples and have become quite familiar with the "good, the bad and the ugly" of samples and hope the tips below on 'Do's and Don'ts' for various steps in the sample handling process can increase the efficiency and quality of insect sampling for its clients. Since most workers who run the traps and submit the samples never see the processing end of the system at BSF, it is difficult for them to envision the results of their efforts in the field. It is our intent to help everyone involved avoid some of these common mistakes.

The protocols suggested below have been developed to accommodate clients with different available resources and to cover a wide variety of field conditions. These are not hard rules, but rather tools to help maximize the quality of the process overall. Therefore, many of the steps can be accomplished in a variety of ways depending on the resources available to each individual worker.

DEFINE THE SITE AND FIX THE DATA - EARLY!!

The data gathered and submitted with a sample serves the purpose of the documentation in space and time of the specimens within that sample. The data initially serves the practical need to identify when and where a specimen of a target taxon was collected, but then follows the residue of that sample on through the identification process. While some of the data may not appear to be useful to the client, it becomes absolutely necessary downstream for the labeling of target and non-target voucher specimens and archiving of the sample residues. The data collected should be in a standardized form to expedite its submission to the BSF system.

Data submission can be accomplished by physically submitting a field data sheet with each sample, but this has caused excessive data handling at BSF during the 2006 field season. By defining the trap sites early and capturing all of the mandatory data in spreadsheet format, the data can be more easily manipulated at BSF. Then, the only "new" data to be sent are the collection dates and possibly collector names. Efforts are underway to

provide automatic data upload access to clients via online connection to the BSF web site.

The following **mandatory** data can be defined and taken at the onset of the trapping season. This eliminates a need, for example, to return to take GPS readings two months into a survey, when they can be locked in at the time of trap installation. The other data fields which can be locked in early are explained below

1) Unique user site code

This coding system for samples is developed by the user, and can be in any format the user prefers, ***as long as a unique code is produced for every sample taken*.**

Always avoid combining trap numbers and sampling dates to produce a two-piece sample data code. Creating a single unique code is easily accomplished by adding an increasing numerical suffix to designate trip numbers to the unique trap number.

For example, for trips **1** to **3** at a trap designated as trap number **PA15**, the codes for these three visits would become **PA15-1**, **PA15-2**, **PA15-3**

2) State or Province

Self-explanatory

3) County

Self-explanatory

4) Specific locality

This field has proven to be the most problematic data submitted to BSF. The specific locality can include a wide variety of information, but providing **concise** data is the goal. Various locality identifiers such as names of national or state forests, state or city parks, industrial parks, company properties, street addresses or a myriad of others can be inserted here *as long as they are carefully defined*.

In the case of traps set in more remote areas that cannot be defined as a specific locale or address, it is preferable to define the site by its distance and directional vector to a locality that can be easily found on a map. For example, **3.7 km SSW Springfield** would be a typical site designation. It is preferred that all distances be given in **meters**.

DO NOT define a trap site by the closest mappable locality **without giving distance/vector information!** We have commonly seen sites identified by a city name, but when the lat/long readings of the trap site were mapped, the site actually was as much as 5 miles away from the reference city!

DO NOT mistake **directions** to a trap site as **locality** data! For example, do not use "*get onto route 31 at the Springfield exit and go about 2 miles, then look for the dirt road by the speed limit sign. Trap is hanging in a gingko tree in a field near the end of the dirt road*."

5) Latitude-longitude coordinates

These can be taken in a variety of formats, but the preferred format is *decimal-degrees.* GPS readings submitted in other formats can be converted, but this creates an extra data handling step as well as a source for error.

	Decimal-degrees	DD/MM/SS	GPS Coordinates
Preferred	**41.818883**		
Acceptable		**41–49-07**	
Disliked			**41° 49.133**

It is highly advisable to map the coordinates taken at the site using an online tool such as Google Map or some similar mapping software to verify that the readings are accurate and that they place the trap at the proper location. This verification need be done only once as long as the trap is not moved.

6) Habitat

Keeping this data concise and restricted to only the pertinent data is important. Long descriptions of the trap site should be avoided. Descriptors such as "*white pine stand*, *hardwood forest*, *maple/beech/oak"* are all acceptable. In the case of traps set in urban or commercial settings, habitat can be defined as "*in warehouse, baggage handling area, lumberyard"* or other pertinent terms.

DO NOT describe habitat as "*hung on fence near dumpster*" or "*in trees at back edge of parking lot*" as these describe the trap **placement** but have no real value in defining the **habitat**.

7) Trap type

This should be a short description of the style of trap used – Lindgren funnel trap, malaise trap, UV light trap, etc.

8) Lure type

This notes the type of lure placed on a trap, and can be abbreviated in a standardized form – *ethanol* (EtOH), *ethanol plus alpha-pinene* (EtOH + a-pinene), *triple Ips lure* (3-Ips), etc. If a trap is unbaited, no entry is necessary.

The following data fields are **mandatory**, but **will change** over time

9) Collection date

If the collection was made on a single day, such as a light trap sample, the *date the trap was set* is all that is necessary.

In most cases the collection date will be a range of dates, from the day that the trap was set or the last sample taken, to the date of the current sampling. When installing a trap, the date of setup should be recorded and clearly defined as **setup** – this indicates that no sample of specimens is associated with the visit. Note that the first s*ample* rarely coincides with the first *trip*, but is usually taken on the *second* trip.

On subsequent visits **the date should be recorded as a continuous range**, from the date of visit number 1 to visit 2, from visit 2 to visit 3, etc. This can be accomplished easily by photocopying the data sheet after each visit and using the copy as the sheet for the next trip. This allows one to build a single data table as the season progresses.

Example:

Trip #	Start date	End date
1	**setup**	3 March 2006
2	3 March 2006	10 March 2006
3	10 March 2006	17 March 2006

10) Collector name(s)

When listing a collector, or collectors, for a sample, give the entire name(s), not just initials. It is expected that one lists multiple collectors if more than one person was involved in taking the sample.

Additional, but not mandatory fields include:

Township

A data field for Township is included in the BSF data base, but it is not a required field. Whenever listing a township, do so as a separate data field, not as part of the Specific locality data field.

Elevation

If elevation is recorded, list it as a separate data field, not as part of the Specific locality. It is preferred that this measurement be given in **meters**.

Usage of data sheets

The use of a preprinted field data sheet is highly recommended and can reduce the chance of lost information as the season progresses. Having a data sheet produced for each trap containing the above mandatory data fields and carried to the site greatly increases efficiency of data collection.

This makes it necessary to record only the date of collection and make any changes to the *collector(s)* field on each trip.

With care, the unique sample code can be produced for each trap prior to each visit to a site and pre-printed labels can be inserted into the sample as it is collected.

A common problem with field data sheets is their protection from the elements. We have seen many such sheets which were filled out and then wetted, either from rain or from leaking preservatives. It is imperative that all data sheets are written in ink that is impervious to alcohol and water (such as a *Pigma* brand pen), or in pencil. **Never** use ball point pens, water-soluble markers or other inks soluble in alcohol.

Preservative usage

Nothing can degrade a trap sample faster than insufficient preservative in the trap. In Lindgren funnel traps the most commonly used preservative is antifreeze. *Propylene glycol* is preferred over *ethylene glycol* due to its lower toxicity. In either case, care must be taken to prevent spillage in the environment.

Antifreeze is not cheap and some consideration must be given to the amount used. A common solution is to recycle the fluid from trip to trip by straining the specimens from the fluid and then returning it to the trap. **DO NOT DO THIS!** Once specimens, debris and sometimes rain have diluted the fluid in the cup, there is no way to be sure of the concentration of the preservative other than by visual inspection, and this is not at all reliable.

The better solution is to dilute the antifreeze to a 50% concentration with water prior to placement in the collection cup, and then completely replace the liquid upon each visit. This assures a known concentration of preservative at the onset of every collecting period. If rain is expected over the duration of the period, play it safe and fill the collecting cup with 100% antifreeze, leaving enough room for additional water in the cup to prevent overflow.

A liquid container should be taken to the trap site upon each visit so that the old preservative from the trap can be collected and later disposed of properly.

Recovery of a trap sample

The quality of a trapping sample depends greatly on how much effort is put into the extraction of the sample from the trap and its subsequent handling. Good trap maintenance begins with having the proper equipment, which will include the following list of items:

conical paint filters

plastic funnel to hold filters

Whirl-Pak storage bags

80% ethanol for preserving the sample

replacement antifreeze for the trap

pre-printed data labels

appropriate pen or pencil

wash bottle with water

disposal container for old antifreeze

Now, we'll look at each of these items and cover some 'Do's and Don'ts'

Choice of filter

The preferred filter is a conical paper paint filter with a nylon or woven cotton screen in the bottom. These come from a variety of manufacturers but are universal in size. All brands work well, although the brands with nylon mesh have some advantage in that specimens don't get snagged as much as in the cotton mesh styles.

DO NOT use other filter types, such as large netting bags or coffee maker filters. These do not provide enough control of the fluid washing the sample into the filter and specimens can be lost. It is also very difficult to remove the samples from these types of filters.

Choice of sample storage container

The preferred storage bag for the samples are Nasco brand Whirl-Paks – plastic sample bags with a built-in twist tie in their upper end. If these are not available, some styles of plastic ZipLoc bags can work in a pinch, but few styles will hold liquids without leaking.

DO NOT USE the zipper-style bags that have a separate sliding plastic zipper for closure. These leak substantially around the plastic slide and will drain dry quickly.

TRANSFER OF A SAMPLE INTO A WHIRL-PAK

When removing a sample from a trap collection cup, **DO NOT** attempt to pre-sort the sample and **DO NOT** remove debris such as leaves, needles or small sticks. Many of the target taxa are very small and can be lost if this material is removed in the field.

To retrieve a sample from the trap collection cup:

1) Place a fully opened paint filter into the plastic funnel and secure over the antifreeze disposal container.
2) Remove the sample collection cup from the trap and carefully pour the contents into the funnel, allowing the antifreeze to drain into the disposal container.
3) Using the wash bottle of water, rinse the trap cup and pour any remaining specimens or debris into the funnel.

4) With the wash bottle, gently rinse the sample down into the point of the filter, and **let the sample drain until all of the excess water has been removed.**
5) Once the sample has drained, gently fold the paint filter vertically into thirds, **taking great care not to press so tightly as to crush the sample** inside.
6) Once these vertical folds are made, fold the top edge of the filter downward upon itself to seal the sample inside – this upper fold should involve only ½ to 1 inch of the filter.
7) Select a Whirl-Pak, and open it by tearing off completely the perforated strip above the yellow twist-tie.
8) Place the folded filter into the bag, pointed end down, and cover it with enough ethanol to completely submerge the specimens. A good rule of thumb for adding the appropriate amount of ethanol is to fill the bag until the entire screen portion of the filter is submerged. This usually results in a bath of ethanol approximately 1.5 to 2 inches deep. If the ethanol level is too low, specimens will quickly begin to rot and disarticulate, making identification impossible.
9) Place a printed sample data label into the Whirl-Pak with the sample. **Take care to place the label such that it can be easily read from the outside of the bag**. This data will need to be clearly visible without opening the Whirl-Pak to be logged when received at the BSF offices. **DO NOT write the unique code number or tape labels onto the outside of the Whirl-Pak as the only identifying mark!** We have seen multiple cases where this was done, and leaking alcohol caused the info to dissolve from the bag, leaving it codeless.
10) Seal the Whirl-Pak by grasping the bag with both hands just above the level of ethanol and slowly **press the air out of the bag** as you move your fingers upward. Some alcohol may spread upward as well – this is good as it will improve the seal by replacing the air with a liquid layer. Once you have reached the top, begin rolling the twist-tie downward onto the bag, **making sure to keep tension so that it rolls tightly** – this is critical in getting a good seal. Roll the bag down to just above the upper edge of the filter, and fold the ends of the twist-tie inward upon the bag to lock it in place.

The sample is now stable for transportation from the field and eventual shipping to the identifiers. Care must be taken to prevent crushing of the samples, so a sturdy plastic container, such as a plastic shoe box, will protect the sample during transit from the field.

Handling and storage of sample until shipping

Once the samples are collected and brought back from the field they should be stored in a freezer if at all possible. Having appropriate ethanol levels on the samples will preserve them for several weeks, but freezing adds another level of protection against degradation of the specimens. If a freezer is not available, placement in a refrigerated or other cool place is advised.

The samples should be examined carefully for loss of preservative as even a small leak can cause ethanol levels to drop quickly. **Insufficient preservative levels can cause samples to rot in a matter of days,** especially in periods of hot weather, making them impossible to screen.

Shipping the samples to the Identification Services lab

The most common problem experienced by the BSF staff during the 2006 season was receiving packages with visible leakage of the ethanol, due to poorly sealed Whirl-Paks or samples being sent in inappropriate bags. If all of the above protocols are followed and care is taken in preparing the Whirl-Pak containing the sample, shipment of the samples through the mail should pose no undue threat.

When ready to ship, place all of the Whirl-Paks containing samples into a large Ziploc bag. Place all associated data sheets in a large ZipLoc bag. Pack the bag containing the samples into a box large enough that the samples are not compressed to avoid crushing specimens. Fill any excess space with absorbent packing such as paper toweling or newspaper to cushion the samples and absorb any possible leakage. Place the data sheets on top of the samples and seal the package.

Shipping can now be done by priority courier such as FedEx, DHL or USPS Priority Mail. If alcohol levels are appropriate, overnight shipping is not necessary and a less expensive 2- or 3-day service can be used. Whether the increased cost of using more alcohol could offset the lowered cost of shipping is a mathematical question better answered by a diligent accountant than by BSF staff …