# TOS PROTOCOL AND PROCEDURE: GROUND BEETLE SAMPLING

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See configuration management system for approval history.

# Change Record

| REVISION | DATE | ECO # | DESCRIPTION OF CHANGE |
|---|---|---|---|
| - | 04/26/2011 | ECO-00160 | Initial Draft Release |
| A_DRAFT | 10/03/2011 | ECO-00280 | Update to new document numbers and template |
| B_DRAFT | 06/25/2015 | ECO-00443 | Adjusted for known issues from 2011 prototype and revised for Domain 3 specific information |
| C_DRAFT | 01/10/2014 | ECO-01138 | Thorough review with many small changes in terms of timing, sampling details, data handling/processing. |
| D | 03/26/2014 | ECO-01674 | Production release, template change, and other changes as detailed in Appendix C |
| E | 04/10/2014 | ECO-01792 | Updated Appendix D with site-specific information |
| F | 12/05/2014 | ECO-02588 | Migration to new protocol template<br>Substantial revision, including:<br>- Clarification to address JIRA tickets and provide more detailed instruction (throughout)<br>- Moved information to appendices and supplementing the information (e.g., Appendix G)<br>- Exported information to (e.g., shipping instructions), and imported information from (e.g., pinning/pointing instructions), related document RD[06] to ease the use of multiple protocols for technicians.<br>- Added tolerances for placement of pitfall traps in plots<br>- Added information for dealing with disturbed and/or empty traps<br>- Added information on handling vertebrate bycatch and morphospecies |
| G | 03/17/2015 | ECO-02749 | Re-imported shipping information in SOP I; added corresponding info and tables (e.g., table 9) throughout |
| H | 05/04/2015 | ECO-02890 | Language requiring that gloves be worn by anyone touching vertebrate bycatch with their hands. Also, technicians performing cervical dislocation must have received prior training.<br>Fixing an error in the figures in response to FOPS-2034. |
| J | 11/23/2015 | ECO-03399 | Fixing workflow compatibility with the WebUI<br>Updated species lists |

| | | | Fixed sample timing section; added site specific estimated start dates<br>Added in language/equipment concerning isoflurane<br>Updated label generation section for consistency<br>Updated workflow to remove 'other carabid' |
| --- | --- | --- | --- |
| K | 01/31/2017 | ECO-04363 | Updated plot relocation criteria; updated text to reflect new data entry application; revised site specific estimated start dates |
| L | 01/26/2018 | ECO-05253 | Add language about barcodes; pinning modification to pin all beetles if less than 10 would be stored together, mobile data entry updates; site specific trap deactivation at STER (due to carrion beetle activity); added 2018 modification to set 3 traps per plot instead of four (all but the north trap), performed as a budget reduction for 2018 only |
| M | 04/11/2019 | ECO-05966 | • Remove all reference to North trap<br>• Clarified when barcodes are needed<br>• Minor updates to conform with fulcrum app changes<br>• Update Appendix D dates based on latest MODIS data<br>• Update Appendix E and F on 2017 expert taxonomy data, where available<br>• Update site specific deviations to document D20 modifications |

| *Title*: TOS Protocol and Procedure: Ground Beetle Sampling | | *Date*: 04/11/2019 |
|---|---|---|
| *NEON Doc. #*: NEON.DOC.014050 | *Author*: Katherine LeVan | *Revision:* M |

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# 1 OVERVIEW

## 1.1 Background

The purpose of the ground beetle abundance and diversity sampling design is to capture inter- and intra-annual variation of the ground beetle (Coleoptera: Carabidae) community in the NEON purview. Ground beetles were chosen as a focal terrestrial invertebrate taxon for several reasons, including that they are easy to sample and well known taxonomically, are relatively common in many habitats, form well-defined species richness gradients across North America, and are useful as indicators of environmental change due to their sensitivity to habitat disturbance. In addition, ground beetles can strongly influence trophic structure, both because many species are predacious (thereby influencing prey populations) and are consumed by other predators (thereby influencing predator populations). As such, changes in ground beetle populations may alter proportions of various other guilds in a community.  A full justification for the inclusion of ground beetle sampling in the NEON framework is provided in the TOS Science Design for Ground Beetle Abundance and Diversity (AD[05]).

The following sub-sections contain protocols that provide detailed guidance for setting pitfall traps in TOS Distributed Plots. Pitfall traps serve to capture ground-dwelling invertebrates (insects and their allies, e.g., spiders, scorpions) that fall into the traps. The animals that fall into the trap become preserved by a liquid mixture of DNA-safe preservative in the bottom of the trap. Animals that are collected in these traps but are not ground beetles are termed "bycatch."  In addition, this protocol describes laboratory processing and storage of the collected animals.

## 1.2 Scope

This document provides a change-controlled version of Observatory protocols and procedures. Documentation of content changes (i.e. changes in particular tasks or safety practices) will occur via this change-controlled document, not through field manuals or training materials.

### 1.2.1 NEON Science Requirements and Data Products

This protocol fulfills Observatory science requirements that reside in NEON's Dynamic Object-Oriented Requirements System (DOORS). Copies of approved science requirements have been exported from DOORS and are available in NEON's document repository, or upon request.

Execution of this protocol procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. These data and samples are used to create NEON data products, and are documented in the NEON Level 1, Level 2 and Level 3 Data Products Catalog (RD[03]).

## 1.3 Acknowledgments

Cara Gibson and Patrick Travers contributed to earlier versions of these protocols.

## 2        RELATED DOCUMENTS AND ACRONYMS

### 2.1        Applicable Documents

Applicable documents contain higher-level information that is implemented in the current document. Examples include designs, plans, or standards.

| AD[01] | NEON.DOC.004300 | EHS Safety Policy and Program Manual |
|---|---|---|
| AD[02] | NEON.DOC.004316 | Operations Field Safety and Security Plan |
| AD[03] | NEON.DOC.000727 | Domain Chemical Hygiene Plan and Biosafety Manual |
| AD[04] | NEON.DOC.050005 | Field Operations Job Instruction Training Plan |
| AD[05] | NEON.DOC.000909 | TOS Science Design for Ground Beetle Abundance and Diversity |
| AD[06] | NEON.DOC.004104 | NEON Science Data Quality Plan |

### 2.2        Reference Documents

Reference documents contain information that supports or complements the current document. Examples include related protocols, datasheets, or general-information references.

| RD[01] | NEON.DOC.000008 | NEON Acronym List |
|---|---|---|
| RD[02] | NEON.DOC.000243 | NEON Glossary of Terms |
| RD[03] | NEON.DOC.002652 | NEON Level 1, Level 2 and Level 3 Data Products Catalog |
| RD[04] | NEON.DOC.001271 | NEON Protocol and Procedure: Manual Data Transcription |
| RD[05] | NEON.DOC.001580 | Datasheets for TOS Protocol and Procedure: Ground Beetle Sampling |
| RD[06] | Available via download of data from NEON portal | NEON Raw Data Ingest Workbook for TOS Ground Beetle Abundance and Diversity |
| RD[07] | NEON.DOC.001953 | Pitfall trap spacers assembly instructions |
| RD[08] | NEON.DOC.001818 | Pitfall trap cover assembly instructions |
| RD[09] | NEON.DOC.001816 | Filter cloth assembly instructions |
| RD[10] | NEON.DOC.001814 | Filter cup assembly instructions |
| RD[11] | NEON.DOC.001271 | Data Management Protocol |

### 2.3        Acronyms

| Acronym | Definition |
|---|---|
| RTE | Rare, Threatened and Endangered |

All other acronyms used in this document are defined in RD[01].

### 2.4        Definitions

None given.

# 3 METHOD

Ground beetle sampling, as detailed here, involves: preparation for sampling (**SOP A**); pitfall trap deployment (**SOP B**) and the collection of ground beetles (**SOP B**); sample processing in the lab to sort target taxa (ground beetles) from invertebrate and vertebrate bycatch (**SOP C - SOP E**); identification of individual ground beetles to species and proper specimen preservation (**SOP E, SOP F**, & **SOP G**); data entry and verification (**SOP I**); and instructions for shipping specimens to a taxonomist (**SOP I**). Additional ground beetle-related data will be gathered when tissues from a subset of specimens are DNA barcoded, with details and rationale provided in the TOS Science Design for Ground Beetle Abundance and Diversity (AD[05]). Any site-specific deviations from this protocol are listed in **APPENDIX G**.

The pitfall trap design consists of two 16 oz. deli containers (7 cm deep with an 11 cm diameter, 540 mL total volume) nestled within one another. The lower container ensures that the trap remains flush with the ground, maintains the integrity of the hole, and enables efficient collection and resetting of the trap. Holes drilled into the base of the lower container allow excess moisture to drain; this also prevents the upper container from floating. The upper container holds a fluid preservative that kills and safeguards beetles from degradation. The contents of the upper container is picked up and changed during each collection event. A square cover (20 x 20 cm) elevated 1.5 cm above the trap entrance protects the container from weather (e.g., dilution from rain, drying from sun) and prevents unintended bycatch of medium to large vertebrates.

Depending on ambient temperatures at a site, technicians will place 150 or 250 mL of preservative into each trap on a biweekly basis (e.g., warmer temperatures or lower humidity will typically require more preservative). Pitfall traps will be labelled with demarcations indicating 150 or 250 mL to allow for efficient resetting of traps. The preserving fluid used in the pitfall traps is a 1:1 mixture of distilled or deionized water and propylene glycol (abbreviated PG). Propylene glycol is non-toxic antifreeze (SDS: mild irritant, non-toxic).

Pitfall traps are placed on the interior edges of the 40m x 40m Distributed Plots (well outside of the 20m x 20m plot interior, where plant sampling will occur). Ten Distributed Plots will each have three pitfall traps (30 traps total per site) located as close as possible to the center of three edges of the plot (20 meters from the center of the plot on the south, east, and west edges). In the diagram below (Figure 1) pitfall traps in a distributed plot are represented by circles with the cardinal direction of the trap location (e.g., 'E' for the east trap). Plots for pitfall sampling will be identified prior to the field season.

Standard Operating Procedures (SOPs), in Section 7 of this document, provide detailed step-by-step directions, contingency plans, sampling tips, and best practices for implementing this sampling procedure. To properly collect and process samples, field technicians must follow the protocol and associated SOPs. Use NEON's problem reporting system to resolve any field issues associated with implementing this protocol.

The value of NEON data hinges on consistent implementation of this protocol across all NEON domains, for the life of the project. It is therefore essential that field personnel carry out this protocol as outlined in this document. In the event that local conditions create uncertainty about carrying out these steps, it is critical that technicians document the problem and enter it in NEON's problem tracking system.

Quality assurance will be performed on data collected via these procedures according to the NEON Science Data Quality Plan (AD[06]).
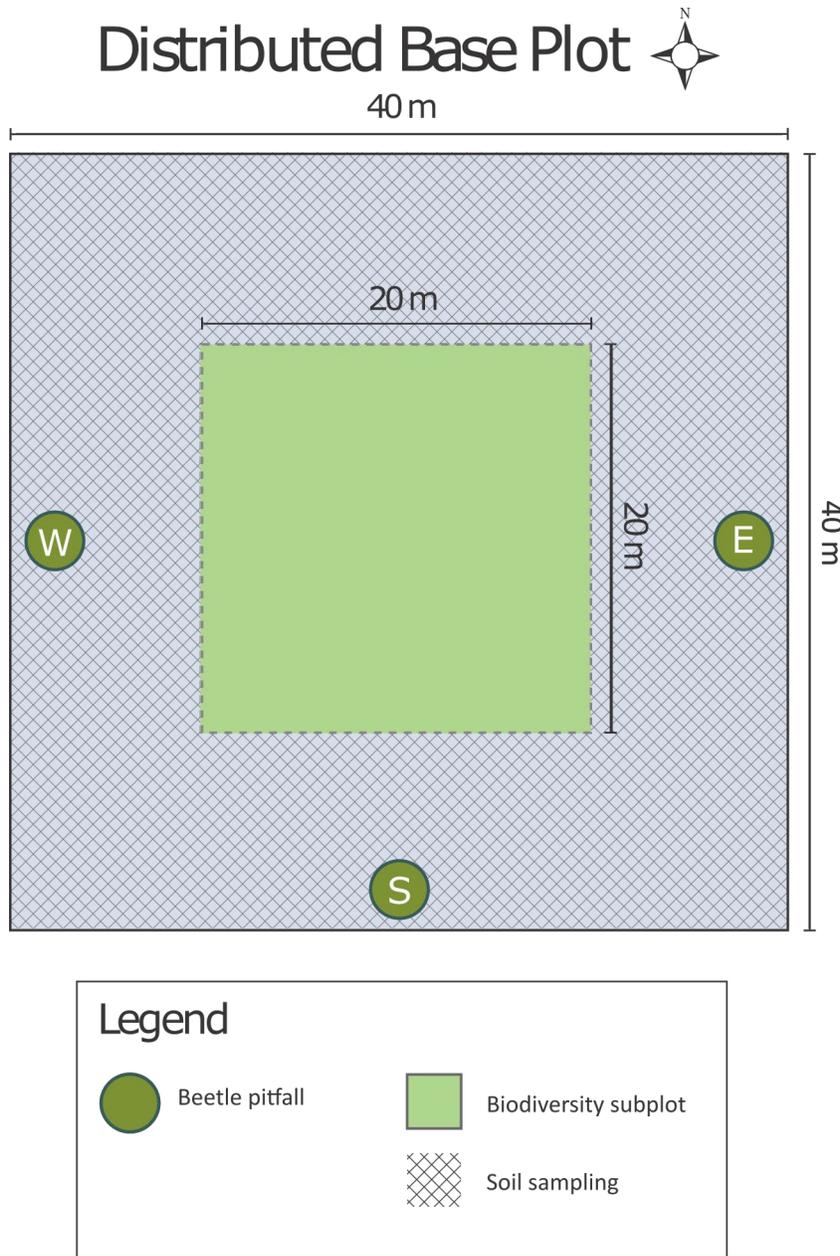


**Figure 1. Standard beetle plot layout. Technicians may not enter the exclusion zone (indicated in green).**

## 4         SAMPLING SCHEDULE

### 4.1        Sampling Frequency and Timing

Pitfall traps will be checked, emptied, and reset every 14 days. For example, if a plot is visited on a Thursday morning for the first sampling bout, it must be visited every other Thursday (morning) subsequently, so that the samples from each bout are directly comparable (i.e., beetles are collected over the same time intervals).  The selected day of the week is discretionary; however, the sampling should occur consistently every 2 weeks for the entire field season, on the same day of the week, and at roughly the same time of day. Sampling schedules that would make a trap collection event fall on a holiday are to be avoided to the extent possible.

### 4.2        Criteria for Determining Onset and Cessation of Sampling

Ground beetles should be sampled during the growing season when biological activity is highest and when minimum temperatures are above 4°C. Estimated dates for onset and cessation of sampling are provided per site in **APPENDIX D**. These dates are based on the average timing of green-up and senescence for each site over the last decade. Field staff at each site will initiate and conclude sampling based on the dates listed in **APPENDIX D**.

Initiation of sampling may be delayed from the estimated start date if there are persistent cold temperatures, which is when the average minimum temperature over the prior ten day period is lower than 4 °C. Likewise, sampling may be concluded earlier than specified by the estimated end date if persistent cold temperatures are observed before the end date occurs. Field staff must issue a problem ticket to the Insect Ecologist if persistent cold temperatures would result in delaying the beginning of sampling or concluding the sampling season early by more than a month of the estimated dates.

*Note*: NEON Science anticipates performing the number of collections (i.e., '**Bouts Expected**') listed in **APPENDIX D** during the date ranges provided for each site. Field Operations staff are not expected to sample substantially outside the date ranges listed in **APPENDIX D**, and should schedule bouts according to the listed dates. If persistent cold temperatures occur (as are described in the paragraph above), the Insect Ecologist will work with the Domain manager to shift scheduled dates such that the number of expected bouts are still performed, if temperatures allow. If temperatures are significantly warmer *outside* the window described in **APPENDIX D** (i.e., the temperature minimums are above 4 °C prior to the start date or after the end date), field staff must issue a problem ticket to the Insect Ecologist to document this discrepancy. However, no additional collections are required above the number of expected bouts. Documented discrepancies will be used to adjust **APPENDIX D** through time, as budget allows.

### 4.3        Timing for Laboratory Processing and Analysis

- Within 24 hours of field sampling:
    - o   pitfall trap samples must be rinsed in ethanol with any vertebrates removed and identified (see **SOP C**)
- Throughout field season:
    - o   sort invertebrate bycatch from the trap (see **SOP D**)
    - o   begin identifying carabids (**SOP E**)
    - o   begin pinning carabids (**SOP F**)
    - o   pool specimens, as needed (see **SOP G**); only after QC on sorting data is complete
- Within 4 months of field season end:
    - o   complete identifying carabids (**SOP E**)
    - o   complete pinning carabids (**SOP F**)
    - o   complete specimen pooling, as needed (see **SOP G**)

### 4.4        Sampling Timing Contingencies

When unexpected field conditions require deviations from the field protocols outlined in this document, contingent decisions, outlined in **Table 1** below, should be followed in the interest of maintaining data quality. The table describes how to respond to delays in the sampling schedule (including delayed trap collection and/or reset) and explains some of the consequences of sampling delays.  It is important to determine the site schedule (e.g. controlled burns, grazing rotation) at the outset of each season to ensure that traps are not damaged by site activities, if possible. Note: this is distinct from site-specific modifications (see **APPENDIX G**).

Occasionally, disruptive events occur during the field season with timing that is impossible to predict at the start of the season, but some notice is given in advance (e.g., the entire site will be tilled or burned, a hurricane coming through the site will destroy all the traps). If advance notification is given of site management activities or other conditions that will definitely compromise traps *in between* normal collection events (i.e., bouts are 14 days long, but the burn management is on day 7 of a bout), collect the specimens early (if staff resources allow) from the plots that would be affected by the disturbance. **DO NOT** redeploy any of the traps until the next regularly scheduled visit. When entering data, the set dates for the bout that was prematurely concluded will be the originally scheduled set date and the collect date will reflect the date the traps were removed. Indicate in the remarks that 'traps were collected early' with the reason that traps that were collected early. For site management activities or natural events use the "Site Management and Event Reporting" data entry application to note the type of disturbance.

**Table 1. Contingent decisions**

| Delay/Situation | Action | Outcome for Data Products |
|---|---|---|
| Collection delayed by 1 day[1] | Collect traps asap. Do not reset trap until all traps can be reset simultaneously (e.g., onset of *next* sampling bout)[2]; note duration & cause; resume standardized sampling (2 weeks) | Data not comparable to standard collection events.<br><br>Cannot calculate diversity indices if trap collections are not comparable. |
| Collection delayed by 2 or more days | Collect traps asap. Do not reset trap until all traps can be reset simultaneously (e.g., onset of *next* sampling bout) [2]; note duration & cause; resume standardized sampling (2 weeks) | Data not comparable to standard collection events; integrity of specimen DNA becomes compromised<br><br>May not be readily able to obtain DNA barcodes; affects Abundance & Diversity measurements |
| Catastrophic event that would damage samples (e.g., tilling through the plot, burning directly in the plot, hurricane at the site) | If event will damage samples *in between* normal collection events (i.e., bouts are 14 days long, but the burn management is on day 7 of a bout), collect the specimens early (if staff resources allow) from the plots that would be affected by the disturbance; note duration & cause in site management application; resume standardized sampling per the schedule as soon as event has ceased | Data not comparable to standard collection events<br><br>Cannot calculate diversity indices if trap collections are not comparable. |

**1** This situation may occur if, for example, technicians went to collect traps, found one or more damaged traps, and did not have the necessary equipment to replace and re-set the damaged traps.

[2]For example, beetle collection normally occurs every other Tuesday. During the 3rd bout, field crews only recover and reset 20 of the traps. On Wednesday, they return to the site and collect the catch from the rest of the traps. However, they *do not* reset any of the 10 belatedly-collected traps. Thirteen days later (the next regularly scheduled Tuesday collection), the contents of the 20 traps are recovered and all 30 traps are reset with new preservative.

## 4.5        Criteria for reallocation of sampling within a site

Beetle sampling will occur on the schedule described above at ten plots within every site (3 traps per plot; 30 per site). Ideally, sampling will occur at those ten plots for the lifetime of the observatory (core

sites) or the duration of site's affiliation with the NEON project (relocatable sites). However, circumstances may arise requiring that beetle sampling within a site be shifted from a particular plot to another location within the site. In general, sampling at a given plot is considered to be compromised when sampling at a location becomes so limited that data quality is significantly reduced. If sampling at a given plot becomes compromised, a problem ticket should be submitted by Field Operations to Science.

There are two main pathways by which sampling can be compromised. Plots can become inappropriately suited to answer meaningful biological questions (i.e., a terrestrial sampling plot becomes permanently aquatic). Alternatively, plots may be located in areas that are logistically impossible to sample on a schedule that that is biologically meaningful. For the ground beetle sampling program, a given plot **must** be sampled at least 50% of the bouts expected for the site (see **APPENDIX D** for the number of expected bouts) over a two-year period. A plot is considered sampled if at a least one trap from the plot is surveyed. Plots that cannot be sampled on this schedule should be considered compromised and a problem ticket should be submitted to NEON Science to receive a list of possible plots for relocation.

# 5        SAFETY

This document identifies procedure-specific safety hazards and associated safety requirements. It does not describe general safety practices or site-specific safety practices.

Personnel working at a NEON site must be compliant with safe field work practices as outlined in the Operations Field Safety and Security Plan (AD[02]) and EHS Safety Policy and Program Manual (AD[01]). Additional safety issues associated with this field procedure are outlined below.  The Field Operations Manager and the Lead Field Technician have primary authority to stop work activities based on unsafe field conditions; however, all employees have the responsibility and right to stop their work in unsafe conditions.

Safety Data Sheets (SDS) are available for the following chemicals used in this work: propylene glycol, ethanol, isoflurane and paradichlorobenzene (moth crystals). Whenever chemicals are used, follow requirements of the site-specific Chemical Hygiene and Biosafety Plan (AD[03]).

## 6        PERSONNEL RESOURCES

## 6.1        Equipment

The following equipment (**Table 2 – Table 8**) is needed to implement the procedures in this document. Equipment lists are organized by task. They do not include standard field and laboratory supplies such as charging stations, first aid kits, drying ovens, ultra-low refrigerators, etc.

Quantities listed are the minimum required to implement protocols. Additional items should be on hand in case of equipment failure.

Table 2. Equipment list - Preparation

| Supplier | Supplier Number | R/S | Description | Purpose | Quantity* | Special Handling |
| --- | --- | --- | --- | --- | --- | --- |
| **Durable Items** | | | | | | |
| Fisher Thomas | 033134F 1709C04 | R | Bottle, 1 L wide-mouth HDPE | Prepare Ethanol (2), DI water (3), PG/DI (3) | 8 | N |
| Fisher | S13507 | R | Jug, 1 gal narrow mouth | Prepare Ethanol | 1 | N |
| **Consumable items** | | | | | | |
| Ben Meadows Forestry Suppliers | 010510-1 49247 | R | All weather copy paper | Print datasheets | | N |
| | | R | Distilled or deionized water | Prepare PG:DI solution, prepare water rinse bottle | 4 L | N |

| Supplier | Supplier Number | R/S | Description | Purpose | Quantity* | Special Handling |
|---|---|---|---|---|---|---|
| Fisher<br><br><br>Thomas | 04355601<br><br><br>**C954K61** | | Ethanol, 190 proof, 95%, 55 gallon<br><br>5 gallons (D05) | Prepare Ethanol bottle | 2 L | Y |
| Bioquip | 1223RB | R | Label paper, ethanol-safe | Print locality and determination labels | | N |
| G2Solutions | 97601958 | R | Propylene Glycol | Prepare PG:DI solution | 3 L | Y |

R/S=Required/Suggested

**Table 3. Equipment list – Initial deployment of pitfall traps**

| Supplier | Supplier Number | R/S | Description | Purpose | Quantity* | Special Handling |
|---|---|---|---|---|---|---|
| **Durable Items** | | | | | | |
| Amazon | B004Q1B3GE | R | Deli container, 16 oz. | Pitfall trap container (half with pre-drilled holes for drainage) | 60 | N |
| | EB07710000 | R | Lexan square with pre-drilled holes | Pitfall trap cover | 30 | N |

| Supplier | Supplier Number | R/S | Description | Purpose | Quantity* | Special Handling |
|---|---|---|---|---|---|---|
| Ben Meadows Forestry Supliers | 122733<br><br>39986 | R | Measuring tape, 100 m | Locate trap installation points | 1 | N |
| Amazon<br><br>B&H Photo | 201965<br>202460<br>SISOK12601 | R | Laser Rangefinder, 0.5yd accuracy, 1500m range | Locate trap installation points | 1 | N |
| Grainger | 6ZFY6 | R | Plastic spike | Secure pitfall trap covers | 120 | N |
| | EG07890000 | R | PVC, ½" schedule 40, 1.75 cm length | Pitfall trap spacers | 120 | N |
| | | S | Washers | Optional; to place between pitfall trap spacers and the ground in loose soils | 120 | N |
| | | R | Trowel or soil knife | Dig holes for traps | 2 | N |
| | | R | Hand mirror | Used to check that traps are flush with the ground | 1 per person | N |

| Supplier | Supplier Number | R/S | Description | Purpose | Quantity* | Special Handling |
|---|---|---|---|---|---|---|
| | | | **Consumable items** | | | |
| | | R | Bottle with 50:50 PG:DI solution, 1 L wide-mouth HDPE | Preserve samples | 6 | N |
| Grainger<br>Forestry Suppliers | 9WKP4<br><br>20101601 | S | Flagging tape | Flag location of trap | 1 roll | N |
| | | | **Resources** | | | |
| RD[05] | | R | Field datasheet | Record data | | |

R/S=Required/Suggested

**Table 4. Equipment list – Sampling beetles at one site for one sampling day**

| Supplier | Supplier Number | R/S | Description | Purpose | Quantity* | Special Handling |
|---|---|---|---|---|---|---|
| | | | **Durable Items** | | | |
| Fisher Thomas | 033134F 1709C04 | R | Bottle, 1 L wide-mouth HDPE | Ethanol waste storage | 2 | N |
| | | R | Hand mirror | Used to check that traps are flush with the ground | 1 per person | N |
| | | R | Cooler | Chill perishable samples in field | 1 | N |
| Amazon | B004Q1B3GE | R | Deli container, 16 oz. | Spare pitfall trap container | 10 | N |
| | EB07680000 | R | Filter assembly | Rinse samples | 3 | N |
| VWR | 89049940 | R | Ice pack, 0°C | Chill perishable samples in field | 2 | N |
| Thomas | 1212W94 | R | Jug, 4 L wide mouth | Store waste Propylene Glycol | 2 | N |
| | EB07710000 | R | Lexan square with pre-drilled holes | Spare pitfall trap cover | 10 | N |
| Grainger | 6ZFY7 | R | Plastic spike | Spare spikes used to secure pitfall trap cover | 40 | N |
| | EG07890000 | R | PVC, ½" schedule 40, 1.75 cm length | Spare pitfall trap spacer | 40 | N |

| Supplier | Supplier Number | R/S | Description | Purpose | Quantity* | Special Handling |
|---|---|---|---|---|---|---|
| | | R | Scissors | Separate locality labels | 2 | N |
| Fisher | 14955102 | R | Specimen cup | Chemical waste containment during field rinse | 3 | N |
| | | R | Tote bag, bucket organizer or other field pack | Carry gear | 2 | N |
| | | R | Trowel or soil knife | Dig holes for traps | 2 | N |
| | | R | Tupperware container | Organize samples | 10 | N |
| Fisher | 0340910E | R | Unitary wash bottle | Rinse samples | 4 | N |
| | | R | Forceps | Push filter into Whirl-Pak bag during rinse | 1 | N |
| Grainger | 3VEP6 | R | Amber bottle, 30 ml with dropper | Administer isoflurane | 2 | N |
| Amazon | B000I1ZZ24 | R | Tea infuser spoon | Administer isoflurane | 2 | N |
| | | S | Funnel | To help balance filter assembly on top of waste container (optional) | 1 per team | N |

| Supplier | Supplier Number | R/S | Description | Purpose | Quantity* | Special Handling |
|---|---|---|---|---|---|---|
| **Consumable items** | | | | | | |
| | | R | Bottle with 50:50 PG:DI solution, 1 L wide-mouth HDPE | Preserve samples | 6 | N |
| | | R | Bottle with distilled or deionized water, 1 L wide-mouth HDPE | Rinse samples | 6 | N |
| Fisher | 033134F | R | Bottle with ethanol, 1 L wide-mouth HDPE | Rinse samples | 2 | N |
| | EB07690000 | R | Filter cloth | Rinse samples | 30 | N |
| Grainger | 9WKP4 | S | Flagging tape | Reflag location of trap | 1 roll | N |
| Fisher | S13507 | R | Jug with ethanol, 1 gal narrow mouth | Store/transport extra ethanol | 2 L | N |
| | | S | Nitrile gloves, powderless | Protect hands; must be used during cervical dislocation and/or isoflurane administration | 1 pair | N |
| Bioquip | 1154F | R | Permanent marker, archival ethanol-safe | Record trapID and date on locality labels | 4 | N |

| Supplier | Supplier Number | R/S | Description | Purpose | Quantity* | Special Handling |
|---|---|---|---|---|---|---|
| | | R | Permanent marker, fine tip | Record plotID and date on resealable plastic bag | 4 | N |
| Grainger | 5CNK5 8YAT5 | R | Resealable plastic bag, 1 gal | Contain Whirl-Pak bags from each plot | 12 | N |
| Thomas | 1303N23 | R | Whirl-Pak bags, 13 oz. | Contain samples | 30 | N |
| MWI Veterinary | 502017 | R | Isoflurane | For administration of anesthesia, in case of moribund vertebrate bycatch | 25 mL | Y |
| Fisher | 19-898-793 22-456-885 | R | Cotton balls | To administer isoflurane | 150 | N |
| | | R | Adhesive barcode labels (Type I) | Labeling sample containers with barcode-readable labels | 1 sheet | N |
| **Resources** | | | | | | |
| RD[05] | | R | Field datasheet from trap deployment | Record data | 10 | N |
| | | R | Locality label | Label samples; 3 per trap | 90 | N |

R/S=Required/Suggested

**Table 5. Equipment list – Laboratory sorting of bycatch from carabids**

| Supplier | Supplier Number | R/S | Description | Purpose | Quantity* | Special Handling |
|---|---|---|---|---|---|---|
| | | | **Durable Items** | | | |
| Fisher | 07200610 | R | Centrifuge tube rack | Organize 15 mL and 50 mL tubes | Variable | N |
| Grainger | THP15 | R | Chemical waste drum (15 gallon) | Store Ethanol and PG waste | 1 | N |
| Lab Essentials | A1502GLG | R | Dual gooseneck light | Illuminate specimens under microscope | 3 | N |
| | | R | Forceps | Manipulate insects | 3 | N |
| Fisher | 11350121 | R | Microscope | Aid in species identification | 2 | N |
| Fisher | 50365047 | R | Petri dish, plastic | Sort specimens under microscope | 2 | N |
| | | R | Secondary containment bin | Spill containment | 1 | N |
| Fisher | 14955102 | R | Specimen cup | Chemical waste containment during rinse | 1 | N |
| | EB07680000 | R | Filter assembly | Rinse samples | 1 | N |
| | | R | Tupperware container | Organize each bout of Whirl-Pak bags | 10 | N |

| Supplier | Supplier Number | R/S | Description | Purpose | Quantity* | Special Handling |
|---|---|---|---|---|---|---|
| | 0340910E | R | Unitary wash bottle | Rinse samples | 1 | N |
| **Consumable items** | | | | | | |
| | | S | Adhesive label | Label sample tubes externally | As needed | N |
| Fisher<br><br>Thomas | 04355601<br><br>C954K61 | R | Ethanol, 190 proof, 95%, 55 gallon<br><br>5 gallons (D05) | Preserve samples | | Y |
| | EB07690000 | R | Filter cloth | Rinse samples | Variable | N |
| Thomas | 1303N23 | R | Whirl-Pak bags, 13 oz. | Contain samples | Variable | N |
| Fisher | 1443222 1495949A | R | Falcon tubes, 50 mL | Contain samples | Variable | N |
| | | R | Jar, 16 oz. wide mouth jars | Contain samples | 50 | N |
| Bioquip | 1223RB | R | Label paper, ethanol-safe | Create sampleID labels | | N |

| Supplier | Supplier Number | R/S | Description | Purpose | Quantity* | Special Handling |
|---|---|---|---|---|---|---|
| | | S | Nitrile gloves, powderless | Protect hands | Variable | N |
| | | R | Permanent marker | Label sample containers | 5 | N |
| Bioquip | 1154F | R | Permanent marker, archival ethanol-safe | Record sampleID number on sampleID labels | 5 | N |
| Amazon | B005IQTSE0 | R | Transfer pipette | Remove ethanol from petri dish and Whirl-Pak during transfer | 1 | N |
| | | R | Tube or container | Contain vertebrate bycatch while sorting | | N |
| | | R | Tube or container | Contain invertebrate bycatch while sorting | | N |
| | | R | Tube or container | Contain carabids while sorting | | N |
| | | R | Adhesive barcode labels (Type I) | Labeling sample containers with barcode-readable labels | 1 sheet | N |
| **Resources** | | | | | | |
| | | R | Determination label | Label samples | Variable | N |
| RD[05] | | R | Sorting datasheet | Record data | | |

R/S=Required/Suggested

**Table 6. Equipment list – Laboratory pinning and pointing of carabids**

| Supplier | Supplier Number | R/S | Description | Purpose | Quantity* | Special Handling |
|---|---|---|---|---|---|---|
| **Durable Items** | | | | | | |
| Lab Essentials | A1502GLG | R | Dual gooseneck light | Illuminate specimens under microscope | 1 | N |
| | | R | Forceps | Manipulate insects | 3 | N |
| John Robert Rose | Pb | R | Insect pinning block | Position point and labels on pin | 1 | N |
| | | R | Microscope | Aid in pinning/pointing specimens | 2 | N |
| Bioquip | 1154F | R | Permanent marker, archival ethanol-safe | Record sample information on labels | 5 | N |
| Bioquip | 1150B | R | Point punch | Create points | 1 | N |
| **Consumable items** | | | | | | |
| Amazon | B002YHHQAY | S | Balsa wood | Hold specimens or pins/points and attach points/labels to pins | 4 | N |
| | | S | Copy paper, white | Aid in pinning/pointing specimens under microscope | 1 | N |

| Supplier | Supplier Number | R/S | Description | Purpose | Quantity* | Special Handling |
|---|---|---|---|---|---|---|
| Bioquip | 1148 | R | Fumigant box | Contain moth crystals within Cornell cabinet | 25 | N |
| Bioquip | 1203B3 | R | Insect pin, No 3 | Mount insects | 10 | N |
| Amazon Grainger | 73299 73299 | R | Moth crystals | Prevent dermestid damage to specimens | 1 lb., ~6.5 g per box | Y |
| Grainger | 6HCH7 | R | Multi-purpose glue | Attach specimens to points | 2 | N |
| Bioqup | 1004 | R | Schmitt box | Store mounted specimens | Variable | N |
| Amazon | B00254AU8Q | R | Strathmore paper | Paper for points | 1 sheet | N |
| Arrow Amazon | WAU22731 B000095S4R | | Yellow cardstock | Print individualID labels | | |
| **Resources** | | | | | | |
| | | R | Determination label | Label samples | 1 per specimen | N |
| | | R | Locality label | Label samples | 1 per specimen | N |
| RD[05] | | R | Pinning datasheet | Record data | 1 | N |

R/S=Required/Suggested

**Table 7. Equipment list - Insect labels**

| Supplier | Supplier Number | R/S | Description | Purpose | Quantity | Special Handling |
|---|---|---|---|---|---|---|
| Locality and Determination Labels |
| Bioquip | 1223RB | R | Label paper, ethanol-safe | Printing labels | Variable | N |
| individualID Labels |
| Arrow Amazon | WAU22731 B000095S4R | R | Yellow cardstock | Printing individual ID labels | Variable | N |
| All Labels |
| Grainger Arrow | 2WFX1 FSK01005037J FSK1294518697WJ SPR39042 | R | Scissors | Cutting apart labels | 1 | N |
| Grainger | 2CJX1 | R | Cutting mat | Cutting apart labels | 1 | N |
| ULINE | H-998 | R | X-acto knife | Cutting apart labels | 2 | N |
| Grainger | 10F265 | R | Ruler, Metal, 18 inch | Cutting apart labels | 1 | N |

**Table 8. Equipment list - Shipping to taxonomic facility**

| Supplier | Supplier Number | R/S | Description | Purpose | Quantity | Special Handling |
|---|---|---|---|---|---|---|
| **Consumable items** | | | | | | |
| Bioquip | 1025PB | R | Cardboard specimen mailers | Organizing specimens for shipment | Variable | N |
| | | R | Cardboard insert with tape tab | Extra stability and protection for pinned specimens during shipment | Variable | N |
| | | S | Packing foam | Fill in extra head space between cardboard insert and specimen mailer lid | Variable | N |
| Bioquip | 1208B3 | R | Insect pin, No 3 | Brace-pinning specimens for shipping | 2 | N |
| | | R | Packaging tape | Handle for cardboard insert AND shipping | Variable | N |
| | | R | Address label | Shipping | Variable | N |
| | | R | Cardboard box | Shipping | Variable | N |
| | | | Cushioning material (e.g. bubble wrap) | Shipping | Variable | N |

## 6.2 Training Requirements

All technicians must complete required safety training and protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[04]).

Training for field techs will include practice analyzing datasheets at various levels of completion or with errors. Training will also include discussions on data interpretation, the importance of uploading data in a timely fashion, and how to recognize the distinction between missing data and true zeroes. For the sorting and pooling SOPs, each technician must be trained by an experienced staff member to meet sorting accuracy requirements before sorting and pooling samples without supervision.

Field technicians (regular and seasonal) who are responsible for leading implementation of this protocol are required to attend all associated training presentations as well as read related documentation (training materials and protocols). This includes all training related to incidental vertebrate bycatch and euthanasia protocols.

## 6.3 Specialized Skills

Prior experience collecting ground beetles or working with related insects (i.e., entomological fieldwork) is desirable but not required. Personnel should have good fine motor skill for handling individual specimens and pinning/pointing.

## 6.4 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, a problem ticket should be submitted. In addition, if sampling at particular plots requires significantly more time than expected, plots may be moved to more accessible locations.

Sampling is estimated to require 2 technicians: 12 min per trap x 30 traps = 360 min = 3 hours per person each day of sampling; 30 min per plot x 10 plots = 300 min = 5 total hours each day of driving; and 15 min x 10 plots = 150 min = 2.5 total hours each day of hiking. Exact drive and hike times will vary by site, and should be considered when deciding whether 1 or 2 teams of 2 technicians are required for sampling.

| SOP | Estimated total time | Suggested staff | Total person hours |
|---|---|---|---|
| A Preparing for sampling | 1 hr/bout | 2 | 2 hrs/bout for each site |
| B Initial Deployment | 0.75 – 1.5 hrs/plot | 2 | 8 – 16 hrs/site |
| C Field Sampling | 1 – 2 hrs/plot | 2 | 2 – 4 hrs/plot |
| D Laboratory processing – Sorting Vertebrate Bycatch | 3 - 6 hrs/bout | 2 | 3 - 6 hrs/bout |
| E Laboratory processing – Sorting Invertebrate Bycatch | 0.25 - 0.5 hrs/trap | 4 | 10 - 20 hrs/bout |
| F Laboratory processing – Sorting Ground Beetles | 0.5 - 2 hrs/trap | 2 - 3 | 20 – 80 hrs/bout |
| G Laboratory processing - Pinning | 5 mins/beetle | 2 | 8 – 40 hrs/site per year |
| H Laboratory processing - Pooling | 10 mins/plot | 3 | 1.5 – 3 hrs/bout |
| I Data entry and verification | 1 - 2 hrs/bout | 1 | 1 - 2 hrs/bout |
| J Sample shipment | 5 - 10 hrs/site (per sample type; pinned individuals vs. vials of ethanol) | 1 - 2 | 5 - 10 hrs/site (per sample type; pinned individuals vs. vials of ethanol) |

**Table 9.** Estimated time required to complete field sampling and lab standard operating procedures

# 7 STANDARD OPERATING PROCEDURES

## SOP A Preparing for Sampling

### A.1 Preparations for the first sampling period (see APPENDIX B, APPENDIX C for quick references and checklists):

1.  Identify the locations of sampling plots and access routes

2.  Work with NEON Permitting to get a list of species and take limits at the start of the season. NEON is not allowed to collect more than the number of individuals (typically small mammals and herptiles) specified in the permit.

3.  Prepare pitfall trap materials, including required labels. Locality labels are preprinted before sampling, cut into strips of the same date/location and inserted into the appropriate whirlpack ahead of sampling.

4.  Prepare final sample containers by affixing one Type I adhesive barcode label to each vial and/or WhirlPak bag used to contain each sample. Adhesive barcode labels should be applied to dry, room temperature containers in advance of their use in the field (at least 30 minutes prior, but may be applied at the start of the season).
    a.  If vials are used, barcode labels should be oriented such that it is possible to scan them; the scanner will not work on a curved surface. This means aligning the barcode lengthwise along a vial, *not* horizontally wrapping around a vial.
    b.  If Whirl-Pak bags are used, the barcode should be affixed to the outside on the upper third of the bag and whirled such that barcode is facing outward, so that the label does not get damaged in the process of being closed. **If the barcode label is bent in the wrong orientation, the barcode is more likely to fall off.**
    c.  Barcode labels must be associated with a unique sample and each barcode must be mapped to one sample in the database. Barcodes are unique, but are not initially associated with a particular sample, so you are encouraged to adhere barcode labels to needed containers in advance.
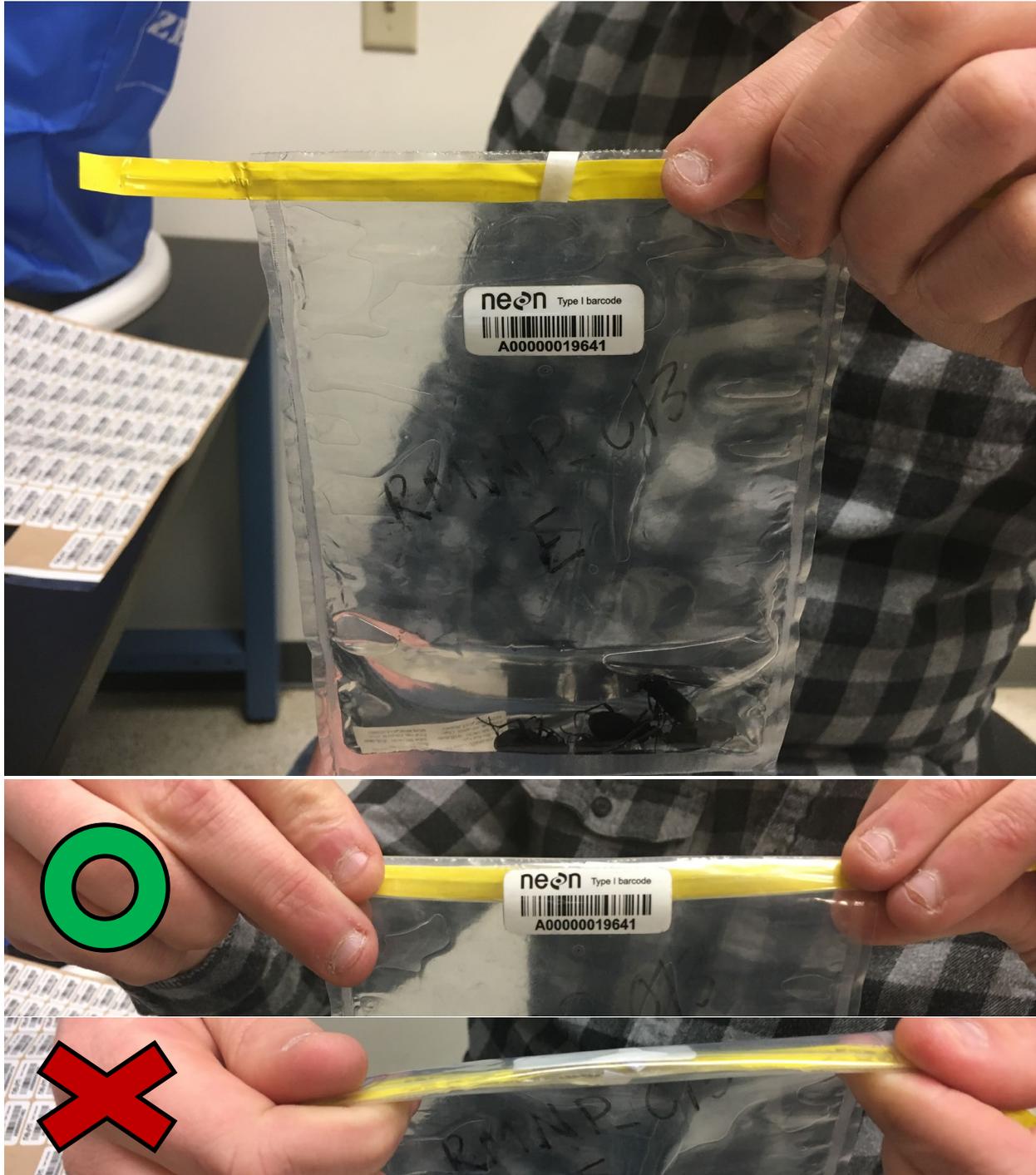
**Figure 2. (top photo) Placement of Type I barcode on Whirl-Pak. (middle photo) Sample whirled in the correct direction; top and bottom edges of barcode bent so that the barcode bulges outward. (bottom photo) Sample whirled in the wrong direction with barcode in a concave form; this will tend to make the barcode peel.**

**Figure 3 An example of a Type I barcode. These large-size, field-tolerant barcodes have a prefix of 'A' followed by 11 numbers.**

5. Cut/drill holes in bottom (exterior) container (16 oz. deli cup)

6. Draw 150 mL and 250 mL fill lines on upper (interior) container

7. Locate other trap materials including pitfall trap spacers to hold up cover and filter squares.

8. Review/prepare checklist of materials needed for pitfall sampling.

*Note: Clean containers in good condition from the previous field season may be reused in subsequent seasons.*

**A.2      Prior to a sampling bout:**

1. Prepare bench and fume hood space in the lab for the preparation of field materials (e.g., ethanol, PG).  Ensure that space in refrigerator or flammable materials cabinet is available for samples. Coordinate with potentially conflicting activities (e.g. soil sieving).

2. Assemble field equipment at least one day prior to field sampling.

3. Create containers for each collected sample by labeling one Whirl-Pak bag with the plotID and trapID of each beetle pitfall using an ethanol-safe marker (30 per bout). If available, also affix one adhesive barcode label to the upper 1/3 of each marked Whirl-Pak or use a Whirl-Pak bag which has already been labelled with a barcode (Step A.1.3).

4. Create organizational bags for each plot by labeling 10 large resealable bags with each plotID and collection date. Place Whirl-Pak bags inside the larger resealable bag according to plotID.

5. Print blank datasheets (see Ground Beetle Field Datasheet in (RD[05]). Verify that the mobile data entry device is charged and synced prior to use.

6. Prepare locality labels (see **APPENDIX J**).

7.  Charge all electronic equipment (e.g., GPS unit, rangefinder, mobile data entry device).

8.  Prepare one liter bottles of propylene glycol: water solution (500 mL PG:500 mL water, either distilled or deionized). A minimum of six 1 L bottles of this solution is required to fill 40 pitfall traps with 150 mL of preserving fluid. Be sure to prepare more of this solution than you think you will need in the field to ensure that there will still be enough to set or reset pitfall traps in the case of accidental spillages, etc. If the weather conditions are hot or dry, bring enough liquid for 250 mL per trap.

9.  In fume hood, prepare 95% ethanol for the field by pouring it into 1 gal narrow mouth and 1 L wide mouth bottles.

10. Prepare distilled or deionized water for the field by pouring it into 1 L wide mouth bottles.


*About barcode uses and placement*

This protocol generates mixed samples from the field and sorts those samples into taxon-specific subsamples to species (e.g., carabids, vertebrates) and group (e.g., non-carabid invert bycatch). Carabids may be pinned and sent for further identification. All sample types have the potential to be pooled at the plot level with other subsamples of the same type to save on space when archiving. Although it is always acceptable to use barcodes, in some cases barcodes are absolutely required. Table 10 provides a quick reference to the types of sample that require barcodes. The rule of thumb is that the primary field sample will ALWAYS need a barcode due to its importance in generating future subsamples. Likewise, the final disposition of all vialed samples must have a barcode affixed to assist in the shipping and receipt of samples destined for the Biorepository.

**Table 10. Sample Types and Barcodes Used**

| Sample Type | Description | Example Identifier | Fulcrum App | Container Type | Barcode Used | Barcode Required? |
|---|---|---|---|---|---|---|
| Field samples | Unsorted samples; may contain vertebrates, carabids and non-carabid invertebrate bycatch | CPER_001.E.20180904 *(plotID.trapID.collectDate)* | BET: TRAP COLLECTION | Whirl-Pak | Type I | **Always Required** |
| Vertebrate subsamples | Vertebrates sorted out of mixed field samples | CPER_001.E.20180904.PEMA.01 *(plotID.trapID.collectDate. taxonID.tubeNumber)* | BET: SORTING | 50 mL vial | Type I | Recommended; required when sample not pooled |
| Carabid subsamples | Carabids sorted out of mixed field samples | CPER_001.E.20180904.PASELO.01 *(plotID.trapID.collectDate. taxonID.tubeNumber)* | BET: SORTING | 50 mL vial | Type I | Recommended; required when sample not pooled |
| Invertebrate subsamples | Non-carabid inverts sorted out of mixed field samples | CPER_001.E.20180904.IB.01 *(plotID.trapID.collectDate. taxonID.tubeNumber)* | BET: SORTING | 50 mL vial | Type I | Recommended; required when sample not pooled |
| Pinned Beetles | Beetles pinned from sorted Carabid subsamples | NEON.BET.D10.000120 *(NEON.BET.domainID.sixDigits)* | BET: PINNING | Pinned specimen | None | Not required |
| Vertebrate subsamples | Vertebrates sorted out of mixed field samples | CPER_001.20180904.PEMA.01 *(plotID.trapID.collectDate. taxonID.tubeNumber)* | BET: POOLING | 50 mL vial | Type I | **Always Required** |
| Carabid subsamples | Carabids sorted out of mixed field samples | CPER_001.20180904.PASELO.01 *(plotID.collectDate. taxonID.tubeNumber)* | BET: POOLING | 50 mL vial | Type I | **Always Required** |
| Invertebrate subsamples | Non-carabid inverts sorted out of mixed field samples | CPER_001.20180904.IB.01 *(plotID. collectDate. taxonID.tubeNumber)* | BET: POOLING | 50 mL vial | Type I | **Always Required** |

### SOP B    Initial Deployment of Pitfall Traps

Pitfall traps are deployed at ground level with the lip of the container flush with the ground.

See **APPENDIX B**, **APPENDIX C** for quick references and checklists pertaining to preparing for and conducting trap deployment.

1. Locate the first plot where sampling is to occur.

2. Find two corners of the plot using the GPS points collected during plot establishment (e.g., the SE and SW corners). Find the mid-point between those corners (approximately 20 meters from each corner) using a Rangefinder or measuring tape and verify that your position is correct. For the Eastern trap, you may move ± 2m to the north, west, or south of the mid-point location if there is a natural feature (e.g., a rock or stream) in the target trap location. *You may not place the trap outside of the plot boundaries, due to permitting constraints.*

3. Dig a small hole with a soil knife to accommodate the pitfall trap (**Figure 4**).  Start small because it is much easier to enlarge the hole than fill in the edges to maintain a tight fit. Outline the container in the dirt and then dig just inside the line to ensure a snug fit.  The exact tools most useful for this step will vary based on soil type, roots, rocks, etc.

4. If the ground is particularly rocky or hard, use a template pitfall trap lid and spikes to pre-bore (with a hammer) the holes for the spikes in the actual trap. Push the bottom container (with holes) into the hole. Slide the top container into bottom container. **Ensure that the lip of the top container is flush with the ground** so no lip is sticking up above the ground surface (this ensures that insects fall into the trap rather than walk around it, and accumulate in the top container only). Also ensure that there is no gap between the containers and the ground.  The containers should fit snugly into the hole.

5. Use the pre-drawn line to fill the top container with 150 mL of PG:water solution. See contingency section (**4.4**) above if you have failed to bring an adequate amount of PG to fill pitfalls. If it is not possible to fill all traps to the 150 mL volume (e.g., spilled in field), revisit traps with additional preserving fluid in the following days.  If re-visitation is not possible, and the amount of PG was insufficient to preserve specimens captured during the bout, the sample will become degraded and it is unlikely that any of the specimens will be identifiable.

   *Note - comparability between samples relies on sufficient preserving solution in each pitfall trap.  Bring more preserving fluid out to the field than you anticipate using. Depending on weather, more solution may be required.*

**Figure 4. Digging a hole for the pitfall trap.**

6. Position cover with four plastic spikes and PVC spacers (**Figure 5-Figure 6**) so that it is visually level and 1.5 cm above the surface of the containers (**Figure 7**).

   a. Label the top of the cover with the name of the plot and S, E or W to indicate the trap location within the plot. This will facilitate matching locality labels with traps during subsequent visits

   b. (OPTIONAL) Occasionally, PVC spacers alone do not adequately maintain the cover 1.5 cm above the ground due loose soils (these soils have high sand content or are waterlogged). As an optional modification, metal washers can be used beneath PVC spacers between the soil and the ground. This provides more surface area and prevents the cover from collapsing on the pitfall cup.

7. Trap cover deployment will vary based on local topography and vegetation cover. Keep the following purposes of the cover in mind while installing the cover. The cover:
   a. Prevents vertebrate bycatch while allowing ground beetles to enter
   b. Shades the trap to lower fluid temperature, thus reducing evaporative loss and decomposition rate

c. Prevents precipitation accumulation in the trap, thus reducing dilution of the preserving fluid



**Figure 5. Positioning cover over pitfall trap. Note the pitfall is not sufficiently dug into the ground in this image. The lip should not stick up from the ground.**



**Figure 6. Installing pitfall lid with spacers. Note that the container is flush with the ground. If washers are used, they should be placed between the ground and the bottom of the spacer.**

**Figure 7. Fastening down pitfall cover. Note the spacer below the cover.**

d. **IMPORTANT**: After fastening the pitfall cover, use a hand mirror to verify that the lip of the cup is still flush with the ground. Simply place the mirror to the side of the pitfall cover at an angle to view the set trap. If the lip of the trap is visible from ground level, the cup is NOT flush with the ground. Remove the cover and adjust the trap as needed to achieve a flush trap level (e.g., excavating a bit more of the hole, pressing dirt into gaps between the cup and ground), using the hand mirror to check the level.

e. Once one trap in one of the cardinal directions has been set, work along the edge of the 40 m² plot to find the next corner, repeating this procedure until all 3 traps are installed. If possible, reuse the same holes for pitfall traps from year to year (backfilled holes can be excavated anew each year).

f. For each trap, record the date in the setDate field in the mobile data entry device using the 'BET: Trap Setting [PROD]' application (or, if unavailable, on the field datasheet). Refer to the 'Manual for Fulcrum Application: TOS Ground Beetle Sampling [PROD]'for details on the mobile data entry application.

**SOP C        Field Sampling**

**C.1        Collecting samples from the trap**

Upon arrival at the trap:

1. Record data in the mobile data entry device 'BET: Trap Collection [PROD]', or (if unavailable) use the same paper datasheet as used when setting the traps (see Ground Beetle Field Datasheet in (RD[05]). See the 'Manual for Fulcrum Application: TOS Ground Beetle Sampling [PROD]' for details on the mobile data entry application.

2. Note the relative fluid level remaining in the container (high, low, ok, or none) in the 'fluidLevel' field, and whether the lid (the 20 x 20 cm trap cover) and/or upper container are missing or have been disturbed (in the 'lidStatus' and 'cupStatus' fields). If the upper container is missing and the bottom container does not obviously contain beetles or fluid, skip to 'Resetting the Pitfall Trap'. Regardless of fluid level (e.g., no preservative, flooded conditions, etc) and even if the trap appears empty, continue to the next steps to collect a sample.

3. [Optional step]  Put on disposable gloves to protect your hands from the materials used. Gloves can be reused.

4. Pick up the upper container containing the sample, leaving the lower container in the ground.

5. Remove any large debris (e.g., sticks, leaves) from the trap prior to collection. Take care that no beetles (especially tiny ones) or bycatch are lost.

6. In the unlikely event that you find live vertebrate bycatch in the trap, attempt to remove and release the animal. Watch the animal for two minutes to verify its ability to move of its own volition. If the vertebrate cannot move and appears injured, euthanize the animal with isoflurane and perform cervical dislocation. All dead vertebrate bycatch should be collected with the rest of the trap contents. Note: Personal protective equipment such as gloves *must* be worn when directly physically handling vertebrate bycatch and performing cervical dislocation. Technicians must be trained in the administration of isoflurane and performance of cervical dislocation prior to implementing this protocol.

7. Select the Whirl-Pak that this sample will be placed into. Verify that the appropriate locality labels are in the Whirl-Pak bag and that the trap and plot information written on the exterior of the Whirl-Pak matches up with the locality labels inside.

8. If filtering directly after collecting each pitfall trap, proceed to the section on filtering samples. If collecting all traps and then filtering simultaneously at the end of sampling, place the PG solution from the pitfall trap directly into the Whirl-Pak container and seal tightly. In

the latter case, the Whirl-Pak should contain any beetles, dead bycatch, and 3 locality labels (which were previously placed into the Whirl-Pak during prep) in the PG solution.

9. As needed: record remaining metadata, including any irregularities in the remarks (e.g. trap was damaged by bears, wind blew cover off, trap flooded with rainwater).

## C.2 Filtering the sample

After each sample is collected, the PG solution needs to be removed and replaced with ethanol that same day. Staff can decide if is easier to filter the samples at each trap between trap collection and trap reset or to place the PG solution from each trap directly into the labelled Whirl-Pak and perform filtering on all 30 traps at the end of collection at the car. If filtering occurs at the end of the collection bout, then place the PG solution with sample from the Whirl-Pak directly into the filter assembly, rather than from the cup as is indicated below.

Here, the process is described as though filtering is occurring prior to trap reset.

1. Filter the contents of each trap using a filter cloth and a filter cup (**Figure 8**).



**Figure 8. 300 μm mesh filter fitted to the top of a Nalgene bottle**

2. Hold the filter above the waste container and pour the PG solution through the filter so that the sample is collected on the filter and the fluid does not spill onto the ground (**Figure 9-Figure 11**).

   a. (Optional) a funnel may be placed between the filter assembly and waste container. This is often useful when picking up samples that are deployed in sloped conditions.

**Figure 9. Preparing to filter out specimens from collecting fluid (pictured left). Optionally, a funnel can be placed between the filter assembly and the Nalgene waste container (pictured right).**



**Figure 10. Filtering specimens from trap. The temporary waste container is below the filter (not visible).**

**Figure 11. Beetle specimens after collecting fluid has been poured off.**

3. After sample collection is finished, dispose of used PG solution back at the lab.

4. Rinse the sample (in the filter cup) using distilled or deionized water (**Figure 12**). Rinse above the waste container so rinse water/PG is efficiently collected instead of spilling onto the ground.


**Figure 12. Rinsing sample with water.**

5. Rinse sample in filter cup with 95% ethanol over the waste container, or directly over the Whirl-Pak bag in which the sample will be stored until processing in the lab (**Figure 13**).



**Figure 13. Rinsing sample with 95% ethanol.**

6. Place insects in a Whirl-Pak bag (**Figure 14**), rinse the filter contents into the same Whirl-Pak bag (**Figure 15**), and add additional ethanol until the contents of the bag are completely covered with 95% ethanol. Finally, remove the filter from the filter cup and place the filter in the Whirl-Pak (**Figure 16**), verifying that the filter cup is free from all sample materials before using it to process another sample. Use forceps to gently push the filter into the Whirl-Pak without tearing the Whirl-Pak.



**Figure 14. Placing sample in Whirl-Pak bag.**

**Figure 15. Two alternative ways to rinse the sample into the Whirl-Pak. After rinsing cover the sample with 95% ethanol. The mesh is also placed in the bag. Note the locality labels in the Whirl-Pak!**



**Figure 16. Checking filter for remaining insects. Place filter in bag with sample.**

7. Scan the barcode label adhered to the Whirl-Pak to associate the identifier with the relevant data record.

Note: one barcode corresponds to a single sample; there should always be a one to one relationship between a scanned barcode and each Whirl-Pak sample such that there is one barcode per trap collection.
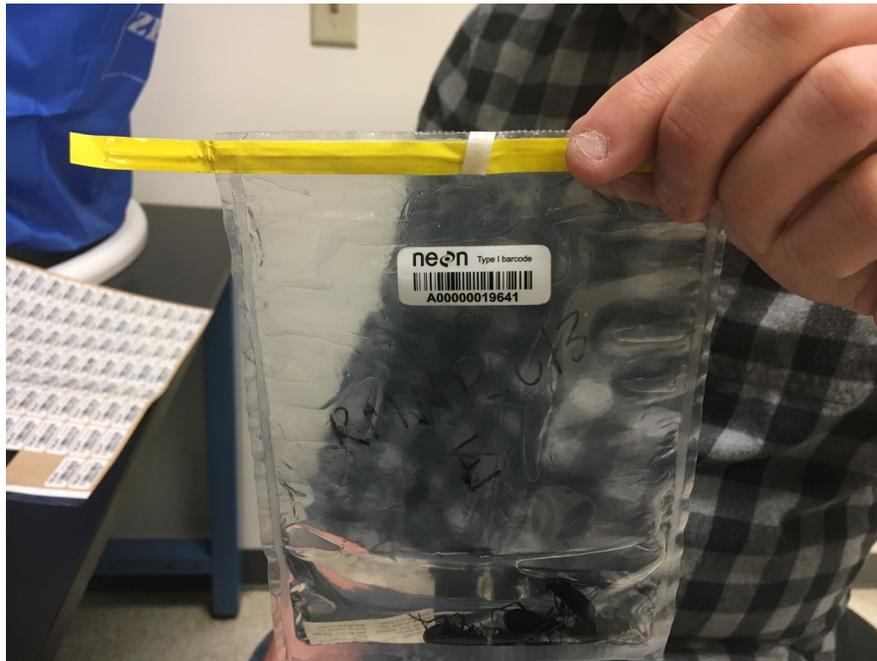


**Figure 17. Proper location for barcode on a Whirl-Pak. Notice there is only one barcode per trap contents.**

8. Roll the Whirl-Pak bag shut and tightly close the bag's twist ties to prevent leaks (**Figure 18**).

**Figure 18. (Above) A packed sample. (Below) Note the barcode in the whirled sample.**

9. Store the Whirl-Pak samples in the shade (e.g., in a backpack) until you return to the field vehicle (see **C.5** for additional instructions).

### C.3        Resetting the Pitfall

1. Using new solution prepared in the lab, refill the PG mixture in the container to 150 mL line. If the PG solution was under the 150 mL line when the trap was collected (more likely when conditions are hot and dry), add PG up to the 250 mL line to prevent potential trap drying.

2. Position cover with four plastic nails so that it is visually level and 1.5 cm above the surface of the containers.

3. **IMPORTANT**: After fastening the pitfall cover, use a hand mirror to verify that the lip of the cup is still flush with the ground. Simply place the mirror to the side of the pitfall cover at an angle to view the set trap. If the lip of the trap is visible from ground level, the cup is NOT flush with the ground. Remove the cover and adjust the trap as needed to achieve a flush trap level (e.g., excavating a bit more of the hole, pressing dirt into gaps between the cup and ground), using the hand mirror to check the level.

4. Record that the trap was reset in the mobile data entry application. This can be done within the trap collection app by clicking 'reset trap' to spawn a new trap record.

5. Repeat this procedure until the contents of all 30 traps have been collected and each trap has been reset.

**C.4        When collecting the final sample of the field season:**

1. Return all trap components to the lab.

2. Backfill holes with local substrate. These same holes will be excavated in following years for pitfall trapping.

**C.5        Sample preservation**

1. Place all 3 Whirl-Pak bags from a single plot into a resealable plastic bag that has been labelled with date and plot ID (includes siteID) before leaving the plot.  You may choose to pre-label these bags in the lab with the plotID and collection date.

2. Place bags into Tupperware container, ensuring that the Whirl-Pak bag openings are upward.

3. Store samples in a cooler (with ice packs lining the bottom) in the field vehicle to prevent exposure to direct sunlight or extremely high temperatures during the remainder of the field work. When transporting coolers back to the lab avoid exposure to heat (e.g., direct sun) and wind to the extent possible.  In the lab, samples must be stored in the fridge prior to removal of vertebrate bycatch. Do **not** use a freezer, as the Type I barcodes may fall off at colder temperatures.

4. Change ethanol within 1 day of collection and remove vertebrate bycatch (see **SOP C**). Document ethanol change date in the Ground Beetle Field Collection application (this information will populate all future sorting records).

5. After vertebrate bycatch are removed, samples containing the remaining invertebrates must be stored at room temperature.

### SOP D          Laboratory Processing – Ethanol Rinse and Sorting of Vertebrate Bycatch

### D.1          Sample processing timing

The ethanol in which each sample is stored must be changed within 1 day of collection (< 24 hours).  At this stage, all vertebrate bycatch <u>must</u> be removed from the sample and sorted, with any rare, threatened or endangered (RTE) species reported to NEON Science and Permitting prior to the following sampling bout.  Invertebrate bycatch may also be removed during this rinse (if time and personnel allows; see **1.1.1.1.1SOP A** for details).

Following the rinse, non-vertebrate specimens can be stored in ethanol for several months at room temperature.  Final processing, including identification and pinning, as well as submission of samples to taxonomists must occur within four months of the end of the field season.  Specimen identifications must be verified and domain voucher collection updated before the beginning of the next field season. See **APPENDIX H** for taxonomic identification resources. Quick reference guides and checklists for processing beetle specimens in the lab can be found in **APPENDIX B - APPENDIX C**.

**NOTE**: A problem ticket must be created when 15 or more individuals *of a given non-RTE vertebrate species* have been collected, <u>cumulatively</u>, within a single plot. NEON Science will review the reported information and scientific literature about the captured species to evaluate possible mitigation measures. Proposed mitigation may be localized (e.g., temporarily deactivating traps from the relevant plot) or generalized (e.g., removing all traps from the site for the remainder of the season). NEON Science will deliver their recommendation to the Battelle IACUC, which will make a determination as to what mitigation measure to implement, if any.  See below for example scenarios that do, and do not, require the issuance of an incident ticket:

> *Example 1*: Fifteen leopard frogs are caught in HARV_001 during bout 1. A problem ticket must be issued to the Insect Ecologist prior to the next sampling bout.

> *Example 2*: Five leopard frogs are caught in HARV_001 during sampling bout 1, two more are caught in HARV_001 during sampling bout 3, and ten more are caught in HARV_001 during sampling bout 7. A problem ticket must be issued to the Insect Ecologist prior to sampling bout 8.

> *Example 3:* One hundred red-backed salamanders are caught in HARV pitfall traps over the whole site, but cumulative bycatch never exceeds 15 salamanders in a single plot. No problem ticket is required.

> *Example 4*: Eight leopard frogs and 8 shrews are caught in HARV_001 pitfall traps over the whole season. No problem ticket is required.

The bycatch of RTE species will involve specialized handling that depends on the permitting at each site. Should any RTE species be caught, consult those permits and notify both NEON Science and NEON

Permitting of the RTE species that was captured. Captures of RTE species may involve limited action, such as the temporary suspension of sampling at the plot in which that species was captured, or extensive remediation, such as inactivation of a whole site for the remainder of the field season. Any response will necessarily be site- and species-specific. Use the vertebrate bycatch tracking sheet in the sorting area to keep all technicians aware of the number of vertebrates caught per plot through the season.

## D.2 Ethanol rinse and removal of vertebrate bycatch

When performing the ethanol (etOH) rinse (a.k.a. 'etOH change'), the date must be recorded in the mobile data entry application 'BET: Trap Collection [PROD]' (or, if unavailable, on the paper sorting datasheet). In addition, all vertebrate bycatch must be removed from the pitfall sample at this time (with their identity and number listed in the sorting datasheet), so that large, soft-bodied animals do not degrade the quality of the etOH in the pitfall sample.

All vertebrate bycatch will be recorded from each trap (i.e., sampleID) such that each *taxon* from each trap has its own line item which accurately reports 1) the sampleID it originated from, 2) the subsampleID of the destination tube, and 3) the number of vertebrates of that species that were transferred from that pitfall trap to the destination tube. In the mobile data entry application, trap-level information is recorded in the 'Ground Beetle Sorting Metadata' section (i.e., sampleID). By selecting 'Click here to enter sorting data', one line item or child record is created for each subsampleID (i.e., describing sample type, subsampleID, taxonID, individual count, etc). If the sorting datasheet is used, each unique subsample receives its own line in the datasheet.

In general, this means that at least **one** child record and one subsample tube will be needed for each taxon/trap combination. However, *extra* child records are required if a species from a single trap is split between two vials. In that case, the 'individual count' for each record is the number of vertebrates of a taxon that contributed to the tube labelled with a given 'subsampleID'. **Figure 19** illustrates the steps for etOH rinse and removal of vertebrate bycatch, with detailed instructions in the following text.

1. [Recommended but optional step]  Put on nitrile gloves.

2. **Find the record for the sample being rinsed in the Field Collection application and enter the ethanol rinse date for the sample in Fulcrum.**

3. For each trap, pour the sample (including the specimens and the initial ethanol added to the Whirl-Pak in the field) through a filter cloth and into an ethanol waste container (**Figure 20**). Remove the used filter cloth from the Whirl-Pak and rinse, with 95% ethanol, over the new filter cloth until there is nothing visible on the filter cloth (**Figure 21**).  Place the filter cloth back into the original Whirl-Pak, keeping the original filter with the sample in case small beetles and bycatch are stuck to it.
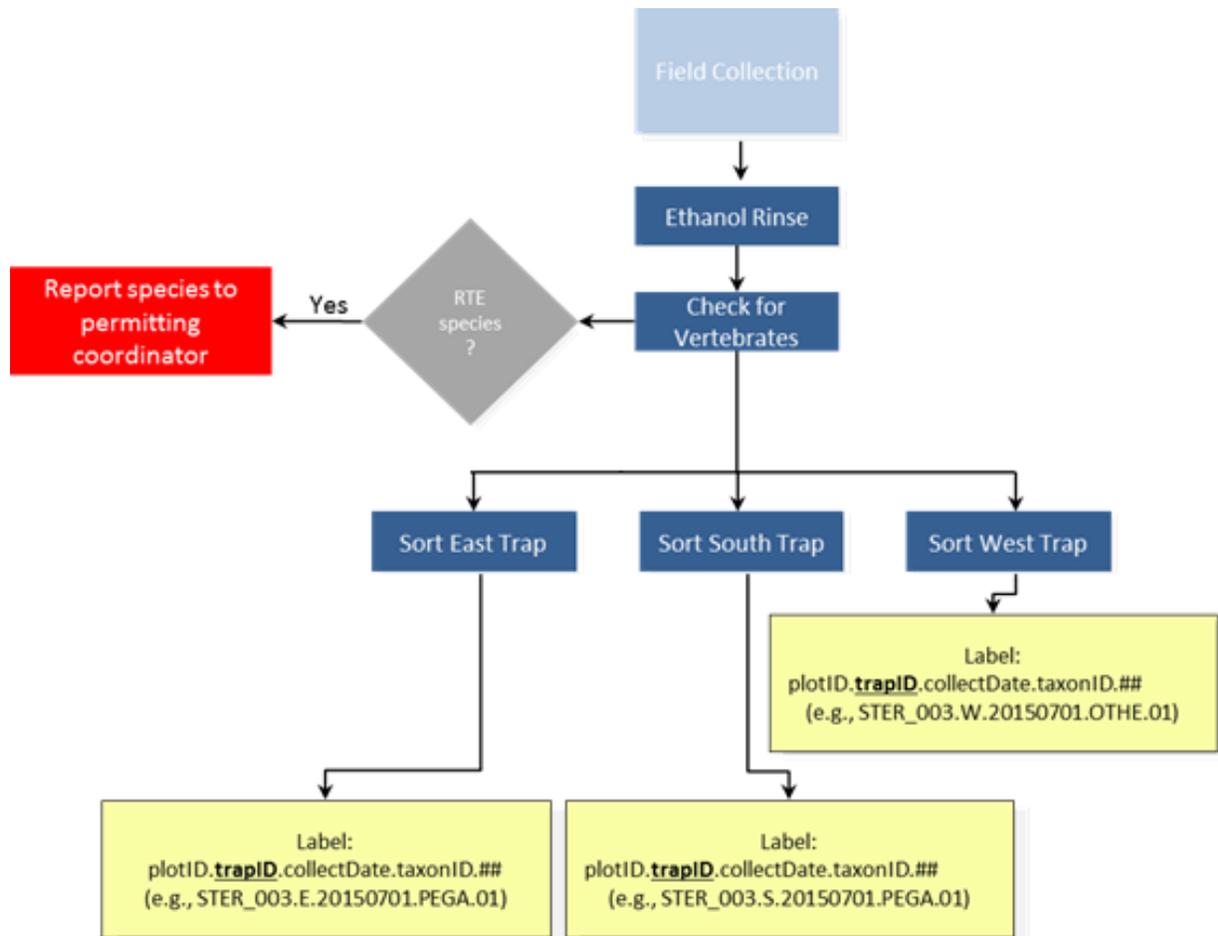
**Figure 19. Workflow of the ethanol rinse and vertebrate bycatch removal processing step. Activities are represented by blue boxes, decisions by grey triangles, and labels by yellow boxes. If the vertebrate is a rare, threatened or endangered (RTE) species, report that collection to NEON Science and Permitting before the next bout.**

4. Remove all vertebrate bycatch and record associated data directly into the Sorting data entry application (**local and/or state permits should be consulted for reporting requirements of vertebrate bycatch**) or (if unavailable) on the sorting datasheet (RD [05]). Refer to the 'Manual for Fulcrum Application: TOS Ground Beetle Sampling [PROD]' for details on appropriate electronic data entry.

**Figure 20. Filtering diluted ethanol off sample into a specimen cup (a temporary waste container).**



**Figure 21. Pouring sample through filter directly into larger waste container.**

5.  Place all individuals of a given taxon, and taken from a single *trap,* into as few 50 mL tubes as are required

    a.  Note: Wrap the top of the tube threads with Teflon tape. This creates a seal between the vial and the lid to prevent ethanol leakage.

6. Fill each tube with enough 95% ethanol that there is at least 1/2 inch of ethanol above the fully submerged specimen(s). If there are too many specimens to allow for 1/2 inch of ethanol above the level of the specimens, split the specimens into multiple tubes to ensure that there is 1/2 inch or more of ethanol above the specimens in each tube.

7. Include a locality label (e.g., label with the location where the specimen was collected; APPENDIX J) and a determination label (e.g., label with the taxonomic ID of the specimen; see APPENDIX K) in each tube. Affix an external label onto each tube on which the following subsample ID is recorded (on an ethanol safe label and using an archival ethanol-safe pen): plotID.trapID.collectDate.taxonID.tubeNumber (where tubeNumber is two digits, starts at 01, and increases for as many tubes as contain that species of vertebrate collected from that trap and date). Note that every tube created from a single trap MUST have a unique tube number so that each subsampleID is unique and not repeated. Each unique subsample requires a data entry record.

> Example: Two tubes are required to hold a vertebrate bycatch species for the plot. SubsampleIDs for these tubes are plotID.trapID.collectDate.taxonID.01 and plotID.trapID.collectDate.taxonID.02.
>
> Note – although subsampleIDs are automatically generated by the mobile application, the application requires that you specify the destination tube number. In this case, it would need two entries to specify how many animals went into tube 01 and tube 02, respectively.

8. Vertebrates from each trap receive their own line item in the sorting data. For each tube:

   a. Scan the barcode on the sample Whirl-Pak and select the plot-level field collection record; select the trap-level record by rescanning the barcode or selecting the sample identifier from the dropdown list (Field Sample ID) – information from the field collection will autopopulate Ground Beetle Sorting Metadata. Ethanol rinse date will autopopulate the sorting record from the field application; if this value is blank, edit the *field collection* record for that sampleID to add ethanol rinse date.

   b. Enter recordedBy.

   c. There is a section labeled 'Click Here to Enter Sorting Data'; For each vertebrate taxon and tube used, record as a new line item with:

      i. The sampleType (vert bycatch – mam or vert bycatch – herp)

      ii. The number of individuals in the tube, in the 'Individual Count' field

iii. The taxonID in the 'taxon ID' field

iv. The morphospecies ID in the 'morphospecies ID' field (if applicable; see **section 7F.2** for instructions on morphospecies naming conventions).

v. Tube number (.01, .02, etc)

vi. All other data that can be filled out (e.g., processing date, date of identification, identification references, etc.) in the mobile data entry device (or on the sorting datasheet if data entry devices are unavailable)

d. If this sample is not pooled (see SOP H for directions on pooling), a barcode must be used. Scan the barcode label of the vial into which the vertebrates have been placed. Take care to make sure that there is congruence between the physical labels and electronic record.

*Example*: If barcode 'A00000000001' is adhered to a vial containing subsampleID CPER_001.W.20171031.PEMA.01, then the barcode needs to be scanned within the record for CPER_001.W.20171031.PEMA.01 *not* CPER_001.W.20171031.PEMA.02 or CPER_001.E.20171031.PEMA.01.
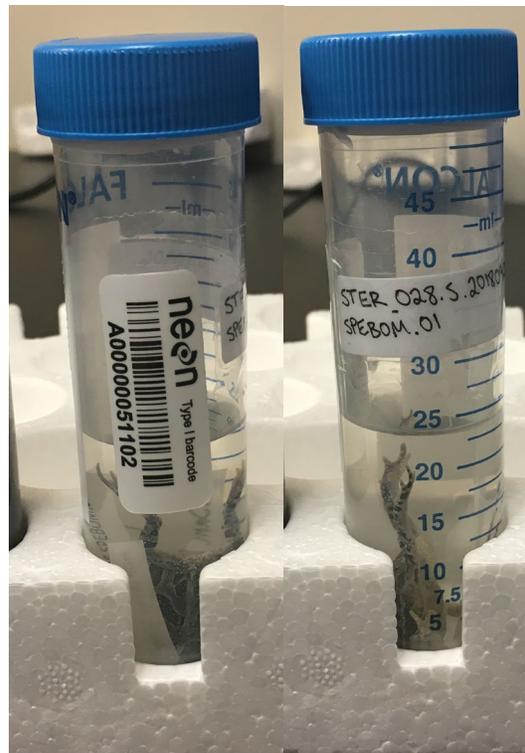


**Figure 22 Barcode and subsampleID labels are placed onto SPEBOM herptile subsample. If this sample is NOT pooled (i.e., this is the final container for this specimen), the tube requires a barcode.**

9.  If a given taxon (e.g. SPEBOM) is found in multiple traps from the same plot and bout (e.g., 2 SPEBOM in CPER_001 West trap, 1 SPEBOM in CPER_001 East trap collected 2018-09-03) – pooling is allowed to save on archival costs. Pooling (see SOP H for directions) should only be done when 1) the specimens are identified to species level, 2) the small mammal lead or beetle lead has confirmed the taxonomic ID, and 3) pooling would *reduce* the number of vials used to archive the sample. Store processed vertebrate subsamples at room temperature in the flammables cabinets unless there is ample space in refrigerator (4°C).

10. Transfer invertebrates and extra locality labels back into the same Whirl-Pak bag from the field.

    a.  Optional step. Traps containing a large amount of mud, silt or sand (a condition common when trapping periods overlap with rain events) can become time consuming to sort later. If time allows during the field season, the invertebrates can be separated from the sand, silt, mud, or debris. The invertebrates and their locality labels can be stored in the original Whirl-Pak bag from the field, while their extraneous debris is discarded. However, extreme care must be taken that no invertebrates are inadvertently part of the discarded sand/silt/mud/etc.

11. Pour fresh 95% ethanol into each Whirl-Pak bag, ensuring that all of the insects are submerged, and close the bag, leaving as little airspace as possible.

12. Confirm that the appropriate labels are still in the Whirl-Pak bag.

13. Keep the Whirl-Pak bags from all traps within a plot together in a small bag and put each sampling bout (all the plots from a single date) into a labeled airtight plastic container or re-sealable bag and seal tightly until further processing (sorting, pinning, etc., detailed in the following steps).

14. Store processed invertebrate samples at room temperature in a flammables cabinet.

**Figure 23. Samples from one sampling bout stored in a sealed container. Label containers using lab tape and permanent markers.**

### D.3    Details about species identifications

The NEON taxon list of vertebrate species codes can be found on the NEON sampling support library within the Ground Beetle Protocol tab (under 'Fauna taxon tables'). Technicians must use ONLY the NEON taxon code on all datasheets. The NEON taxon lists also include codes for instances when identification below a given taxonomic rank (e.g., family, genus) cannot be made. These are indicated by a 'sp.' or 'spp.' in the scientific name, where the former is used when only one unknown species is involved and the latter when the group of individuals in question might belong to more than one species. When one of these taxa is selected, an identification qualifier is not needed, unless the lowest taxonomic rank indicated (e.g., family, genus) is uncertain.

Read the section on identification and taxonomic uncertainty for more information about the use of identification qualifiers, identification codes, and morphospecies designations (**Handling uncertainty in species identifications**). Use these qualifiers and morphospecies codes for the vertebrates exactly as you would for the carabids.

**SOP E        Laboratory Processing – Sorting Invertebrate Bycatch**

**E.1        Preparation for Sorting**

1. Clear lab bench space for beetle sorting and processing.  Coordinate with potentially conflicting activities (e.g. soil sieving).  Work beside fume hood/extractors to diminish inhalation of ethanol fumes.

2. Secure access to:

    a. Propylene glycol and ethanol waste storage.

    b. Sink for washing materials.

    c. At least one dissecting microscope

    d. Storage space for samples (e.g., cold storage for tubes of individuals)

    e. Storage space for pinned insects in Cornell drawers or Schmitt boxes.

3. Determine listed rare, threatened or endangered (RTE) invertebrate species that occur at the sites being sampled by the domain support facility. Place the list in a conspicuous location in the area commonly used for sorting invertebrates. Relatively few invertebrate species are listed (especially compared to vertebrate species) and are unlikely to appear in pitfalls due to their rarity; however, each domain must know all their special status species and be alert for their presence.

**E.2        Sorting Invertebrate Bycatch**

NEON is interested in adult beetles of the family Carabidae. All other invertebrates, including non-Carabid beetles and larval beetles of any kind, should be stored in separate, invertebrate bycatch containers. Do not begin sorting ground beetles from other invertebrates in the pitfall sample unless certain that the process can be completed for all samples in a plot. It is important that each sample remains clearly labeled and is not left unattended for any length of time.  Never separate samples from their labels.  Sorting should only be conducted under the microscope.

It is not uncommon for parts of beetles to become dissociated during the time that they are in pitfall traps in the field. If this happens, dissociated beetle parts should be treated as invertebrate bycatch **unless** all three pieces of the main body (head, pronotum, AND abdomen) are present, these pieces can **confidently** be identified as belonging to a single specimen, AND the beetle can be identified as a member of the family Carabidae.

*A note about entering records electronically*

The parent-child structure of the Sorting application allows for information that is repeated for all related records (i.e., the sampleID of the trap Whirl-Pak, ethanol change date) to be entered once and apply to all linked child records (i.e., any subsampleIDs entered in the record); this allows for saved time when entering data. However, ALL electronic records are pulled into the database on a rolling basis based upon the date they are *created* and locked for editing in the data entry application.

Invertebrate bycatch subsample information may be entered as a child records within the same parent record along with any vertebrate bycatch and/or carabids sourced from the same trap. Entering multiple subsamples of all types derived from the same trap is the *ideal* outcome (such that one Whirl-Pak = one parent record). However, technicians should be aware that using this approach may limit their QC window if a sorting record is created early in the season (i.e., vertebrate subsamples documented in March), but invertebrates are sorted later in the season. If invertebrates are sorted close to when the trap was collected, it is recommended that invertebrate subsample records be entered in the same parent record as any vertebrates derived from the same trap. If invertebrates are sorted at the end of the season, it is acceptable to make a new record for the subsample.

In the mobile data entry application, trap-level information is recorded in the 'Ground Beetle Sorting Metadata' section (i.e., sampleID, ethanol change date). By selecting 'Click here to enter sorting data', one line item or child record is created for each subsampleID derived from the original sample (i.e., describing sample type, subsampleID, etc). If the sorting datasheet is used, each unique subsample receives its own line in the sorting datasheet. If multiple tubes are required from the same trap, generate additional locality labels and place a locality label in each tube. A separate data entry child record is required for each tube that is created.

Steps for sorting invertebrate bycatch are detailed in **Figure 24**, with instructions in the following text.

1. Sort each sample individually, from one trap at a time.

2. Suspend specimens in ethanol in the Whirl-Pak bag so that they are uniformly floating throughout. Jostle the bag and use wash bottle to add 95% ethanol if necessary.

3. Pour specimens into a large Petri dish marked with a grid (hereafter referred to as the sorting dish). Remove or add 95% ethanol as needed to keep specimens submerged in the sorting dish, using a transfer pipette and a secondary container for used ethanol (removal) or wash bottle of ethanol (addition).

4. Remove the filter cloth from the Whirl-Pak and inspect it for any small invertebrates that may be attached. Rinse invertebrates into the sorting dish, with 95% ethanol, and then discard the filter. Due to the potential for DNA contamination, filters cannot be reused.

5. Remove all non-beetle invertebrates, larval beetles, and beetle body parts that cannot be confidently identified as belonging to an individual carabid, and place these into a temporary container marked 'invert bycatch'. Make sure this container always contains enough 95% ethanol to fully submerge its contents, and place a locality label (taken from the locality labels in the original pitfall trap) into the temporary holding container.
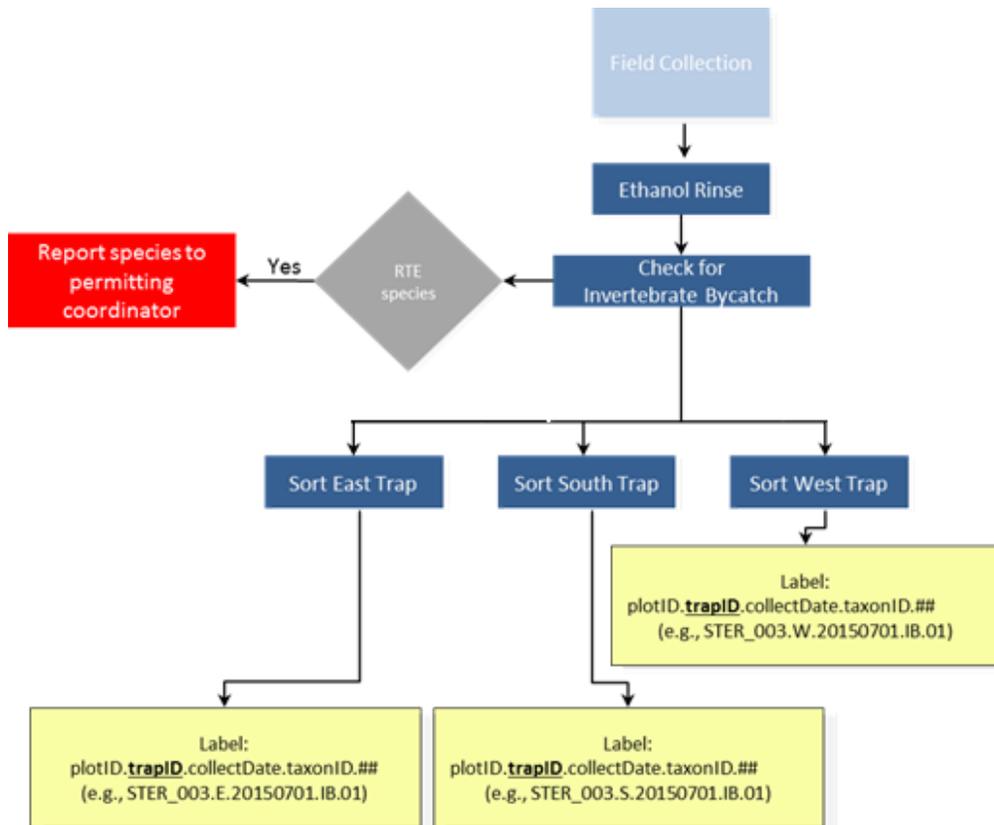


**Figure 24. Workflow for processing invertebrate bycatch. Activities are represented by blue boxes, final products by red boxes, decisions by grey triangles, and labels by yellow boxes. The taxonID for invertebrate bycatch is always IB.**

6. Using a dissecting microscope, sort all remaining beetle specimens into either the temporary 'invert bycatch' container OR a second temporary container that is marked 'carabids' and into which a single locality label, from those in the initial pitfall trap, has been placed (see **APPENDIX H** and **APPENDIX I** for guides for delineating which beetles are carabids). If a specimen cannot be confidently identified to the family Carabidae, but is a member of the suborder Adephaga, treat as if it is a carabid.

7. If special status or endangered non-carabid invertebrates are incidentally collected, such invertebrates must be maintained in their own vial *separate from any other invertebrate bycatch*. Use the subsampleID for this vial when communicating capture of RTE species to NEON permitting staff. **Local and/or state permits should be consulted for further reporting requirements of RTE invertebrate bycatch**.

8. Some samples may contain debris that will be stored with the bycatch (large items, e.g., leaves or twigs), that may be removed and discarded, but be careful that small invertebrates are not attached to them.

9. When all non-carabid invertebrates have been separated from all carabids, they are ready to be placed into a prepared vial. Depending on quantity, this final vial may contain 1) all the invertebrate bycatch from a single trap, 2) a portion of the invertebrate bycatch from a single trap, OR 3) invertebrate bycatch from up to three traps collected from the same plot and date.

   If the sorted invertebrate bycatch is not pooled with other bycatch from the same plot (situations 1 and 2 above), then:

   a. Invertebrate bycatch taken from a single *trap* is placed into as few 50 mL tubes as are required to hold the sample (make sure there is ½ inch of space between the top of the bycatch and the tube lid); these subsamples are placed into tubes that have had a Type I barcode applied.

   b. Fill each tube of invert bycatch with enough 95% ethanol that there is at least 1/2 inch of ethanol above the fully submerged specimen(s). If there are too many specimens to allow for 1/2 inch of ethanol above the level of the specimens, split the specimens into multiple tubes to ensure that there is 1/2 inch or more of ethanol above the specimens in each tube.

   c. Include a locality label (e.g., label with the location where the specimen was collected; APPENDIX J) in each tube. Affix an external label onto each tube on which the following subsample ID is recorded (on an ethanol safe label and using an archival ethanol-safe pen): plotID.trapID.collectDate.IB.tubeNumber (where tubeNumber is two digits, starts at 01, and increases for as many tubes as contain invertebrate bycatch collected from that trap and date). Note that every tube created from a single trap MUST have a unique tube number so that each subsampleID is unique and not repeated. Each unique subsample requires a data entry record.

   d. Wrap the threads on the top of the tube with Teflon tape. This creates a robust seal between the vial and the lid to prevent ethanol leakage.

**Figure 25. Bycatch samples stored in a 50 mL centrifuge tubes, the amount of ethanol in a bycatch sample will vary by sample size.**
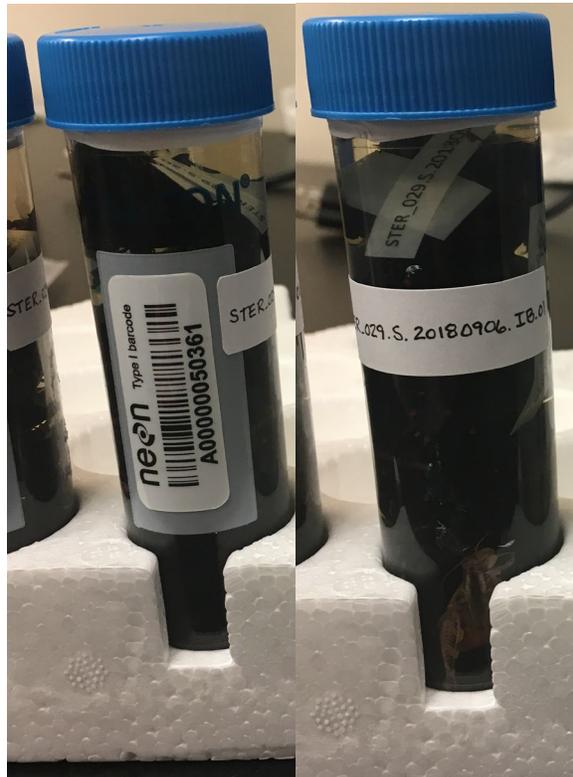


**Figure 26. Sorted invertebrate bycatch. Samples have a barcode and external sample identifier.**

When invertebrate bycatch from the sorted subsample **will** be pooled with invert bycatch from other traps in the same plot:

e. The sorted subsample may be held in a temporary container until one or two other subsamples from the same plot and date of collection have been sorted and are available for pooling (SOP H).

---

**IMPORTANT**: Pooled invertebrates will be placed into a pooled archive vial that has a barcode affixed with locality labels from each source trap. It will also need an external pooled identifier. Even if the sample is pooled, **a sorting data record is required** to document the subsampling process. Do not scan the pooled archive vial barcode in the sorting application, it will be scanned later in the Pooling application.

Pooling is allowed to save on archival costs, but should only be done when pooling would *reduce* the number of vials used to archive the sample (e.g., a small amount of inverts in CPER_001 present in West trap, a small amount of inverts in CPER_001 present in East trap collected 2018-09-03). See SOP H for more information about pooling.

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10. Record data directly into the data entry application 'BET: Sorting [PROD]' or (if unavailable) on the sorting datasheet (RD [05]). Refer to the 'Manual for Fulcrum Application: TOS Ground Beetle Sampling [PROD]' for details on appropriate electronic data entry.

    a. If vertebrates were removed from the sample, invertebrate subsamples may be added to the existing record (searchable with the Whirl-Pak sample barcode from the field collection). Otherwise, create a new record in the Sorting application by scanning the barcode on the field sample Whirl-Pak and selecting the plot-level field collection record; select the trap-level record by rescanning the barcode or selecting the sample identifier from the dropdown list (Field Sample ID) – information from the field collection will autopopulate Ground Beetle Sorting Metadata. Ethanol rinse date will autopopulate the sorting record from the field application; if this value is blank, edit the *field collection* record for that sampleID to add ethanol rinse date.

    b. Enter recordedBy.

There is a section labeled 'Click Here to Enter Sorting Data'; for each tube used to contain the invert bycatch, record as a new line item with: sample type ('invert bycatch') and processingDate fields. *Neither counts nor taxonomic identifications are expected for invertebrate bycatch specimens.*

11. Unless the sample was immediately pooled, record the subsample barcode from the vial into which the invertebrates were placed in the mobile data entry application. Take care to make sure that there is congruence between the physical labels and electronic record.

    Example: If barcode 'A00000000001' is adhered to a vial containing subsampleID CPER_001.W.20171031.IB.01, then the barcode needs to be scanned within the record for CPER_001.W.20171031.IB.01 *not* CPER_001.W.20171031.IB.02 or CPER_001.E.20171031.IB.01.

    > Example: Two tubes are required to hold invertebrate bycatch for a trap E. SampleIDs for these tubes are plotID.E.collectDate.IB.01 and plotID.E.collectDate.IB.02.
    >
    > Note – subsampleIDs are automatically generated by the mobile application when the Sample Type is 'invertebrate bycatch', but the tubeNumber for each tube must be entered

12. Store processed samples at room temperature in the flammable storage cabinets.

13. Transfer carabids (if any) from the temporary container marked 'carabids' and one locality label back into the original Whirl-Pak bag from the field.

*Note: Any 50 mL tubes that were used as temporary storage may be rinsed and reused, assuming they are still in good condition.*

**SOP F      Laboratory Processing - Sorting Ground beetles**

Ideally, samples will be recovered from the field and undergo an initial sorting phase within 24 hours where any vertebrate bycatch will be removed (discussed in SOP D). Invertebrate bycatch (discussed in SOP E) can be separated from unidentified carabids (sorting procedure discussed below; all samples maintained at the trap level until definitive IDs have been made) anytime during the field season, as staff time and resources allow.

At the end of the field season, carabid beetles will be further sorted to species and either pinned/pointed (instructions in **SOP F**) or stored in 95% ethanol. The final destination of these specimens will include: 1) a domain's teaching collection or 2) an archive facility. This SOP provides instructions for beetle processing up to the pinning or pointing stage.

Carabid beetles should be initially sorted from invertebrate bycatch, as described in SOP E. After non-Carabidae invertebrates are removed from Whirl-Pak, the carabids (at this point still not identified to species) are returned to their original, trap-level Whirl-Pak (see 13 from above). During the season, only the initial sorting phase discussed in SOP E will be conducted and data concerning invertebrate bycatch generated from separating bycatch from carabids may be directly entered into the mobile application. Information about carabids themselves (e.g., individual counts, etc) are input into the sorting datasheet at the end of the field season when either the species or morphospecies is known. Species-level sorting occurs on a trap-by-trap basis so that specimens from individual traps are kept separate through the entire sorting process.

During the sorting process specimens will be identified to species and grouped by species in vials of ethanol at the **_trap-level_**. At the end of sorting, a subset of beetles will be individually pinned or pointed. For domain support facilities with multiple sites, identification of beetles should happen for one site at a time, completing the identification of all beetles at one site before moving on to another site. This cuts down on the number of temporary vials that are in storage at any one time. Only **after** an entire site has been processed can groups of the same species be pooled by plot and collection date (see SOP G). The general process of sorting carabid beetles is illustrated in Figure 27, with detailed instructions in the following text. Record any carabid sort information into the data entry application as samples are processed and species identified.

*A note about entering records electronically*
The parent-child structure of the Sorting application allows for information that is repeated for all related records (i.e., the sampleID of the trap Whirl-Pak, ethanol change date) to be entered once and apply to all linked child records (i.e., any subsampleIDs entered in the record); this allows for saved time when entering data. However, ALL electronic records are pulled into the database on a rolling basis based upon the date they are *created* and locked for editing in the data entry application.

Carabid subsample information may be entered as child records within the same parent record along with any vertebrate and/or invertebrate bycatch sourced from the same trap. Entering multiple

subsamples of <u>all</u> types derived from the same trap is the *ideal* outcome (such that one Whirl-Pak = one parent record). However, technicians should be aware that using this approach may limit their QC window if a sorting record is created early in the season (i.e., vertebrate subsamples documented in March), but carabids are sorted later in the season. If carabids are sorted close to when the trap was collected, it is recommended that carabid subsample records be entered in the same parent record as any bycatch derived from the same trap. If carabids are sorted at the end of the season, it is acceptable to make a new record for all carabid subsamples derived from a particular trap.
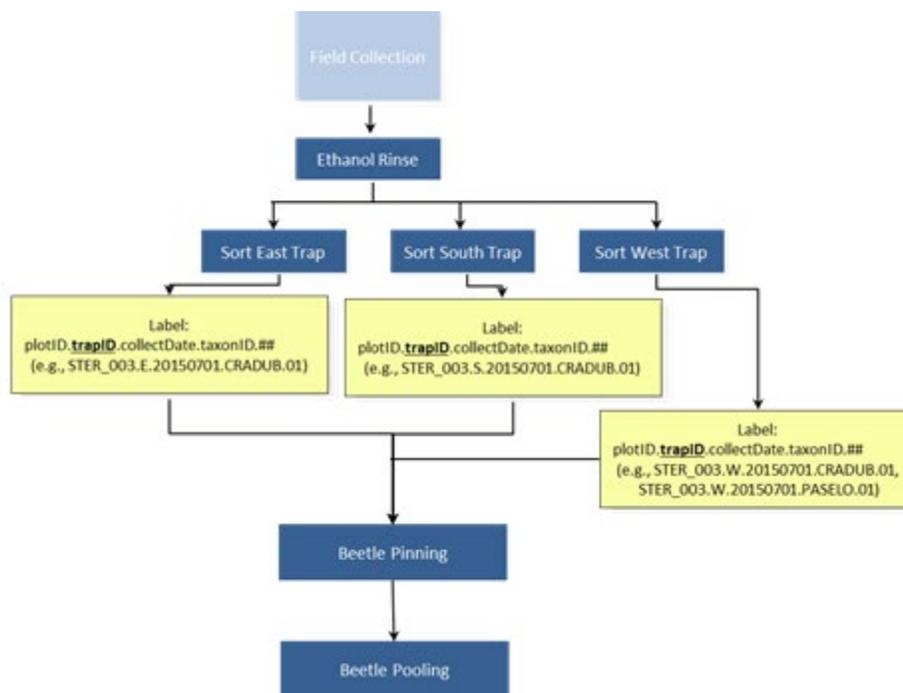


**Figure 27. Workflow for processing carabid beetles. Carabids are sorted by species within each trap, pinned as necessary. At the end of the season, more pinning may be required to get a representative mixture of variation within the pool of beetles. Only *after* all identifications and pinning are complete, *then* carabids are pooled as necessary. Activities are represented by blue boxes and labels by yellow boxes.**

1. Separate each group of carabids into groups of the same species or morphospecies. Work on only one trap at a time and sort through the carabids when there is enough time to identify all the specimens in a trap.

2. When all carabids from the trap are placed in conspecific groups, they are ready to be placed into a prepared vial. Depending on carabid quantity, this vial may contain either all the carabids of a single species from a single trap OR a portion of the carabids of a single species from a single trap (if they do not all fit into a single vial). Do **not** pool samples until all pinned carabids have been allocated; pinned specimens require known trap-level information.

a.  Place all carabids from the <u>same</u> trap of the *same species or morphospecies,* into as few 50 mL tubes as required.

    i.  Wrap the top of the tube threads with Teflon tape. This creates a seal between the vial and the lid to prevent ethanol leakage.

b.  Fill each tube with enough 95% ethanol that there is at least 1/2 inch of ethanol above the fully submerged specimen(s). If there are too many specimens to allow for 1/2 inch of ethanol above the level of the specimens, split the specimens into multiple tubes to ensure that there is 1/2 inch or more of ethanol above the specimens in each tube. Each tube will require a unique subsampleID, which is created by incrementing the tube number.

c.  Include a locality label (e.g., label with the location where the specimen was collected; APPENDIX J) and a determination label (e.g., label with the taxonomic ID of the specimen; see APPENDIX K) in each tube.

3.  For each tube, record directly into the data entry application (or on the paper sorting datasheet; only if the data entry application is unavailable). Refer to the 'Manual for Fulcrum Application: TOS Ground Beetle Sampling [PROD]' for details on appropriate electronic data entry.

a.  Scan the barcode on the sample Whirl-Pak and select the plot-level field collection record; select the trap-level record by rescanning the barcode or selecting the sample identifier from the dropdown list (Field Sample ID) – information from the field collection will autopopulate Ground Beetle Sorting Metadata. Ethanol rinse date will autopopulate the sorting record from the field application; if this value is blank, edit the *field collection* record for that sampleID to add ethanol rinse date.

b.  Enter recordedBy.

c.  There is a section labeled 'Click Here to Enter Sorting Data'; For each vertebrate taxon and tube used, record as a new line item with:

    i.  The sampleType (carabid)

    ii.  The number of individuals in the tube, in the 'Individual Count' field (this is the number of carabids in the tube before any were removed for pinning per SOP G)

    iii.  The taxonID in the 'taxon ID' field

    iv.  The morphospecies ID in the 'morphospecies ID' field (if applicable; see **section 7F.2** for instructions on morphospecies naming conventions).

    v.  Tube number (.01, .02, etc)

vi.    Processing date (date that carabid(s) were initially identified; will only differ from identified date if identification is updated later)

vii.    All other data that can be filled out (e.g., date of identification, identification references, etc.) in the mobile data entry device (or on the sorting datasheet if data entry devices are unavailable)

d.    If multiple tubes are required from the same trap, generate additional locality labels and place a locality label in each tube. A separate data entry child-record is required for each tube that is created.

4.    Externally label each subsample on an ethanol-safe label with its unique subsampleID using an archival ethanol-safe pen in the following format: plotID.trapID.collectDate.taxonID.tubeNumber (where tubeNumber is two digits, starts with 01, and increases for as many tubes as contain the same taxon).

Verify that the taxonID corresponds to the correct identification. Misuse of taxonID decreases data quality and takes extra time to remedy (e.g., *Pasimachus sublaevis* and *Pasimachus subsulcatus* are designated PASSUB1 and PASSUB2, respectively. Double checking the taxonID list prevents errors where PASSUB1 is used incorrectly for an identification of *P. subsulcatus*)

> Example: Two tubes are required to hold a particular carabid species for trap W. SubsampleIDs for these tubes are plotID.W.collectDate.taxonID.01 and plotID.W.collectDate.taxonID.02.
>
> Note – subsampleIDs are automatically generated by the data entry application when the Sample Type is 'carabid'; make sure that the subsampleID in the application is the name used for the vial.

5.    Close the lid on the tube(s) so no ethanol leaks out (can result in smeared labels).

Note: a subset of carabids may be selected for pinning according to the guidelines in SOP G from each tube**.** Once all carabids that are being pinned have been removed from a vial, *verify that the 'individualCount' number in the sorting data includes all individuals of that species which came from that trap regardless of whether they were pinned*. Ground beetle abundance will later be calculated by adding up the number of beetles of a particular species from each record in the sorting database. Pinning records will **only** be used for the tracking of archived samples.

*Example*: If the sorting datasheet indicates that sampleID CPER_001.W.20160401.PASSUB1.01 has an individualCount of 10 and the pinning data indicate that 2 *Pasimachus sublaevis* were

pinned from CPER_001.W.20160401.PASSUB1.01, then that will be interpreted as 10 beetles being present in the West trap of plot 001 at CPER on the 1st of April 2016.

6. If this sample will not be pooled (see SOP H for directions on pooling), a barcode must be used. Scan the barcode label of the vial into which the carabids have been placed. Take care to make sure that there is congruence between the physical labels and electronic record.

   Example: If barcode 'A0000000001' is adhered to a vial containing subsampleID CPER_001.W.20171031.PASELO.01, then the barcode needs to be scanned within the record for CPER_001.W.20171031.PASELO.01 not CPER_001.W.20171031.PASELO.**02** or CPER_001.**E**.20171031.PASELO.01.

   Do not scan the barcode of a <u>pooled</u> vial into the subsample barcode field in the sorting app; only enter a barcode in the subsample barcode field if that is the final container for that sample. It is acceptable to hold off on applying a barcode on a sorted carabid sample until pinning and pooling decisions have been made. At that later time, a barcode should be added and scanned into the record for the sample.

7. Store processed samples at room temperature in the flammables cabinets.

**F.1      Equipment maintenance, cleaning, and storage**

1. After all samples have been sorted:

   a. Empty, wash, and dry all waste receptacles (e.g. ethanol and PG containers).

   b. Return all flammables to the appropriate cabinets.

   c. Pack equipment and consumables for subsequent field work and store neatly.

*Note: Any 50 mL tubes that were used as temporary storage may be rinsed, washed with soap, and reused, assuming they are still in good condition.*

**F.2      Handling uncertainty in species identifications**

All specimens must have a taxonID associated with them. However, taxonomic identifications based on morphological features can involve uncertainty for a variety of reasons. When specimens are badly damaged such that key features or body parts missing (i.e., because insufficient preservative was in the trap or trap collection was delayed beyond the standard interval), then taxonomic identification can be compromised such that species-level identifications are impossible and coarser taxonomic assignments are unavoidable. Identifications of damaged specimens should be as specific as possible using the features that are present without being inaccurate (i.e., identifications may be to genus—*Myodes* sp.— or tribe—Harpalini sp.).

Specimens that are in good condition should be identified to the species-level, where possible. However, there will be instances where accurate identification to species is not feasible *even* for a well-preserved sample. Some features may be reliable morphological markers, but require high-powered microscopy, extensive dissection, or a decade's worth of experience to identify properly. In these cases, technicians can indicate the finest known level of taxonomic information in one of two ways: 1) recording an identification qualifier and a taxonID with finer taxonomic resolution or 2) assigning a morphospecies and a taxonID with coarser resolution.

An identification qualifier contains information that indicates the taxonomic level at which there is uncertainty. If there is confidence about the genus of a specimen and uncertainty in the species identification, then 'cf. species' or 'aff. species' indicates that the provided species identification is possibly incorrect (Table 11). If a specimen is definitively of a particular tribe (i.e., Pterostichini) and the technician is uncertain in their assignment of genus (i.e., *Pterostichus*), then 'cf. genus' or 'aff. genus' could be used to indicate uncertainty in the genus-level assignment. If there is no uncertainty associated with lowest taxonomic rank specified, the identification qualifier field should be left blank. An inappropriate usage of the qualifier occurs when the level of the selected identification qualifier does not match the given identification of the specimen. For example, if the scientific name of a specimen is *Harpalus* sp., then only the genus is known; it is inappropriate to use the 'cf. species' identification qualifier because that is saying the *species* ID is uncertain without having provided species level information.

Identification qualifiers are preferred when the specimen's identity can be narrowed down to one of a few choices. If the genus or subgenus of a specimen is obvious and the specimen is one of a few species (3 species maximum), assign that specimen the taxonomic identification with which it most closely aligns and the identification qualifier at the appropriate level. For example, a domain collects 10 specimens that are either *Pterostichus pensylvanicus* or *Pterostichus adstrictus*. Based on morphological features, the identifying technician feels that two seem more like *Pterostichus pensylvanicus* and the other eight are more closely aligned with *Pterostichus adstrictus*. The technician would use the 'cf. species' identification qualifier, because these specimens are all definitely *Pterostichus* but the species identifications are uncertain. In the remarks, **briefly** indicate possible other species identifications and reason for rejecting them. In this example, the remarks might say "ID either *P. pensylvanicus* or *adstrictus;* identification based on number of clip setae on protibia".

**Table 11. Codes for identification qualifier entries**

| idQ Code | Identification Qualifier Description* |
|---|---|
| **CS** | cf. species |
| **AS** | aff. species |
| **CG** | cf. genus |
| **AG** | aff. genus |

| | |
|---|---|
| **CF** | cf. family |
| **AF** | aff. family |

\* cf. roughly equals "not sure"; aff. roughly equals "similar to, but is not"

Morphospecies designations must be used when the specimen is in good condition, but technicians cannot narrow the field of possible identifications to just two or three choices. Keep notes in the morphospecies tracking sheet that include descriptive information about the specimen plus any known taxonomic information. A good description might be: "similar to or is either *Harpalus fuscipalpus* or *Harpalus carbonatus*. Approximately 10 mm long. Basal fovea more convex. Sparsely punctate. Posterior pronotal corners rounded but defined with small overlap onto elytra". This example description contains taxonomic information (it is within the genus *Harpalus*) and detailed morphological descriptions (see all the information about the pronotum, elytra, etc). This still requires that a taxonID be assigned, but it can be of a coarser resolution than at the species level (e.g., a morphospecies within the *Pterostichus* genus would use taxonID 'PTESP23' for *Pterostichus* sp., a herptile morphospecies that is only known to phylum would use taxonID 'OTHE' which corresponds to *Chordata* sp.).

If domain staff are able to subsequently identify the morphospecies, the full scientific name associated with that ID must be provided with the datasheet, either via annotation (see RD[04]) or by attaching a key to each datasheet. If domain staff are not able to identify a given morphospecies prior to data entry, the morphospecies ID and description from the morphospecies tracking datasheet must be transcribed into the appropriate spreadsheet on the NEON intranet on the beetle-specific sampling support library.

Cryptic species issues arise when two species that are morphologically indistinguishable in the field co-occur (or might co-occur) at a site. NEON intends to add these species pairs (e.g., 'slash' taxa) to the master taxon lists to account for this. If a cryptic species pair is not currently available in the master list, the proposed species pair must be submitted for consideration by NEON Science via the 'Taxon list additions and updates (Field Science request)' form on Service Now.

Finally, the mobile application currently limits technicians such that only species thought to be <u>present</u> in a domain are available for selection. With climate change and species introductions, it is likely that technicians will observe species within their site that represent the first ever records of that species in that area. When this happens, the mobile application will not have the scientific name of that species available for selection. In that case, technicians must use the taxon code "OTHE" for carabids that are new to their domain. In the remarks field, write the scientific name of the identified beetle. NEON will revise that record to reflect the correct scientific name after confirmation of identification. The taxon table will be updated for the subsequent year to make that species name available to technicians within that domain.

### F.3 About morphospecies designations

A morphospecies is a temporary designation for an individual or group of beetles that are of the same group (ideally that group is a species; morphospecies only requires that all individuals look the same). A subset of each morphospecies will be sent for later taxonomic identification by an expert taxonomist.

Sometimes morphospecies designations turn out to contain multiple species. While this is unavoidable for certain species complexes that are difficult to resolve, in most cases this can be avoided by designating multiple morphospecies for similar (but not identical) unknown specimens. Should these morphospecies be assigned the same species designation by the taxonomist, the two morphospecies can later be merged into the same species designation.

- If a species assignment cannot be made based on the teaching collection or other identification resources and identification qualifiers are not useful (could be one of more than 3 options), give a morphospecies name to that type of ground beetle.
- As a general rule, split groups that look similar but not identical into different morphospecies, focusing on features like: size, color, proportions, bristle placement and the shape of the pronotum. It is easy to lump them together later, but difficult to later split them into multiple species.
- If it is unclear whether a newly captured specimen is the same as individuals from a previously assigned morphospecies, a new morphospecies ID should be assigned (it is better to have the same species designated as different morphospecies than to have multiple different species designated as the same morphospecies).
- Ground beetle morphospecies are prioritized for identification by taxonomic experts, who will return identified specimens that should then be added to the teaching collection at the domain lab.
- The format of a morphospecies ID includes: the domainID where the specimen was captured, the year of capture, and the word "Morph" followed by one or more unique letters.  For example, "D15.2014.MorphA" would be the first morphospecies from domain 15 that was captured in 2014.
- The letter at the end of the morphospecies ID (e.g., "A") should *never* be repeated for any other morphospecies than that for which it was originally designated, in a given year. If more than 26 morphospecies are encountered in a given domain in a given year, the 27th morphospecies ID should include two letters at the end (e.g., the 27th morphospecies in domain 15, for 2014, would be "D15.2014.MorphAA"). For every additional 26 morphospecies, a new letter will be added (i.e., the 54th morphospecies would be "D15.2014.MorphAAB")
  *Note: Because domain 13 is split across two support facilities, the MorphA assigned by the Boulder office will not be the same as the MorphA assigned by the support facility in Utah. To avoid confusion, the Utah domain support facility will put an extra letter ("Z") between "Morph" and their unique letter combinations. Unique letters will be used as described above. The first*

*morphs would be called MorphZA, MorphZB, MorphZC, etc. The 27th morphospecies at the Utah facility will be MorphZAA.*

Record and describe each morphospecies on the morphospecies datasheet, along with the year and name of the person who designated it and place this sheet in the location where identification is performed. When identifying carabids or vertebrate bycatch, morphospeciesIDs and descriptions can be entered directly into the 'sorting' data entry application the first time they are described. The morphospeciesID will be available to be selected for subsequent samples where it is found.

## SOP G      Laboratory Processing – Pinning and Pointing Ground Beetles and Sample Preservation

### G.1      Pinning and pointing large and small ground beetles

Pinning and pointing involve mounting specimens directly onto a pin (if they are large enough) or onto a small paper triangle on a pin (if they are very small). The individuals selected for pinning and pointing should represent different plots and sampling bouts throughout the entire sampling period (e.g., individuals should be selected from all plots, and from samples representing all bouts in which they were captured). Additionally, these individuals should exhibit the range of morphological variation encountered for the species in question (e.g., species with varying degrees of red patterning on their elytra should have representatives of the range of variation for this patterning).

The number of pinned individuals from a species that will be sent for secondary identification by taxonomic experts will depend on its abundance and the level of uncertainty in the identification. Species are considered abundant if more than 100 are found per site in a season, moderately common if 21 to 100 individuals occur per site in a season, and uncommon if 20 or fewer occur per site in a season. Species-level identifications that contain more uncertainty will require more individuals of that species group to be pinned. Decision trees in Figure 28 and Figure 29 describe the minimum number of specimens that must be pinned in different situations.

The objective of pinning is three-fold. The foremost reason for pinning beetles, is that only pinned individuals are available for secondary identification by taxonomic experts; thus, the teaching collection (the reference guide of known specimens derived from field operations collections) can only be supplemented based on the availability of pinned individuals. Additionally, only pinned individuals will be available as DNA barcoding candidates. During initial operational sampling at a site, more pinning will be required to bolster the teaching collection and for observatory commissioning. Future versions of this protocol will provide revised guidelines with reduced pinning requirements based on demonstrated success in identifications and the availability of mature teaching collections.

The other rationale for pinning beetles (vs. storing individuals in ethanol), is that pinned individuals are *substantially* cheaper to archive. If fewer than 10 carabids would be slated for long-term storage in a single vial of ethanol, it is cheaper to archive pinned individuals than to archive carabids in ethanol. EXCEPTION: if a specimen is in too poor a condition to be pinned, it can be archived in ethanol even if there are fewer than 10 carabids of that species. See below for example scenarios that do, and do not, require pinning of carabids:

> *Example 1*: Fifteen *Cratacanthus dubius* are caught in HARV_001 W trap during bout 1. Because more than 10 are present in one subsample and all 15 fit into one vial, these specimens can be maintained initially in ethanol.

> *Example 2*: During bout 1, five *Cratacanthus dubius* are caught in HARV_001 W trap, two more are caught in HARV_001 E trap, and four more are caught in HARV_001 S trap. A total of 11

*Cratacanthus dubius* were collected for that plot & date, and can be pooled later according to SOP H into a single vial. Because more than 10 will be present in one archiveVialID, these specimens can be maintained initially in ethanol.

*Example 3:* In bout 6, three *Pasimachus elongatus* are captured in E trap of plot HARV_001, but are not present in any other traps for plot HARV_001. Pin all 3 *Pasimachus elongatus* individuals.

*Example 4*: In bout 4, four *Pasimachus elongatus* are captured in each trap of plot HARV_001 (12 individuals total). However, only 5 *Pasimachus elongatus* fit into a vial. Pin all 12 individuals, because they cannot be consolidated into a single vial of ethanol that would have more than 10 individuals in it.

*Example 5*: In bout 2, five *Cratacanthus dubius* are captured in East trap of plot HARV_001. However, two are in very poor condition and cannot physically withstand pinning or pointing. Pin the three good-condition individuals, but maintain the two poor condition *Cratacanthus dubius* in ethanol.

In addition to the requirement that all beetles be pinned if fewer than 10 individuals are being consolidated into a single vial of ethanol, more individuals may need to be pinned according to the guidelines below. If these guidelines are already met by the initial round of pinning (described above), then no further pinning is required.

During initial operations, pinning or pointing is required for:

1. Up to 20 individuals per species per site per year of uncommon species (see Figure 28: directions for pinning moderately common to uncommon species).

2. At least 20 individuals, if available, for each easily identified species (**APPENDIX E**).

3. At least 100 individuals, if available, for each species or genus on the list of difficult taxa (**APPENDIX F**). Note: At the discretion of the technician, fewer individuals (i.e., as few as fifty) may be pinned if at least 20 individuals of the species in question are already present in the teaching collection.

4. If species is moderately common to abundant, does not occur on any list of species (**APPENDIX E, APPENDIX F**), and the technician is confident in their identification, then pin at least 20 but no more than 100 (see Figure 28 and Figure 29 for more detailed instructions).

   *Note – In order to ensure that temporal, spatial, and morphological variation is accounted for in the selection of beetles to be pinned and pointed, attempts should be made to pin one or two individuals of each species from each bout. Ideally, individuals will be selected for pinning across all plots in which that species is found to occur.*

Data for each pinned or pointed specimen should be recorded directly into the pinning data entry application 'BET: Pinning [PROD]' (or if unavailable on the pinning datasheet (RD[05])). See the 'Manual for Fulcrum Application: TOS Ground Beetle Sampling [PROD]' for additional details about the data entry application. Most pinning data is directly available from the labels in the tubes from which the specimens are obtained, or in the record in the sorting data that corresponds to this tube.
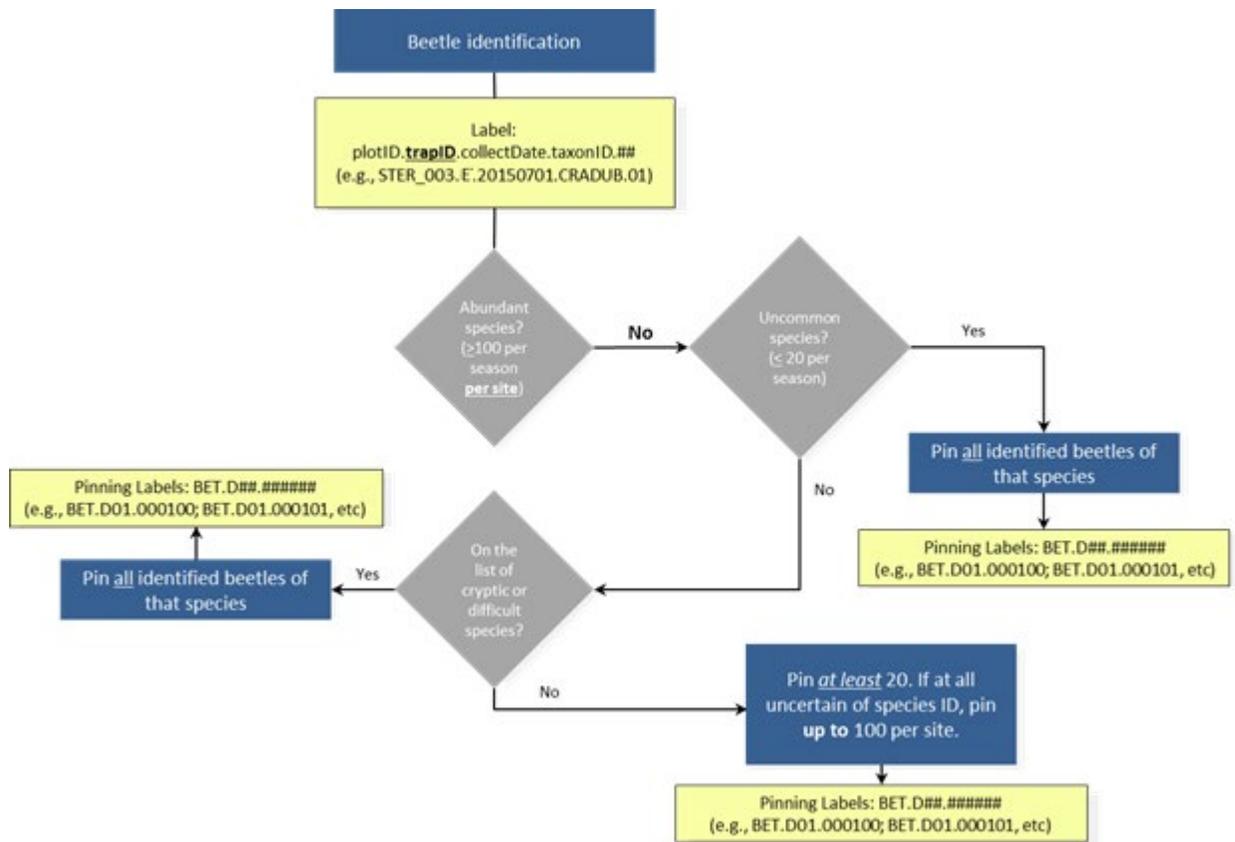


**Figure 28. Pinning instructions for moderately common and uncommon species (less than 100 over the season)**
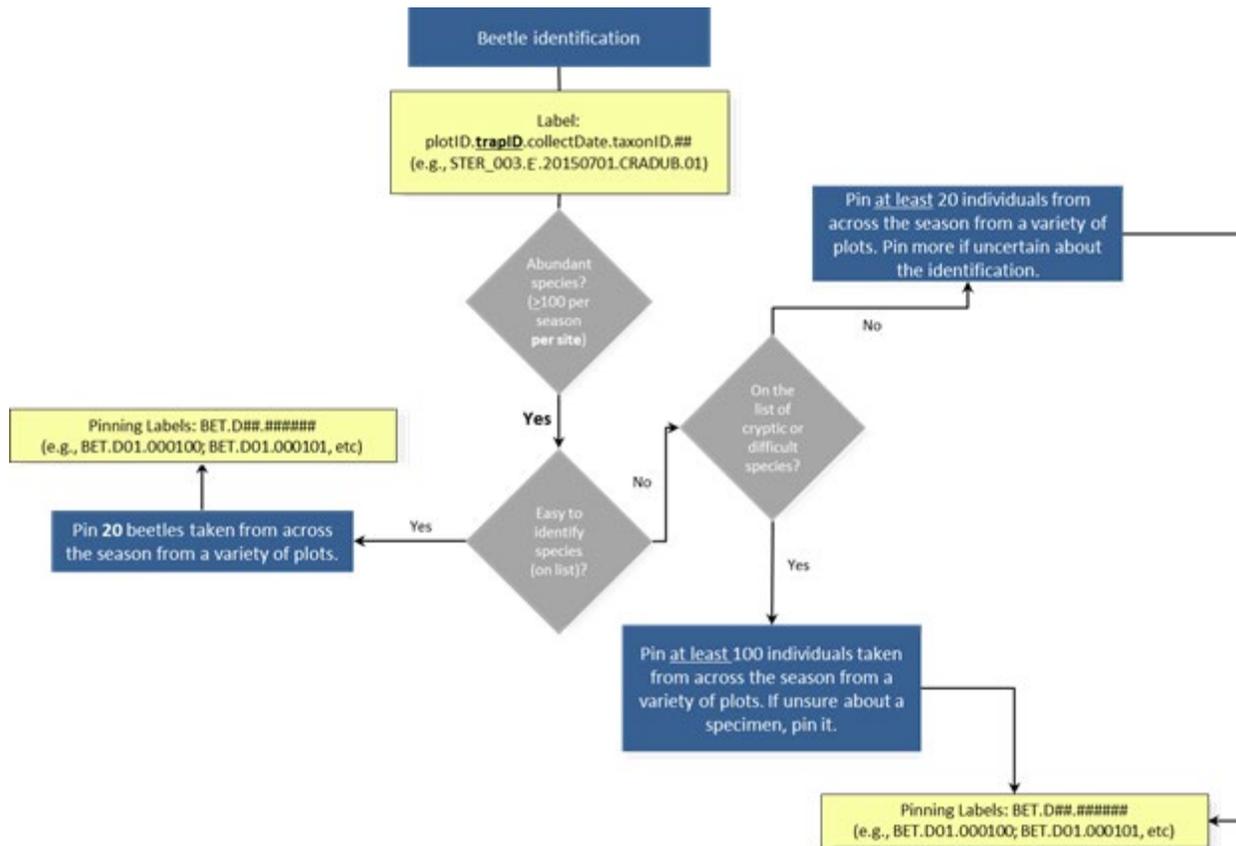
**Figure 29. Instructions for pinning abundant species (more than 100 present at a site over the entire season).**

### G.2 Determine whether to pin or point the specimen

1. Generally specimens 5 mm and greater are pinned, and specimens smaller than 5 mm are pointed (**Table 12**; **Figure 30**).

2. Pointing is a good default option for any specimen you are concerned will be destroyed by pinning (**Table 12**; **Figure 30**).

**Table 12. Summary of beetle mounting criteria**

| Specimen size and criteria | Mounting Method |
|---|---|
| ≥ 5mm | Pinning |
| ≥ 5 mm but likely to be damaged by pinning | Pointing |
| < 5 mm | Pointing |

**Figure 30. Pinned (left) and pointed (right) beetle specimens**

### G.3       Prepare points (if pointing)

1.  Prepare a batch of points prior to beginning pointing specimens.

2.  Using 100% rag cotton paper (Bristol or equivalent), prepare ~40 triangles using the point punch.

3.  Over a balsa wood surface, for each point, press a No. 3 archival quality pin into the point at the center of the point's base (see the "X" in **Figure 31**). Ensure that the point is at a 90 degree angle from the pin and neither angled up nor down.



**Figure 31. Materials for pointing including a schematic of a point showing where to place pin**

4.  Use a pinning block (**Figure 32**) to push the point up the pin until it is 15 mm from the top (non-pointed end) of the pin. This leaves enough space above the insect for the pin to be

handled, and below for labels. Additionally, specimens at a uniform height are easier to examine and compare with one another.
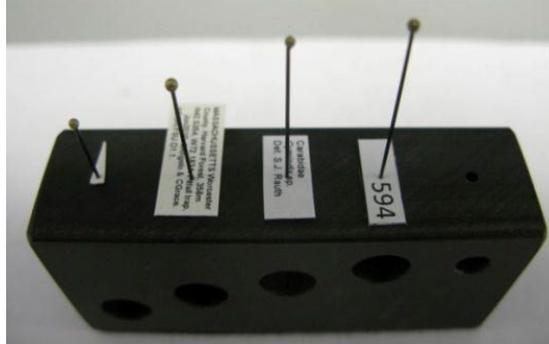


**Figure 32. A pinning block with a point and the various labels that will be affixed to a pinned or pointed specimen. This shows the heights at which points and various labels should be placed on a pin.**

### G.4        Prepare labels

1. See **APPENDIX J** for instructions on creating locality labels. These are created for all mounted beetle specimens during field preparation and will be placed on the pin below each specimen.

2. See **APPENDIX K** for instructions on creating determination labels. These are created for all mounted beetle specimens during field preparation and will be placed on the pin below the locality label located below each specimen.

3. See **APPENDIX L** for instructions on creating insect individual ID labels. These will be created for all mounted beetle and specimens at the time of preparation and will be placed on the pin below the determination label (which is below the locality label) located below each specimen.

It is important to consider how you will attach the correct locality and determination labels to specimens as you are mounting them. ***A specimen dissociated from its labels is worthless***.

### G.5        Mount Specimens

1. For pointed beetle specimens:

    a. Pointing should be done under the dissecting microscope so that the point is attached to the correct place on the beetle. For efficiency, work with several specimens (from the same pitfall trap or sampling bout) at a time.

b. After removing a specimen from the tube, allow it to dry for a few minutes. It is easier to work with dried specimens when pointing.

c. Use forceps to put the beetle specimen on its left side on a slip of plain white paper, so that its head is facing the right and its legs are toward the edge of the bench (**Figure 33**)



**Figure 33. A ground beetle on its side in preparation for pointing**

d. Slide the slip of paper with the beetle to the center of the microscope stage.

e. Examine the slope of the ground beetle's thorax to determine if the tip of the point needs to be bent to ensure that the specimen will lie flat on the point. Use forceps to bend the point if needed (**Figure 34**).

    i. **NOTE**: The sides of a beetle's thorax can slope inward to differing degrees, therefore, the tip of the point can be bent down with forceps to accommodate the size and shape of each specimen. For example, a specimen that has a flat-bottomed thorax can be mounted on a flat (unbent) point. A specimen on which the sides of the thorax are vertical must have the tip of the point bent downward at a right angle. See **Figure 34** for thorax slope scenarios and **Figure 35** - **Figure 36** for examples of well-pointed and poorly-pointed specimens, respectively.
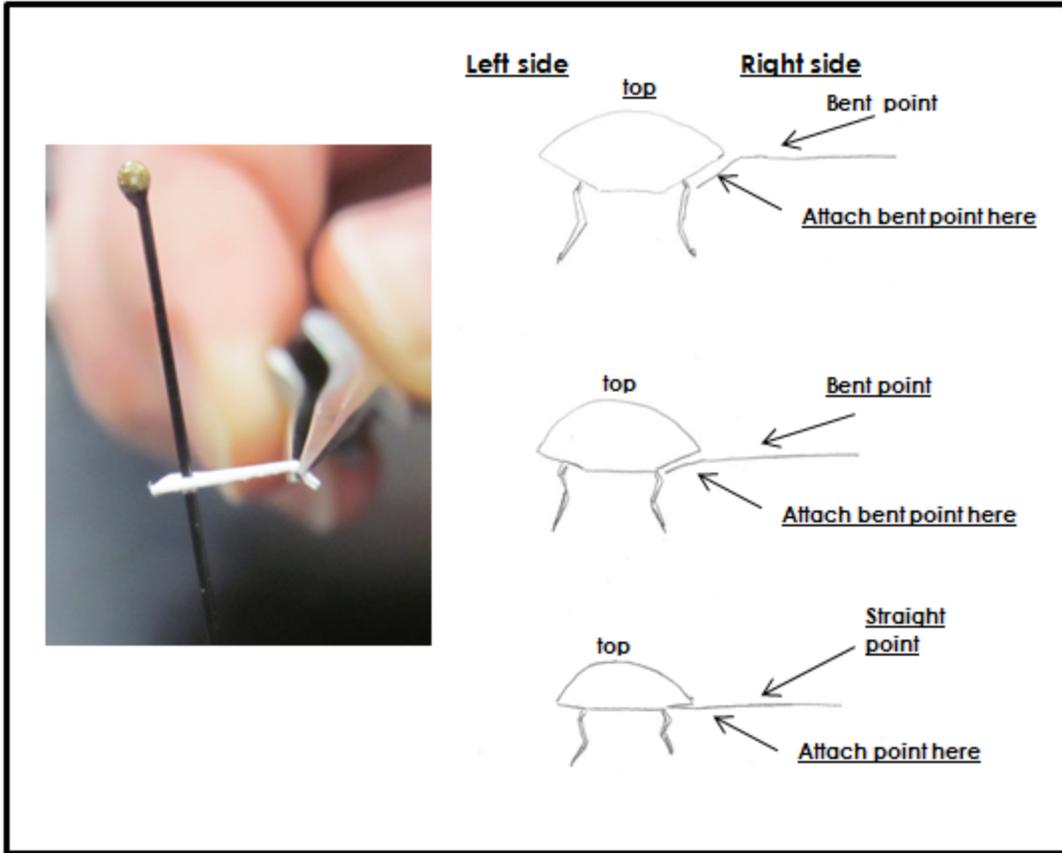
**Figure 34. Examples of bending a point tip to maintain a level body position of the specimen**
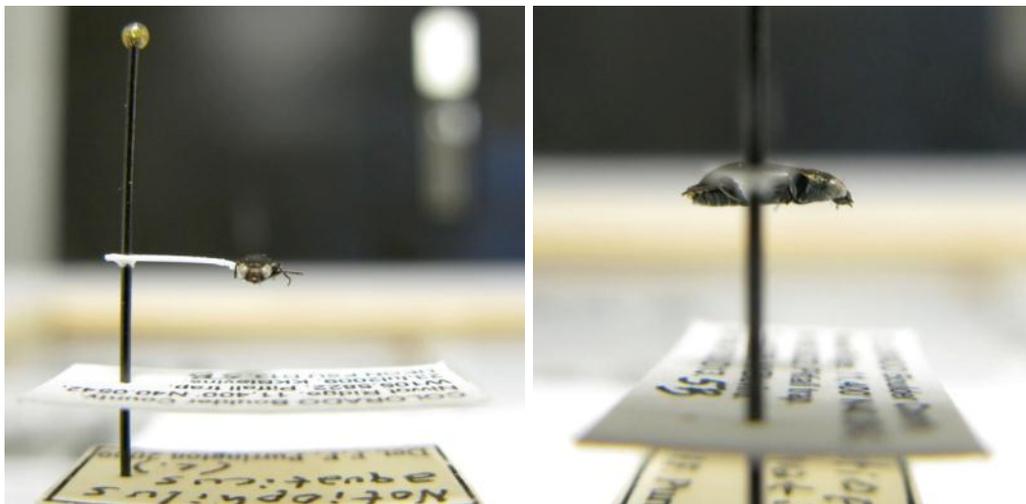


**Figure 35. A well-pointed beetle specimen, with associated labels**
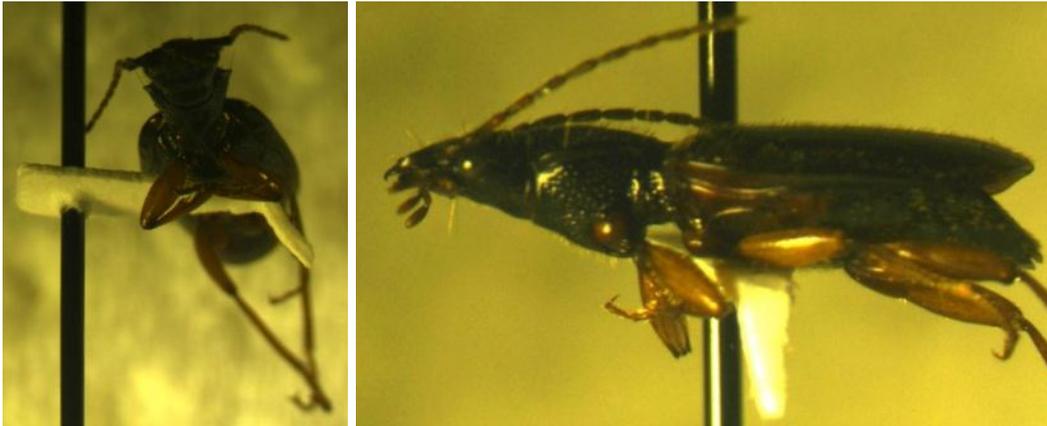
**Figure 36. A poorly pointed beetle specimen**

f.  Take the prepared point and touch the tip of the point in a bit of glue that has been squeezed out onto a paper towel or piece of paper (**Figure 37**). Use the minimum amount of glue needed in order to attach the specimen firmly to the point.



**Figure 37. Preparing to glue a specimen to a point by dipping the tip of the point in glue.**

g.  Under a microscope, zoom in on the target location for the tip of the point, this being on the **RIGHT** side (from a dorsal perspective) of the specimen's thorax and between the bases of the 2nd and 3rd pairs of legs (see "X" on **Figure 38**). Gently press the glue-covered tip of the point to the beetle's body (**Figure 39**), and wipe off excess glue in order to avoid obscuring any part of the beetle with large amounts of glue.

    i.  **Note**: Although the point and glue will obscure one side of the thorax, the other side needs to remain unobscured for examination. Neither the point nor the glue should extend onto the top or the bottom of the beetle, nor touch the head, abdomen, or wings.

    ii.  **Note:** Be sure to place the point on the *right* side of the specimen
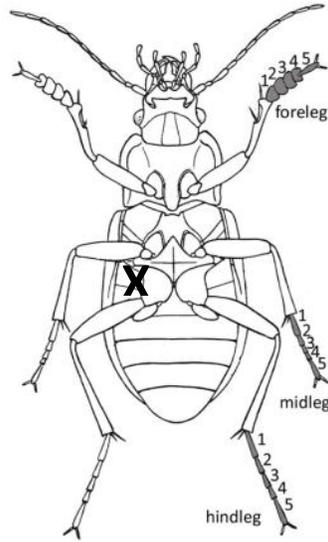
**Figure 38. Diagram of ventral side of a beetle, with and X indicating where to place point**
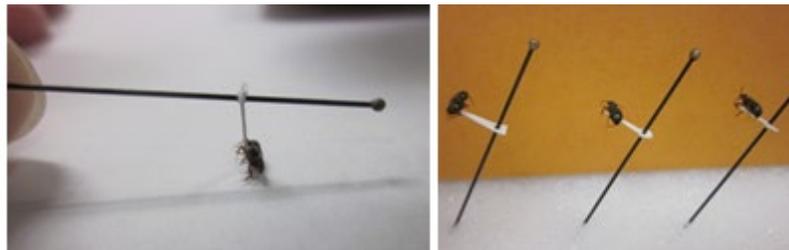


**Figure 39. Attaching a specimen to a point, and pointed specimens left to set.**

h.  Wait for the glue to set slightly, so that the beetle stays relatively still when the pin is moved. Then lift and angle the pin so that the weight of the specimen is leaning directly into the point and the specimen is properly aligned on the point, as in **Figure 34 - Figure 35**.

i.  Place the pin in a block of Styrofoam such that gravity is holding the specimen down on the point at the correct angle.

j.  Adjust the specimen as needed with forceps or a pin to ensure the specimen binds firmly to the point. A heavy specimen that rotates on the point should be straightened as the glue thickens but before it completely hardens.

k.  Slide the locality label (instructions in **APPENDIX J**) onto the pin below the specimen, and use the second-deepest hole in the pinning block to position the

label at the correct height (**Figure 40**).  Position the specimen perpendicular to the text on the label, with the back edge of the point slightly inset from the short edge of the label and the specimen centered between the two longer edges of the label (**Figure 40**).

   i. **Note**: Now is a great time to ensure that the label information is correct.



**Figure 40. Attaching a locality label below a pointed beetle.**

l. Attach the determination label (instructions in **APPENDIX K**) below the locality label. Use the third-deepest hole in the pinning block to position the label at the correct height. Orient the label in the same manner as the locality label.

m. Attach the insect individual ID label (instructions in **APPENDIX L**) below the determination label using the fourth-deepest (shallowest) hole. Orient the label in the same manner as the locality label.

n. When all of the specimens are mounted and labeled, examine them under the microscope to confirm that they are all attached securely to the points. To test this, place the specimens in a Schmitt box or unit tray and gently tap the box or tray. The specimens should remain affixed. Re-attach any specimen that do not stay affixed to the points, as long as it is clear which locality label belongs with the specimen.

2. For pinned beetle specimens:

a. Note: if you are new to pinning, start with larger specimens as they are easier.  Keep specimens in ethanol until ready to pin.

b. Use No. 2 or No. 3 archival quality pins. Finer pins are too flexible and vibrate in the specimen, destroying them from within. Thicker pins damage specimens as they are inserted.

c. Place the specimen directly on the pinning block over the deepest hole. Hold the beetle in position it so that it is straight in your fingers and the legs are facing downward.

d. Insert the pin vertically through the top layer of the specimen's body (**Figure 41**).

   i. **Note: It is important to insert the pin in the correct location in the beetle's body**. A properly placed pin is positioned on the **RIGHT** side of the beetle (when viewed dorsally), halfway between the midline of the beetle's body and the edge where the elytra (wing shells) begin to round behind the thorax (see the "X" in **Figure 41**).

   ii. **Note:** Be sure to place the pin through the *right* side of the specimen
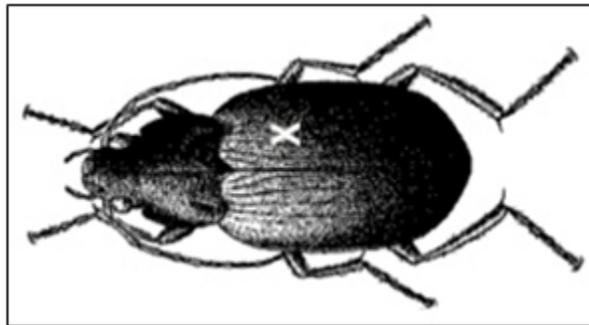


**Figure 41. Ground beetle pinning schematic showing where to place the pin (marked by an "X")**

e. Gently slide the pin through the body of the specimen. Push the pin slowly and with constant pressure.

f. Ensure that the pin is at a right angle to the beetle's body and push the specimen up the pin until its top surface is 15 mm from the top of the pin. This leaves enough space above the specimen for the pin to be handled and below for labels. Additionally, specimens at a uniform height are easier to examine and compare with one another. See **Figure 42** for examples of well-pinned and poorly-pinned specimens. A well-pinned specimen will have its legs positioned in such a way that the maximum number of features are clearly visible when dry. If time allows, care should be taken to move legs in position when pinning (especially for larger specimens over 25 mm) because legs are not easily repositioned when the specimen is dry on a pin. However, this is an optional step only to be undertaken if time and staff are available.
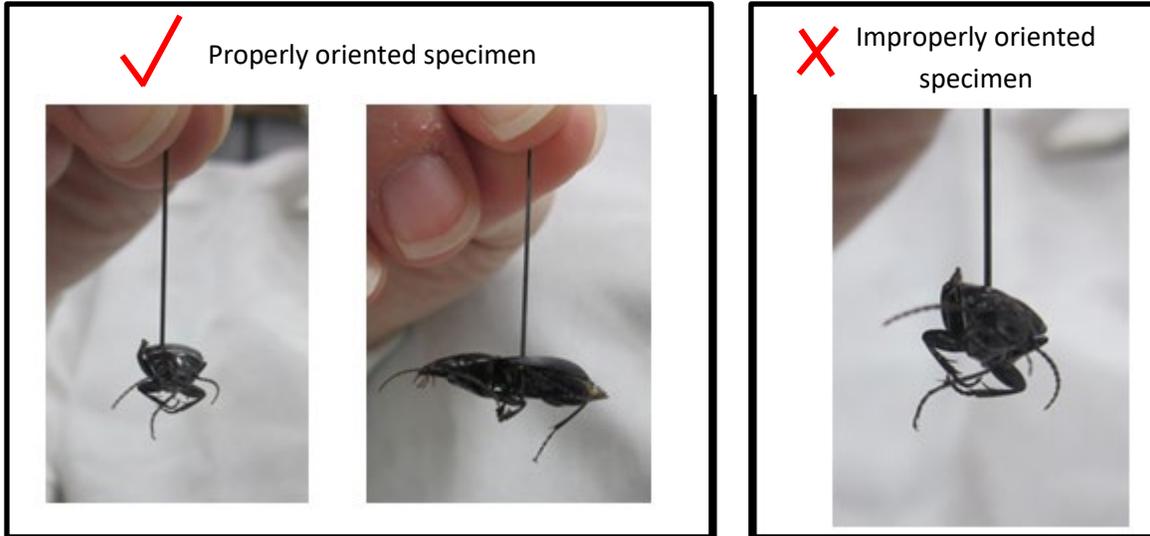
**Figure 42. Correctly and incorrectly pinned beetle specimens**

g. Slide the locality label (instructions in **APPENDIX J**) onto the pin below the specimen, and use the second-deepest hole in the pinning block to position the label at the correct height (**Figure 43**). Position the specimen in the middle of the locality label and with the head pointing to the left side. The specimen should be lined up with the long axis of the body parallel to the print on the label (**Figure 43**).

    i. **Note**: Now is a great time to ensure that the label information is correct.



**Figure 43. Attaching a locality label below a pinned ground beetle**

h. Attach the determination label (instructions in **APPENDIX K**) below the locality label. Use the third-deepest hole in the pinning block to position the label at the correct height. Orient the label in the same manner as the locality label.

i.  Attach the insect individual ID label (instructions in **APPENDIX L**) below the determination label using the fourth-deepest (shallowest) hole. Orient the label in the same manner as the locality label.

### G.6        Loss of Body Parts

1.  If a part of a specimen, such as a leg, falls off during pinning, save it and re-attach it to the beetle (provided you are certain which specimen the body part belongs to; **Figure 44**). To do this:

    a.  Hold the part being reattached with forceps.

    b.  Touch the top of the body part to a bit of glue.

    c.  Place the body part back into correct location and orientation and hold until the glue has set.



**Figure 44. A leg being inserted back into its socket, using forceps and a dab of glue**

### G.7        Sample Preservation

1.  Pinned or pointed ground beetle specimens are to be stored in airtight Schmitt boxes or in unit trays in Cornell cabinet drawers, with a small amount of moth crystals (≈6.5 g) in a cardboard fumigant box.

2.  After a specimen has been pinned or pointed, and any glue has dried, it should be placed in a box or tray so that locality labels are oriented in the same direction as those of the other specimens in the box or tray (

3.  Figure 45).

Heads of pinned specimens point toward the back of the box/tray

Heads of pointed specimens point toward the right side of the box/tray

**Figure 45. Proper orientation of mounted specimens in unit trays within a Cornell drawer.**

4. Specimens should be grouped into unit trays within each Cornell drawer or Schmitt box by species or morphospecies, leaving space to place specimens mounted at a later date/from different sampling bouts. If there are empty areas in the Cornell drawer, fill them with empty unit trays to keep those holding specimens from sliding around.

5. If there are not enough unit trays, containing specimens, to fill a Schmitt box or Cornell drawer, add empty unit trays so that filled trays are unable to slide around.

6. A fumigant box should be secured in the corner of one of the unit trays with pins, so that it does not slide around and damage specimens (**Figure 46**).



**Figure 46. Fumigant box filled with moth crystals and secured in the corner of a unit tray with pins.**

7. Specimen storage containers and workspaces should be kept clean to prevent pests. Dermestid beetles are common pests in insect collections. If you notice dermestid beetle activity, such as debris under a specimen or larval skins of dermestid beetles, freeze the drawer/box for two weeks and clean up the damage.

8. Check/replace moth crystals monthly.

9. Samples can be stored in this manner indefinitely.

10. Maintain fumigant in every drawer, including those without specimens. The fumigant acts as a repellent, but will not kill pests if they get into the collection.

11. Never leave boxes or drawers sitting out, put them away when you are not working on them.

12. Keep counters clean, always wipe down with ethanol before putting a box or drawer on a countertop.

13. Minimize amount of time lids are off drawers; never put drawers or lids on the floors.

14. Keep all dead insects away from light, e.g. lights and windowsills. If sticky traps are used to capture pests in/around the boxes or drawers, replace these traps frequently.

## SOP H  Laboratory Processing - Pooling samples

After all sorting and pinning data have been entered for a site, specimens of the same species (or type, in the case of invert bycatch) may be pooled from traps collected within the same plot and bout. Pooling is done to save on archiving costs and space, but all sorting and pinning data entry relies on reporting trap level data. For this reason, pooling within plot **may *not* be conducted** until <u>all</u> subsamples to be pooled have had their trap-level data entered into the data entry application 'BET: Sorting [PROD]'.

Individuals of a species are often unevenly distributed across the plot (i.e., East trap has 20 individuals of species A and West trap has 1 individual of species A) or may be present in just one trap. Vials from traps of a particular species (or in the case of invert bycatch, sample type) may only be pooled if pooling will actually result in fewer tubes to archive. Carabids and vertebrates may only be pooled within the same species and <u>only if the identification is to the species or subspecies level</u>. If invertebrate bycatch from each trap is substantial enough for a given plot that they cannot be pooled into a smaller number of tubes, pooling should not occur for that plot. If it is not possible to reduce the amount of materials used by pooling, then it is *desirable* to keep tubes filled with a single trap's contents.

If subsample vials are to be pooled, record associated data directly into the data entry application 'BET: Pooling [PROD]' or (if unavailable) on the pooling datasheet (RD [05]). Refer to the 'Manual for Fulcrum Application: TOS Ground Beetle Sampling [PROD]' for details on appropriate electronic data entry. Making a pooling record is only necessary if two subsamples are being placed together (i.e., don't make a pooled record for a single subsample). DO NOT POOL SUBSAMPLES FROM DIFFERENT PLOTS OR BOUTS. Pooling only occurs *within* a plot from same-taxon subsamples derived from the three traps collected during the same bout.

1. For each set of tubes to be pooled, make a record in the mobile data entry application (or if unavailable, on the paper sorting datasheet).

   a. Enter recordedBy

   b. Look up one of the subsamples to be pooled. This can be entered by scanning the barcode of a subsampleID or entering the sample identifier a subsampleID.

   c. Enter the processing date (date of pooling)

   d. If the subsamples have barcodes applied, scan the barcode of each sorted subsample to be pooled. Each sorted subsample will appear in the list of pooled subsamples. If barcodes are not present on one or more of the subsamples to be pooled, then provide the subsample type (e.g., invert bycatch, etc) and select the sorting record that corresponds to each subsample being pooled.

   e. Specify the archiveVialID tube number (1, 2, 3, etc)

i. The archiveVialID will be autogenerated and this should be written on the side of the vial of pooled specimens.

ii. Apply a barcode to the archiveVialID. Record the barcode label of associated with the archive vial into which all specimens have been placed. Take care to make sure that there is congruence between the physical labels and electronic record.

Example: If barcode 'A00000000001' is adhered to a vial containing archiveVialID CPER_001. 20171031.PASELO.01, then the barcode needs to be scanned within the record for CPER_001.20171031.PASELO.01 not CPER_001.20171031.PASELO.**02** or CPER_001. 20171031.**PEMA**.01.

f. On the paper datasheet (to be used only when the unusual circumstance where the mobile application is not available):

i. Enter plotID and collection date

ii. Circle the trapIDs of the tubes to be pooled

iii. The taxonID in the 'taxon ID' field

iv. The tube numbers (.01, .02, etc) for each subsampleID (corresponding to trapID on the same line)

v. The archiveVialID

g. All other data important taxonomy metadata is filled out (e.g., date of identification, identification references, etc.) in the sorting data.

2. As each subsample is added to the digital record, that subsample is added to the physical archive vial.

3. For each new vial used (for a particular plotID/collectDate/taxonID combination) increase the tubeNumber by 01. The tube number listed in the 'archiveVialID' should indicate the final destination of the specimen.

4. Fill each tube with enough 95% ethanol that there is at least 1/2 inch of ethanol above the fully submerged specimen(s). If there are too many specimens to allow for 1/2 inch of ethanol above the level of the specimens, split the specimens into multiple tubes to ensure that there is 1/2 inch or more of ethanol above the specimens in each tube. Each pooled tube requires its own record, but pooling is not required if it does not result in a saving of materials.

5. Include a locality and determination label inside the archive vial from each of the subsamples that were included in the pool. Externally write the archiveVialID in the format

**plotID.collectDate.taxonID.tubeNumber** on an ethanol-safe label placed on the outside of the vial in archival ink.
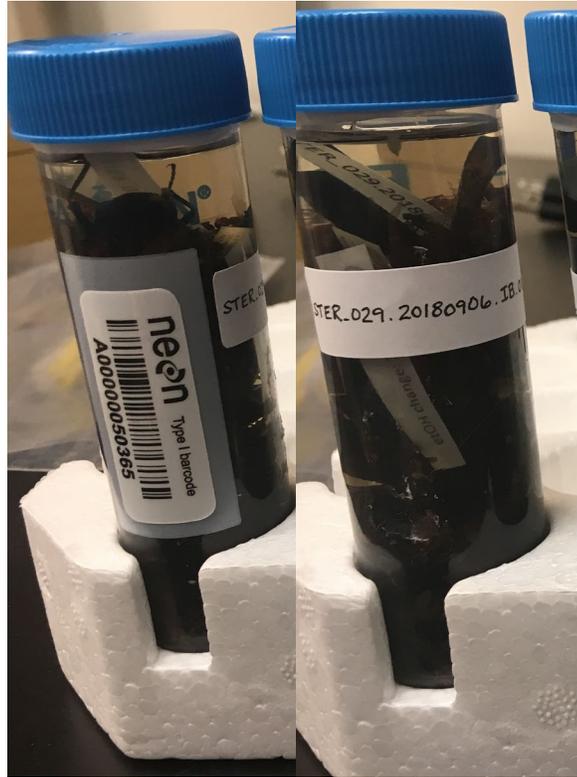


**Figure 47. Pooled invertebrate bycatch. Samples have a barcode and external sample identifier (STER_029.20180906.IB.01). A locality label from each subsample should be included in the vial.**

## SOP I        Data Entry and Verification

The importance of thorough, accurate data transcription cannot be overstated; the value of the efforts in the field is only manifested once the data are properly entered for delivery to NEON's end users. Mobile applications are the preferred mechanism for data entry. Data should be entered into the protocol-specific application as they are being collected, whenever possible, to minimize data transcription and improve data quality. For detailed instructions on protocol specific data entry into mobile devices, see the NEON Internal Sampling Support Library (SSL). Mobile devices should be synced at the end of each field day, where possible; alternatively, devices should be synced immediately upon return to the Domain Support Facility.

However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times. As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable).  Vertebrate sorting takes place within 24 hours of trap collection, thus digital transcription should happen within 14 days of collection. Invertebrate bycatch may not be processed until the end of the field season; digital data transcription of *invertebrate* bycatch is therefore not required until 14 days after <u>sorting</u> of invertebrates occurs.

 See RD[04] for complete instructions regarding manual data transcription.

If paper datasheets are used, the procedure is as follows:

1. Enter data from field datasheets and the number of vials generated from sample processing into the pertinent Beetle mobile application, according to instructions in the NEON Protocol and Procedure: Manual Data Transcription (RD[04]).

2. Scan datasheets and save in PDF file format.

3. Save paper copy of datasheets.

Before entering data, all personnel must read RD[04] for complete instructions regarding manual data transcription. Prior to entering data via the mobile application, each technician shall enter a plot (or subplot) of data from one bout into the protocol-specific mobile data application housed on the Training portal, as described in RD[04].

Be sure to enter data for all plots within a bout that were visited even if traps were not set as scheduled, due to unforeseen circumstances. However, if an entire bout is missed then no data need to be entered.

*Quality Assurance*

Data Quality Assurance (QA) is an important part of data collection and ensures that all data regarding observations and samples are accurate and complete. This protocol requires that certain QA checks be conducted in the field (i.e., before a field team leaves a plot or site), while others can be conducted at a later date in the office (typically within a week of collection). Field QA procedures are designed to prevent the occurrence of invalid data values that cannot be corrected at a later time, and to ensure that data and/or sample sets are complete before a sampling window closes. Incomplete data and/or sample sets cannot be supplemented by subsequent sampling efforts if the sampling window has closed. Invalid meta-data(e.g. collection dates, plotIDs) are difficult to correct when field crews are no longer at a sampling location. Office QA procedures are meant to ensure that sampling activities are **consistent** across bouts, that sampling has been carried out to **completion**, and that activities are occurring in a **timely** manner. The Office QA will also assess duplicative data to maintain data **validity** and **integrity**.

All QA measures needed for this protocol are described in the Data Management Protocol (RD[11]) and included in the QAQC Checklist in Appendix C.

*Sample Labels & Identifiers*

By default each sample or subsample produced by this protocol is assigned a human-readable sample identifier which contains information about the location, date, and/or taxonomy of the collected sample. Each sample may also be associated with a scannable barcode, which will not contain information specific to sample provenance, but will reduce transcription errors associated with writing sample identifiers by hand.

If available, adhesive barcode labels should be applied to dry, room temperature containers in advance of their use in the field (at least 30 minutes prior, but may be applied at the start of the season). Barcodes are unique, but are not initially associated with a particular sample, it is encouraged to make these up in advance. Use the appropriate barcode label type with each container (i.e., cryo-safe barcode labels only used for samples that are stored at -80°C, etc).

Barcodes are scanned into the mobile application when the sample is placed into the container; only one barcode may be associated with a particular sample. Do not reuse barcodes. If a barcode is associated with multiple samples, the data ingest system will throw an error and refuse to pull in entered data. Thus, one barcode must always be associated with one container (sampleID, subsampleID or archiveVialID).

**Data and sample IDs must be entered digitally and quality checked prior to shipping samples to an external lab.**

**SOP J       Sample Shipment**

Select beetle specimens will be shipped to a taxonomic facility for professional identification to the species level and/or verification of identifications made at the domain facility. Those not selected for shipment to a taxonomist should continue to be stored in the domain facility, many of them in a teaching collection, until they can be shipped to a long term storage facility. Specimens are to be shipped to taxonomists only after they have been pinned or pointed, and identified as thoroughly as possible by domain parataxonomists. Specimens **MUST**, however, be sent out *no later than* the 1$^{st}$ of February in the year following the field season in which the specimens were collected. This is due to the time required for the taxonomist to receive and process samples before sending them back, and the need for domain lab teaching collections to be updated and identification verifications completed prior to processing the next years' specimens.

Information included in this SOP conveys science-based packaging, shipping, and handling requirements, not lab-specific or logistical demands. For that information, reference the NEON CLA Intranet site (available through the sampling support library) and the Domain Chemical Hygiene Plan and Biosafety Manual (AD[03]).

**J.1       Choosing specimens for shipment**

Each year, up to 260 ground beetle specimens *per site* may be sent to a taxonomist for identification. Specimens must be intact, as a badly damaged specimen (e.g., one with a missing head) is not identifiable, even by experts. Specimens should be preferentially selected for shipment to a taxonomist based on the following criteria, and in the following order:

1. At least five (if available), and up to 20% of all captured individuals of species that have not been identified to species by domain staff (i.e., morphospecies, specimens with identification qualifiers of 'cf. species'). These specimens need to be in good condition, as stated above.
2. At least five (if available), and up to 20% of all uncommon species (≤ 20 individuals captured, combined and across all plots, over the course of the field season).
3. At least five (if available), and up to 10% of captured individuals from the list of taxa that are difficult to identify (as determined by domain staff).
4. Up to 10 specimens from species that are <u>not</u> represented in the teaching collection.
5. Additional specimens from species that are represented by fewer than 10 individuals in the teaching collection (enough individuals to bring the total in the teaching collection to 10).
6. At least five, and up to 10% of individuals of species that have been identified in the domain facility, are commonly encountered, and for which 10 individuals are already present in the teaching collection.

*Note - Individuals selected should represent spatial (e.g., from the entire range of plots sampled), temporal (e.g., from all sampling bouts throughout the field season), and morphological variation*

Use the pinning data (downloaded from data entry application) to create a "pick list" of specimens to be shipped to taxonomists. This can be done by sorting the sheet by scientificName (or taxonID) or morphospeciesID, and sampleID, and choosing a variety of specimens that represent samples from all plots and times of collection.

If all specimens meeting the criteria above have been sent to a taxonomist and the limit of 260 total individuals per site has not been met, a problem ticket should be issued to the Insect Ecologist so that budgets can be properly allocated between domains with low versus high capture rates. In future revisions of this protocol, the maximum number of specimens to send to the taxonomist may be adjusted as teaching collections become more complete.

### J.2 Preparing for shipment

1. No hazardous materials or chemical substances will be included in this shipment. The fumigant box containing moth crystals (paradichlorobenzene) shall be **REMOVED** prior to shipment. In addition, failure to remove the fumigant box can result in serious damage to the specimens (unsecured box will bump and <u>destroy</u> specimens).

2. **Supplies/Containers:  See Table 8 for a complete list of shipping materials.**

3. Timelines: Beetles must be sent no later than the 1st of February in the year following collection, although more expedited shipment is recommended as other steps in the ground beetle processing workflow are contingent upon the samples being returned to the domain facility. Prior to shipment, dried, pinned/pointed specimens may be stored in an air tight container with paradichlorobenzene (<u>to be removed prior to shipping</u>) without detriment to the integrity of the specimens.

### J.3 Creating a shipping manifest

1. Navigate to the "Shipping Information for External Facilities" document on [CLA's NEON intranet site](). Check whether there are items such as permits or cover letters required to include in the shipment. *Check this document often as instructions are subject to change.*

2. Print out required documents (if needed) to include in shipment box.

3. Prepare a shipping inventory detailing the contents of the shipment, using the Shipment Creation and Shipment Review applications. Include a printed copy of the inventory in the shipment box (downloaded from the Stork Shipment Verification Tool).

   Complete packing slip, address shipment, and ship <u>ground</u> to the destination(s) specified in the CLA "Shipping Information for External Facilities" document.

**J.4        Packaging specimens for shipment**

1. Ship prepared dry (pinned/pointed) beetle specimens to the taxonomic ID facility with a hard copy of the Taxonomy Shipping Manifest (created using the Stork Shipment Verification Tool).  Email a digital copy of Taxonomy Shipping Manifest to the taxonomy lab and NEON's CLA contact by submitting the shipment in the Stork Shipment Verification Tool.
2. Place specimens in a mailer box so that locality labels are oriented in the same direction and the specimens are neatly aligned (**Figure 48**)



**Figure 48. Specimens prepared for shipping**

3. Large beetles (typically 1.5 cm or larger, but this step may also be required for smaller specimens with heavy bodies) must be brace-pinned on either side of the label to prevent them from spinning on their pin during transit (**Figure 49**).

**Figure 49. Large beetle that is brace-pinned for shipment**

4.  When the mailers are ready for shipping, take a photo of each box (with specimens visible) for tracking purposes (**Figure 48**). Save these photos to the domain's local drives for future reference.

5.  **Do NOT include moth crystals in shipments.**

6.  Examine specimens before sealing the box. Make sure all specimens are appropriately labeled. Ensure that all of the specimens are firmly glued to their points by gently tapping the box to test. A specimen dissociated from its locality label is worthless. Finally, make sure that all pins with specimens are as vertical as possible.

7.  Place four pins (#3 size) in the four corners of the specimen mailer, and four more at the midpoint of each of the four sides. These pins will support the cover board (cardboard insert).

8.  Cut a cardboard insert to size to place on top of specimens (resting on pins). This cover board should fit PRECISELY inside the box with a snug fit. The cover board will contact ALL pins in the box. Make a packing tape tab to easily pull off the cardboard insert (otherwise it can become wedged in the box and difficult to remove; **Figure 50**). The cardboard insert must fit exactly to the dimensions of the box because a slightly smaller insert will chatter over the pin heads and ultimately become a source of specimen breakage during shipment.

9.  Fill in any additional head space between the cover board and the lid of the specimen mailer with packing or bubble-foam so tightly that it slightly bulges the top of the box when filled.

10. Secure the lid of the box with a rubber band, tape, or other method that will firmly push the lid downward onto the cover board. Gently break off the metal tabs that come as part of the box. The secondary securement backs up the metal tabs which are prone to break off after

several uses. If they break during shipment, they are prone to rattling around among the specimens.



**Figure 50. Cardboard insert that serves as extra protection for pinned/pointed specimens, and helps fills dead space between specimens and mailer lid.**

11. Ship prepared dry material in cardboard mailers inside of larger cardboard boxes with ample packing material to ensure that the specimens are not jostled (**Figure 51** -**Figure 52**).



**Figure 51. A mailer placed inside a larger box for shipping**

**Figure 52. Mailer within a shipping box, with adequate padding to keep mailer from moving in transit**

12. Outer boxes should be clearly labeled with 'Fragile' and 'Dead Insects for Scientific Study'.

### J.5        Creating shipping inventory

Whenever samples are shipped, they must be accompanied by a hard-copy Taxonomy Shipping Manifest enclosed within the shipping container. In addition, a corresponding electronic version of the Taxonomy Shipping Manifest (csv file) must be emailed to the taxonomic ID facility and NEON's CLA contact using the Stork Shipment Verification Tool as soon as possible after the samples have been shipped. For locations to which to ship beetle specimens, and CLA contract information, please reference CLA's NEON intranet site, available through the sampling support library.

### J.6        Laboratory contact information and shipping/receipt days

See CLA's NEON intranet site, available through the sampling support library.

### J.7        Return of shipping materials

Taxonomists will return ONLY specimens that are to be added to the domain lab teaching collection if there is a return shipping label in the original shipment. The format for this is found on CLA's NEON intranet site, available through the sampling support library.

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ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 16, 2018. Subset obtained for MOD13Q1 product at 17.9695N,66.8687W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [GUAN]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 17, 2018. Subset obtained for MOD13Q1 product at 42.5369N,72.1726W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [HARV]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 15, 2018. Subset obtained for MOD13Q1 product at 63.8756N,149.2133W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [HEAL]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 16, 2018. Subset obtained for MOD13Q1 product at 31.1948N,84.4686W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [JERC]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 15, 2018. Subset obtained for MOD13Q1 product at 32.5906N,106.8425W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [JORN]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 16, 2018. Subset obtained for MOD13Q1 product at 39.1104N,96.6129W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [KONA]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 16, 2018. Subset obtained for MOD13Q1 product at 39.1007N,96.563W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [KONZ]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 16, 2018. Subset obtained for MOD13Q1 product at 18.0212N,67.0769W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [LAJA]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 15, 2018. Subset obtained for MOD13Q1 product at 31.8538N,88.1612W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [LENO]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 17, 2018. Subset obtained for MOD13Q1 product at 37.3782N,80.5248W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [MLBS]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 15, 2018. Subset obtained for MOD13Q1

product at 38.2483N,109.3882W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [MOAB]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 15, 2018. Subset obtained for MOD13Q1 product at 40.0542N,105.5823W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [NIWO]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 17, 2018. Subset obtained for MOD13Q1 product at 46.7697N,100.9153W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [NOGP]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 15, 2018. Subset obtained for MOD13Q1 product at 35.4105N,99.0587W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [OAES]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 15, 2018. Subset obtained for MOD13Q1 product at 40.1775N,112.4524W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [ONAQ]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 17, 2018. Subset obtained for MOD13Q1 product at 35.9641N,84.2826W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [ORNL]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 16, 2018. Subset obtained for MOD13Q1 product at 29.6892N,81.9934W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [OSBS]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 15, 2018. Subset obtained for MOD13Q1 product at 19.553N,155.3173W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [PUUM]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 16, 2018. Subset obtained for MOD13Q1 product at 40.2759N,105.5459W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [RMNP]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 16, 2018. Subset obtained for MOD13Q1 product at 38.8929N,78.1395W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [SCBI]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 16, 2018. Subset obtained for MOD13Q1 product at 38.89N,76.56W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [SERC]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 15, 2018. Subset obtained for MOD13Q1 product at 37.1087N,119.7322W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [SJER]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 15, 2018. Subset obtained for MOD13Q1 product at 37.0333N,119.2621W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [SOAP]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 15, 2018. Subset obtained for MOD13Q1 product at 31.9106N,110.8354W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [SRER]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 20, 2018. Subset obtained for MOD13Q1 product at 45.5089N,89.5863W, time period: 2005-01-01 to 2014-12-31, and subset size: 2.25 x 2.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [STEI]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 16, 2018. Subset obtained for MOD13Q1 product at 40.4619N,103.0293W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [STER]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 17, 2018. Subset obtained for MOD13Q1 product at 32.9504N,87.3932W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [TALL]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 15, 2018. Subset obtained for MOD13Q1

product at 37.0058N,119.006W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [TEAK]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 15, 2018. Subset obtained for MOD13Q1 product at 68.661N,149.3704W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [TOOL]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 16, 2018. Subset obtained for MOD13Q1 product at 45.4936N,89.5857W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [TREE]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 17, 2018. Subset obtained for MOD13Q1 product at 39.0404N,95.1921W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [UKFS]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 16, 2018. Subset obtained for MOD13Q1 product at 46.2338N,89.5372W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [UNDE]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 17, 2018. Subset obtained for MOD13Q1 product at 47.1282N,99.2413W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [WOOD]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 15, 2018. Subset obtained for MOD13Q1 product at 45.8204N,121.9519W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [WREF]

ORNL DAAC. 2018. MODIS and VIIRS Land Products Global Subsetting and Visualization Tool. ORNL DAAC, Oak Ridge, Tennessee, USA. Accessed August 15, 2018. Subset obtained for MOD13Q1 product at 44.9534N,110.5391W, time period: 2005-01-01 to 2014-12-31, and subset size: 10.25 x 10.25 km. https://doi.org/10.3334/ORNLDAAC/1379 [YELL]

## APPENDIX A    Datasheets

The following datasheets are associated with this protocol:

**Table 13. Datasheets associated with this protocol.**

| NEON Doc. # | Title |
|---|---|
| NEON.DOC.001580 | Datasheets for TOS Protocol and Procedure: Ground Beetle Sampling |

These datasheets can be found in Agile or the NEON Document Warehouse.

**APPENDIX B    Quick References**

**Part 1: Beetle Workflow**

# Quick Reference: Getting Ready for Field Sampling

**STEP 1** – Charge all electronic devices (e.g., GPS units, mobile data entry device)

**STEP 2** – Upload waypoints into GPS or get maps of trap locations

**STEP 3** – Print locality labels

**STEP 4** – Prepare chemicals

**STEP 5** – Pack extra materials: pitfall trap parts, chemicals, storage container

**Properly installed pitfall trap has:**

- Lip of top cup flush with ground

- No gap between top and bottom cups

- No gap between bottom cup and ground

- Minimal disturbance of surrounding vegetation

**Adjust height of lid as needed, to ensure that:**

- Ground beetles can enter

- Precipitation cannot get into traps and dilute PG solution

- Lid provides shade, slowing evaporation and decomposition of captured specimens



Properly installed pitfall trap. Lip of *top container* is flush with the ground



Improperly installed pitfall trap. Lip of top container is above the ground surface

# Quick Reference: Collecting Insects from Trap

**STEP 1** – Record date and trapID on three locality labels

**STEP 2** – Place three locality labels in top container

**STEP 3** – Remove top container

**STEP 4** – Filter contents over waste container

**STEP 5** – Rinse sample through filter with distilled or deionized water

**STEP 6** – Rinse sample through filter with 95% ethanol

**STEP 7** – Transfer **ALL** organisms and locality labels to Whirl-Pak bag, fill with 95% ethanol until all contents are submerged.

**STEP 8** – Rinse filter apparatus with ethanol, over Whirl-Pak bag, to ensure that no organisms are left behind.

**STEP 9** – Place mesh filter into Whirl-Pak bag (with specimens and locality labels)

**STEP 10** – Record all metadata and any irregularities on datasheet.

**STEP 11** – Scan the barcode into the electronic record

[**ALTERNATIVELY**, complete steps 1-3 and 11. Place contents of trap into Whirl-Pak. Reset trap, complete steps 4-9 at the car on all 30 traps in series]



Rinsing filter with ethanol, over Whirl-Pak, to ensure no specimens are left behind

# Quick Reference: Initial Processing and Bycatch Removal

**Ethanol Rinse (within 24 hours of sample collection)**

**STEP 1** – Filter contents of Whirl-Pak bag (specimens, labels, and mesh filter), discard ethanol waste, and record date of ethanol rinse on sorting datasheet

**STEP 2** – Remove vertebrate bycatch, record relevant information in the mobile data entry application (or if unavailable, on sorting datasheet), and store vertebrate bycatch in 50 mL tubes with locality and determination labels. Fill tubes with enough 95% ethanol to submerge their contents.

**STEP 3** – Place vertebrate bycatch in labeled 50 mL tubes, with locality and determination labels. Fill tubes with enough 95% ethanol to submerge their contents. (Be sure to associate a barcode with each subsampleID by scanning it into the record)

**STEP 4** – Transfer trap contents (specimens, labels, mesh filter) back into original Whir-Pak bag

**STEP 5** – Fill Whirl-Pak bag with enough 95% ethanol to completely cover its contents, and seal the bag (leaving as little air space as possible)

**STEP 6** – Store: 1) 50 mL tubes and 2) Whirl-Pak bags from the same sampling bout (in an airtight container)

**Sorting Ground Beetles from Invertebrate Bycatch**

**STEP 1** – Transfer contents of Whirl-Pak bag into 'sorting' dish

**STEP 2** – Rinse mesh filter with 95% ethanol, over sorting dish; discard mesh filter when clean

**STEP 3** – Sort specimens into two temporary containers, one marked 'invertebrate bycatch' and the other marked 'carabids'. Make sure each container contains locality labels and enough 95% ethanol to keep its contents submerged

**STEP 4** – Place invertebrate bycatch in labeled 50 mL tubes, with locality labels (Be sure to associate a barcode with each subsampleID by scanning it into the record)

**STEP 5** – Return carabid beetles to the original Whirl-Pak bag until ready for sorting, pinning and identification.

**APPENDIX C       Reminders and Checklists**
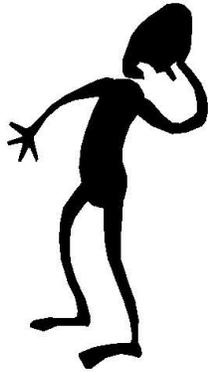
Avoid looking like this guy

Make sure you have everything needed for field sampling (including extras of just about everything)

Gather supplies and prepare chemicals at least one day before the beginning of a sampling bout

# <u>Checklist: Preparing for Field Sampling</u>

**Locality labels: Be sure to…**

☐   Print labels with correct location information

☐   Cut labels into strips

**Equipment: Do you have…**

☐   Trap replacement parts

☐   Ice packs in the cooler

☐   Extra chemicals (PG and 95% ethanol)

☐   Distilled or deionized water

☐   Map and coordinates uploaded onto GPS

☐   Ground beetle data sheets and mobile data entry device

# Checklist: Collecting Quality Samples

**Sample collection: Be sure to…**

☐ Use waste containers to catch PG solution

☐ Double check that your actual location matches the one on the locality label

     o plotID & trapID are written on the trap cover

☐ Remove twigs, leaves and debris from trap

☐ Rinse the sample – first with distilled or deionized water, then with 95% ethanol

☐ Rinse the filter apparatus with 95% ethanol to ensure that all beetles and bycatch are transferred to the Whirl-Pak bag

☐ Check Whirl-Pak bag for locality labels and mesh filter

☐ Record all metadata (plotID, date, field staff, barcode etc.) on the datasheet or mobile data entry device

☐ Record any irregularities or deviations from procedure that may impact data, e.g., trap damage, flooding in the area, alterations to the trap cover, etc.

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**Before leaving trap: Check that…**

☐ Top cup is flush with ground (use a mirror!)

☐ There is no gap between the trap cups and the ground

☐ There is no gap between the bottom and top cups

☐ Lid spacers are in place

☐ There is a 1.5 cm gap between the lid and the ground

---

**Transporting samples: Make sure…**

☐ Whirl-Pak and Ziploc bags are sealed and upright

☐ Cooler is out of direct sunlight and away from extreme temperatures

☐ Cooler is secured in vehicle, so that if cannot tip over during driving

☐ Samples are transferred promptly to the laboratory upon return from the field

# Checklist: Processing Specimens in the Lab

**Ethanol rinse: Check that…**

☐ Ethanol rinse is completed within 24 hours of sample collection

☐ No specimens were lost during filtering

☐ Date of rinse is recorded in the sorting datasheet or mobile data entry application

☐ Locality labels are with specimens (in Whirl-Pak bag)

☐ Specimens are stored in airtight containers so the ethanol does not leak out

**Sorting beetles and bycatch: Be sure to…**

☐ Only work with one Whirl-Pak bag at a time

☐ Examine Whirl-Pak bags, mesh filters, and sorting dishes under a microscope, to check for tiny organisms

☐ Ask for a second opinion, use reference collections, and refer to species/photo lists if unsure whether an insect is a ground beetle. Still unsure? Call it a ground beetle for now

☐ Keep specimens and associated locality labels together at all times and create new locality labels (with date and trapID) as needed

☐ Place locality labels (with collection date and trapID) in each 50 mL tube and/or jar, and any temporary container used while working

☐ Completely cover all organisms with 95% ethanol at all times, and use multiple storage tubes as needed

☐ Record sorter's name and the date of the sorting event (processingDate) on the sorting datasheet or in the appropriate mobile data entry application

### APPENDIX D    Estimated Dates for Onset and Cessation of Sampling

The dates in **Table 14** are based on MODIS (Moderate Resolution Imaging Spectroradiometer) EVI phenology data from NASA records from the most recently available decade (2005-2014). The season is bounded by increasing green-up as the start date and the mid-point between decreasing greenness and minimum greenness as the end date. If sites experience two peak greens, the start date is based on the first cycle of greening and the end date is based on the second cycle. Estimates for the start and stop dates of sampling are provided for each site.

These dates are <u>estimates</u> and local conditions may vary. If the listed start date passes and temperatures remain persistently below 4 °C, then the start of the sampling season should be delayed until temperatures rise above that threshold. If temperatures fall persistently below the 4 °C threshold, the sampling season may be concluded in advance of the estimated end date. If initiating or completing sampling at a site differs by a more than one month from the listed estimated dates below, issue a problem ticket on Service Now before executing trap deployment or final trap removal.

Note: MODIS data are of limited utility for tropical sites (i.e., D04, D20). For these locations, a six-month window of sampling has been selected based on patterns of precipitation at the site.

**Table 14. Estimated seasonal start and end dates based on patterns of historical 'green-up'**

| Domain | Site | Start | End | Bouts Expected |
|---|---|---|---|---|
| 1 | BART | 29-Apr | 16-Sep | 10 |
| 1 | HARV | 23-Apr | 20-Sep | 11 |
| 2 | BLAN | 22-Mar | 19-Sep | 13 |
| 2 | SCBI | 28-Mar | 27-Sep | 13 |
| 2 | SERC | 18-Mar | 1-Oct | 14 |
| 3 | DSNY | 5-Mar | 17-Sep | 14 |
| 3 | JERC | 24-Mar | 17-Sep | 13 |
| 3 | OSBS | 6-Mar | 19-Sep | 14 |
| 4 | GUAN* | 14-Apr | 13-Oct | 13 |

| 4 | LAJA* | 14-Apr | 13-Oct | 13 |
|---|---|---|---|---|
| 5 | STEI | 30-Apr | 10-Sep | 10 |
| 5 | TREE | 28-Apr | 12-Sep | 10 |
| 5 | UNDE | 1-May | 9-Sep | 9 |
| 6 | KONA | 4-Apr | 16-Sep | 12 |
| 6 | KONZ | 3-Apr | 17-Sep | 12 |
| 6 | UKFS | 23-Mar | 28-Sep | 14 |
| 7 | GRSM | 3-Apr | 20-Sep | 12 |
| 7 | MLBS | 18-Apr | 23-Sep | 11 |
| 7 | ORNL | 18-Mar | 23-Sep | 14 |
| 8 | LENO | 10-Mar | 26-Sep | 14 |
| 8 | DELA | 2-Mar | 16-Sep | 14 |
| 8 | TALL | 17-Mar | 24-Sep | 14 |
| 9 | DCFS | 1-May | 4-Sep | 9 |
| 9 | NOGP | 19-Apr | 2-Sep | 10 |
| 9 | WOOD | 6-May | 6-Sep | 9 |
| 10 | CPER | 30-Mar | 12-Oct | 14 |
| 10 | RMNP | 10-May | 7-Sep | 9 |
| 10 | STER | 28-Mar | 9-Aug | 10 |
| 11 | CLBJ | 28-Feb | 6-Oct | 16 |
| 11 | OAES | 10-Mar | 25-Nov | 19 |
| 12 | YELL | 6-May | 15-Aug | 7 |

| 13 | MOAB | 16-Mar | 8-Oct | 15 |
|---|---|---|---|---|
| 13 | NIWO | 31-May | 2-Sep | 7 |
| 14 | JORN | 22-Mar | 10-Oct | 14 |
| 14 | SRER | 2-Mar | 10-Oct | 16 |
| 15 | ONAQ | 18-Mar | 29-Jul | 10 |
| 16 | ABBY | 19-Apr | 6-Sep | 10 |
| 16 | WREF | 22-Apr | 8-Sep | 10 |
| 17 | SJER | 8-Oct | 6-May | 15 |
| 17 | SOAP | 31-Mar | 10-Sep | 12 |
| 17 | TEAK | 5-May | 3-Sep | 9 |
| 18 | BARR | 27-Jun | 4-Aug | 3 |
| 18 | TOOL | 7-Jun | 11-Aug | 5 |
| 19 | BONA | 14-May | 17-Aug | 7 |
| 19 | HEAL | 19-May | 18-Aug | 6 |
| 19 | DEJU | 13-May | 19-Aug | 7 |
| 20 | PUUM* | 13-Oct | 14-Apr | 13 |

\* sites where precipitation data were used in lieu of MODIS data

**APPENDIX E    Site Specific List of Easy to Identify Ground Beetle Species**

Lists of easily identified ground beetle species will be available after the first year of sampling and expert review within each domain. Species will be **added** to this list on an annual basis when: 1) 20 or more individuals identified to a particular species by NEON parataxonomists are reviewed by an expert taxonomist and 2) NEON staff have a 95% accuracy rate (or better) compared to the expert. Species will be **removed** from this list on an annual basis for species where accuracy rates fall below the 95% threshold for any number of specimens verified from that species. For each species, a site list is provided with locations from which each species was found by NEON staff and verified by a taxonomic expert.

Because NEON seeks high accuracy at the species-level, specimens will be considered 'correctly identified' when the species-level identification match even if the expert taxonomist can resolve the specimen to the subspecies level or the parataxonomists incorrectly assigned a subspecies identification. For the purposes of parataxonomists identification, any specimen identified to subspecies may consider that subspecies as 'easily identified' if the taxon is named at the species-level in the following tables (and is not explicitly called out as difficult to identify in Appendix F). Any discrepancies in identifications to subspecies will be remedied in future field seasons through updates made to training materials and teaching collections.

**E.1    DOMAIN 1**

Table 15. D01 easily identified ground beetle species. Based (in part) on 2017 identifications.

| Site | Species |
|---|---|
| BART\|HARV | *Agonum retractum* |
| HARV | *Carabus goryi* |
| HARV | *Carabus serratus* |
| BART\|HARV | *Cymindis cribricollis* |
| BART\|HARV | *Cymindis neglecta* |
| BART\|HARV | *Notiophilus aeneus* |
| BART\|HARV | *Pterostichus adoxus* |
| BART\|HARV | *Pterostichus coracinus* |
| BART | *Pterostichus lachrymosus* |
| BART\|HARV | *Pterostichus pensylvanicus* |
| BART\|HARV | *Pterostichus rostratus* |
| BART\|HARV | *Pterostichus tristis* |
| BART\|HARV | *Sphaeroderus canadensis* |
| BART\|HARV | *Sphaeroderus stenostomus* |
| BART\|HARV | *Synuchus impunctatus* |

### E.2    DOMAIN 2

**Table 16. D02 easily identified ground beetle species. Based (in part) on 2017 identifications.**

| Site | Species |
|---|---|
| SERC | *Amara aenea* |
| BLAN\|SCBI | *Anisodactylus dulcicollis* |
| BLAN\|SCBI | *Anisodactylus ovularis* |
| BLAN\|SCBI\|SERC | *Anisodactylus rusticus* |
| BLAN\|SCBI | *Bembidion pedicellatum* |
| BLAN\|SCBI | *Calathus gregarius* |
| BLAN\|SCBI\|SERC | *Chlaenius aestivus* |
| BLAN\|SCBI\|SERC | *Chlaenius emarginatus* |
| BLAN\|SERC | *Chlaenius tomentosus* |
| BLAN\|SCBI | *Cyclotrachelus furtivus* |
| BLAN\|SCBI\|SERC | *Cyclotrachelus sigillatus* |
| BLAN\|SCBI\|SERC | *Dicaelus dilatatus* |
| BLAN\|SCBI\|SERC | *Dicaelus furvus* |
| SERC | *Elaphropus incurvus* |
| SERC | *Elaphropus xanthopus* |
| BLAN\|SCBI\|SERC | *Galerita bicolor* |
| BLAN\|SCBI\|SERC | *Harpalus pensylvanicus* |
| BLAN\|SCBI\|SERC | *Notiophilus aeneus* |
| SERC | *Poecilus chalcites* |
| BLAN\|SCBI\|SERC | *Poecilus lucublandus* |
| SERC | *Polyderis laeva* |
| SCBI | *Pterostichus coracinus* |
| BLAN | *Pterostichus permundus* |
| BLAN\|SCBI\|SERC | *Pterostichus stygicus* |
| BLAN | *Pterostichus trinarius* |
| BLAN\|SCBI\|SERC | *Scarites subterraneus* |
| BLAN\|SCBI\|SERC | *Trichotichnus autumnalis* |
| BLAN\|SCBI\|SERC | *Trichotichnus fulgens* |

### E.3    DOMAIN 3

**Table 17. D03 easily identified ground beetle species. Based (in part) on 2016 identifications.**

| Site | Species |
|---|---|
| OSBS | *Pasimachus marginatus* |
| DSNY\|OSBS | *Pasimachus strenuus* |

| JERC\|OSBS | *Pasimachus subsulcatus* |
|---|---|

## E.4    DOMAIN 5

**Table 18. D05 easily identified ground beetle species. Based (in part) on 2017 identifications.**

| Site | Species |
|---|---|
| STEI\|TREE\|UNDE | *Agonum gratiosum* |
| TREE | *Carabus maeander* |
| STEI\|TREE\|UNDE | *Carabus nemoralis* |
| STEI\|TREE\|UNDE | *Cymindis cribricollis* |
| STEI\|TREE | *Harpalus providens* |
| STEI\|TREE\|UNDE | *Platynus decentis* |
| TREE\|UNDE | *Platynus mannerheimii* |
| STEI\|TREE\|UNDE | *Pterostichus melanarius* |
| STEI | *Pterostichus novus* |
| STEI\|TREE\|UNDE | *Pterostichus pensylvanicus* |
| STEI\|TREE\|UNDE | *Sphaeroderus stenostomus* |
| STEI\|TREE\|UNDE | *Synuchus impunctatus* |

## E.5    DOMAIN 6

**Table 19. D06 easily identified ground beetle species. Based (in part) on 2017 identifications.**

| Site | Species |
|---|---|
| KONZ\|UKFS | *Brachinus alternans* |
| KONA\|KONZ\|UKFS | *Cyclotrachelus sodalis* |
| KONZ | *Cyclotrachelus substriatus* |
| KONZ\|UKFS | *Dicaelus elongatus* |
| UKFS | *Dicaelus purpuratus* |
| UKFS | *Dicaelus sculptilis* |
| KONZ\|UKFS | *Galerita atripes* |
| KONA\|KONZ\|UKFS | *Harpalus caliginosus* |
| KONZ\|UKFS | *Poecilus lucublandus* |
| KONA\|KONZ\|UKFS | *Pterostichus permundus* |
| KONZ\|UKFS | *Tetracha virginica* |

## E.6    DOMAIN 7

**Table 20. D07 easily identified ground beetle species. Based (in part) on 2016 identifications.**

| Site | Species |
|---|---|
| GRSM\|ORNL | *Carabus goryi* |

| GRSM\|ORNL | *Chlaenius emarginatus* |
|---|---|
| GRSM | *Cyclotrachelus freitagi* |
| ORNL | *Cyclotrachelus fucatus* |
| GRSM\|ORNL | *Cyclotrachelus sigillatus* |
| GRSM\|ORNL | *Dicaelus dilatatus* |
| GRSM | *Dicaelus teter* |
| GRSM\|ORNL | *Galerita bicolor* |
| GRSM\|ORNL | *Pasimachus punctulatus* |
| GRSM | *Pterostichus acutipes* |
| GRSM | *Pterostichus moestus* |

## E.7    DOMAIN 8

Table 21. D08 easily identified ground beetle species. Based (in part) on 2017 identifications.

| Site | Species |
|---|---|
| DELA\|LENO | *Brachinus alternans* |
| DELA\|LENO | *Chlaenius aestivus* |
| DELA\|LENO | *Chlaenius erythropus* |
| DELA\|LENO\|TALL | *Cyclotrachelus convivus* |

## E.8    DOMAIN 9

Table 22. D09 easily identified ground beetle species. Based (in part) on 2016 identifications.

| Site | Species |
|---|---|
| WOOD | *Amara neoscotica* |
| WOOD | *Brachinus cyanochroaticus* |
| NOGP | *Cyclotrachelus torvus* |
| NOGP\|WOOD | *Harpalus opacipennis* |
| NOGP\|WOOD | *Harpalus somnulentus* |

## E.9    DOMAIN 10

Table 23. D10 easily identified ground beetle species. Based (in part) on 2016 identifications.

| Site | Species |
|---|---|
| CPER\|STER | *Agonoleptus conjunctus* |
| STER | *Agonum placidum* |
| CPER\|STER | *Amara convexa* |
| STER | *Anisodactylus carbonarius* |
| CPER\|STER | *Anisodactylus merula* |
| CPER\|STER | *Anisodactylus rusticus* |

| | |
|---|---|
| CPER\|STER | *Chlaenius tomentosus* |
| CPER\|STER | *Cicindela punctulata* |
| CPER\|STER | *Cratacanthus dubius* |
| CPER\|STER | *Cyclotrachelus torvus* |
| CPER\|STER | *Discoderus parallelus* |
| CPER\|STER | *Euryderus grossus* |
| CPER\|STER | *Harpalus amputatus* |
| CPER\|STER | *Harpalus caliginosus* |
| CPER\|STER | *Harpalus desertus* |
| CPER\|STER | *Harpalus pensylvanicus* |
| STER | *Harpalus reversus* |
| CPER\|STER | *Pasimachus elongatus* |
| CPER\|STER | *Piosoma setosum* |
| STER | *Poecilus lucublandus* |
| STER | *Poecilus scitulus* |
| CPER\|STER | *Selenophorus planipennis* |
| STER | *Stenolophus comma* |

## E.10     DOMAIN 11

**Table 24. D11 easily identified ground beetle species. Based (in part) on 2017 identifications.**

| Site | Species |
|---|---|
| CLBJ | *Chlaenius emarginatus* |
| OAES | *Pasimachus californicus* |
| OAES | *Selenophorus pedicularius* |

## E.11     DOMAIN 13

**Table 25. D13 easily identified ground beetle species. Based (in part) on 2016 identifications.**

| Site | Species |
|---|---|
| NIWO | *Amara alpina* |
| NIWO | *Amara quenseli* |
| NIWO | *Calathus advena* |
| NIWO | *Carabus taedatus* |
| MOAB\|NIWO | *Cicindela punctulata* |
| NIWO | *Cymindis unicolor* |
| MOAB | *Euryderus grossus* |
| MOAB | *Piosoma setosum* |
| MOAB\|NIWO | *Selenophorus planipennis* |

### E.12    DOMAIN 14

**Table 26. D14 easily identified ground beetle species. Based (in part) on 2016 identifications.**

| Site | Species |
|---|---|
| JORN | *Tetragonoderus pallidus* |

### E.13    DOMAIN 15

**Table 27. D15 easily identified ground beetle species. Based (in part) on 2016 identifications.**

| Site | Species |
|---|---|
| ONAQ | *Amara quenseli* |

### E.14    DOMAIN 16

**Table 28. D16 easily identified ground beetle species. Based (in part) on 2016 identifications.**

| Site | Species |
|---|---|
| ABBY | *Pterostichus pumilus* |
| ABBY | *Scaphinotus angusticollis* |

**APPENDIX F      Domain Specific List of Species that are Difficult to Identify**

Some species of ground beetles are inherently difficult to parse because the morphological characters that are useful for telling these species apart vary across that beetle's range. There are also cryptic species or small species that are fundamentally difficult to differentiate. Although these lists contain many of the more difficult ground beetle taxa, they are by no means exhaustive. Each list will continue to grow after the first year of sampling within each domain based on review of material by a taxonomic expert. Species will be **added** to this list on an annual basis when: 1) 20 or more individuals identified to a particular species by NEON parataxonomists are reviewed by an expert taxonomist and 2) NEON staff have less than an 80% accuracy rate compared to the expert. Species will be **removed** from this list on an annual basis for species where accuracy rates rise above the 80% threshold (given 20 or more specimens). For each species, a site list is provided with locations from which each species was thought to occur by NEON staff, along with a list of species with which the difficult taxon is confused (based on expert taxonomist identifications). The column 'Present in Teaching Collection?' refers to whether there are individuals of the difficult taxon present in the relevant domain teaching collection.

Because NEON seeks high accuracy at the species-level, specimens will be considered 'correctly identified' when the species-level identification match even if the expert taxonomist can resolve the specimen to the subspecies level or the parataxonomists incorrectly assigned a subspecies identification. For the purposes of parataxonomists identification, any specimen identified to subspecies may consider that subspecies as 'difficult to identify' if the taxon is named at the species-level in the following tables. Any discrepancies in identifications to subspecies will be remedied in future field seasons through updates made to training materials and teaching collections.

**Table 29. Difficult taxa**

| Domain | Site(s) | Difficult Taxon | Mistaken for these species | Present in Teaching Collection? |
|---|---|---|---|---|
| D04 | LAJA | *Selenophorus integer* | *Athrostictus paganus* <br> *Discoderus sp.* <br> *Selenophorus propinquus* <br> *Selenophorus sp.* | No |
| D04 | LAJA | *Selenophorus puertoricensis* | *Discoderus sp.* <br> *Selenophorus propinquus* | No |
| D05 | STEI\|UNDE | *Pterostichus luctuosus* | *Pterostichus caudicalis* <br> *Pterostichus mutus* <br> *Pterostichus tenuis* | Yes |
| D06 | KONA\|KONZ | *Cyclotrachelus* | *Cyclotrachelus deceptus* | Yes |

| | | | | |
|---|---|---|---|---|
| | \|UKFS | *alternans* | *Cyclotrachelus sodalis*<br>*Cyclotrachelus torvus* | |
| D06 | KONA\|KONZ | *Cyclotrachelus torvus* | *Cyclotrachelus deceptus*<br>*Cyclotrachelus substriatus* | No |
| D06 | KONA\|KONZ | *Harpalus erraticus* | *Harpalus hatchi*<br>*Harpalus pensylvanicus* | No |
| D06 | KONZ\|UKFS | *Harpalus pensylvanicus* | *Harpalus compar*<br>*Harpalus faunus*<br>*Harpalus paratus* | Yes |
| D06 | KONA | *Harpalus vagans* | *Harpalus hatchi*<br>*Harpalus pensylvanicus* | Yes |
| D06 | KONZ | *Pasimachus californicus* | *Pasimachus depressus*<br>*Pasimachus elongatus* | Yes |
| D06 | KONA\|KONZ | *Pasimachus elongatus* | *Pasimachus californicus*<br>*Pasimachus depressus* | Yes |
| D06 | KONA\|KONZ\|UKFS | *Pterostichus sculptus* | *Pterostichus permundus*<br>*Selenophorus opalinus* | No |
| D06 | KONA\|UKFS | *Scarites vicinus* | *Scarites subterraneus* | Yes |
| D07 | GRSM | *Sphaeroderus canadensis* | *Sphaeroderus stenostomus* | Yes |
| D08 | DELA\|LENO | *Cyclotrachelus sodalis* | *Cyclotrachelus hypherpiformis* | No |
| D08 | LENO\|TALL | *Dicaelus dilatatus* | *Dicaelus furvus*<br>*Dicaelus sculptilis* | Yes |
| D08 | DELA\|LENO | *Harpalus protractus* | *Harpalus sp.* | No |
| D08 | DELA\|LENO | *Scarites subterraneus* | *Scarites quadriceps* | Yes |
| D11 | CLBJ\|OAES | *Anisodactylus rusticus* | *Anisodactylus dulcicollis*<br>*Anisodactylus haplomus*<br>*Anisodactylus merula*<br>*Anisodactylus ovularis* | Yes |
| D13 | MOAB\|NIWO | *Amara sinuosa* | *Amara sp.* | No |
| D14 | SRER | *Apristus tuckeri* | Apristus sp.<br>Selenophorus sp.<br>Staphylinidae sp. | No |

| D14 | JORN | *Discoderus robustus* | Apristus sp.<br>Discoderus sp.<br>Pasimachus californicus | No |
|---|---|---|---|---|
| D17 | SJER | *Pterostichus californicus* | Notiophilus semiopacus<br>Pterostichus ordinarius<br>Pterostichus panticulatus | No |
| D06, D08 | All | *Loxandrus* sp. | | Yes |
| D10 | All | *Microlestes* sp. | | Yes |

Cryptic species issues arise when two species that are morphologically indistinguishable by NEON parataxonomists co-occur (or might co-occur) at a site. NEON intends to add these species pairs to the master taxon lists to account for this. If a cryptic species pair is not currently available in the master list, the proposed species pair may be submitted as a problem ticket to NEON Science.

**APPENDIX G     Site-Specific Protocol Modifications**

*DOMAIN 07*

At Great Smoky Mountain National Park (core site), a few modifications will be implemented in response to site-specific permitting requirements around bear activity. However, unless detailed in this paragraph, sampling will occur at Great Smoky in a way that is identical to sampling conducted at any other site. At Great Smoky Mountain National Park, electrified fencing will be placed around each plot for the duration of the sampling season. At the end of the sampling season, the fencing will be removed.

*DOMAIN 10*

At Sterling (relocatable site), one modification to sampling timing will be implemented in response to a seasonal pulse of carrion beetles. At this site, carrion beetles typically become super-abundant in the first two weeks of August. During this time, carrion beetles consume pitfall trap contents – rendering the identification of collected material difficult to impossible and disrupting accurate assessment of diversity and abundance. Thus, staff at the domain support facility will monitor carrion beetle activity through the field season and will deactivate pitfall traps when carrion beetles are super abundant. Deactivating pitfall traps entails: removal of the interior pitfall cup (such that only the exterior cup with holes in it remains), putting the deli cup lid on top of the interior pitfall cup, removing the PVC spacers, and closing the 20 x 20 cm pitfall cover down flush to the ground.

Staff will deactivate traps for two full bouts during highest carrion beetle activity and redeploy traps for the remainder of the season following this hiatus.

*DOMAIN 12*

At Yellowstone (core site), one modification will be implemented in response to site-specific permitting requirements around bear activity and vertebrate bycatch. However, unless detailed in this paragraph, sampling will occur at Yellowstone in a way that is identical to sampling conducted at any other site. The National Park Service has a requirement concerning 'trap predation', which is any disturbance and/or excavation of the cup trap that appear to derive from an animal (i.e., a bear) trying to eat the contents of the trap. If three or more instances of trap predation are observed during collection (out of 40 total traps possible), the park liaison will be notified within 24 hours. Five or more instances of trap predation within a single collection bout will trigger temporary trap closures across the entire site for two bouts (28 days).

*DOMAIN 20*

At PUUM (core site), a modification will be implemented in response to site-specific permitting requirements around *Corvus hawaiiensis* ('Alalā or Hawaiian crow) activity. However, unless detailed in this paragraph, sampling will occur at PUUM in a way that is identical to sampling conducted at any

other site. At PUUM, 19 gauge galvanized steel hardware cloth boxes (dimensions: 61 cm x 61 cm x 35.6 cm) will be placed around each trap cup for the duration of the sampling season. The bottom two inches of the wire cage will be open to insect movement.  At the end of the sampling season, the fencing and cup traps will be removed.

Affects plots: 002, 003, 006, 008, 013, 014



**Figure 53. Hardware cloth enclosures are placed outside traps at certain PUUM plot locations.**

**APPENDIX H    Insect Taxonomic Identification Aids**

In addition to the teaching collection, one or more of the following keys may be useful aids in identifying beetles in a given domain. Resources and keys for difficult taxa are also posted on the Field Operations Intranet site in the TOS – Sampling Support Documentation folder (see the Identification References and Beetle Identification Resources folders). Lists of endangered invertebrates and keys will also be posted on the Intranet site in the Beetle Identification Resources folder.

**H.1    GENERAL KEYS**

- [Catalogue of Geadephaga (Coleoptera, Adephaga) of America, north of Mexico](#)
  Bousquet Y. 2012. ZooKeys 245: 1–1722.
- [The ground-beetles (Carabidae, excl. Cicindelinae) of Canada and Alaska, parts 1—6](#)
  C.H. Lindroth. 1961. Opuscula Entomologica Supplementa XX, XXIV, XXIX, XXXIII, XXXIV, XXXV.
- Pearson, D.L., Knisley, C.B., and Kazilek, C.J. 2006. A Field Guide to the Tiger Beetles of the United States and Canada: Identification, Natural History, and Distribution of the Cicindelidae. Oxford University Press, New York, NY. Available from: [http://www.amazon.com/Field-Beetles-United-States-Canada/dp/0195181565](http://www.amazon.com/Field-Beetles-United-States-Canada/dp/0195181565)
- Tiger Beetles of the United States (website: [http://www.npwrc.usgs.gov/resource/distr/insects/tigb/index.htm](http://www.npwrc.usgs.gov/resource/distr/insects/tigb/index.htm)). Coordinated by W. Wyatt Hoback and John J. Riggins
- Arnett Jr., R. H., and M. C. Thomas. 2001. American Beetles Archosemata, Myxophaga, Adephaga, Polyphaga: Staphyliniformia. 1st Vol. (C. Press, Ed.). 1st Ed. Boca Raton, FL.
- [The Photo-Annotated Guide to Tribes, Guide to Subgenera of Amara, and Guide to Harpalini](#) (based on Arnett Jr., R. H., and M. C. Thomas. 2001.)

**H.2    REGIONAL KEYS**

- [Illustrated Identification Guide to Adults and Larvae of Northeastern North American Ground Beetles (Coleoptera: Carabidae)](#) Yves Bousquet. 2010. Pensoft Publishers.
- [An annotated checklist of Wisconsin ground beetles (Coleoptera: Carabidae)](#)
  Peter W. Messer. 2009. The Great Lakes Entomologist 42(1-2): 30-61.
- [Key to Florida Ground Beetles](#)
- [Ground Beetles and Wrinkled Bark Beetles of South Carolina](#)
  Janet Ciegler. 2000. Clemson University.
- [University of New Hampshire Insect and Arachnid Collections](#)
- [Checklist of the Coleoptera of Oklahoma](#)
- [California Beetle Project](#)
- [The Beetles of the Pacific Northwest](#) Hatch, M. 1953. University of Washington publications in biology, Volume 16. University of Washington Press, Seattle, Washington.
- Choate, P.M. 2001. Manual for the Identification of the Ground Beetles (Coleoptera: Carabidae) (including tiger beetles) of Florida [http://www.entnemdept.ufl.edu/choate/florida_Carabidae_new.pdf](http://www.entnemdept.ufl.edu/choate/florida_Carabidae_new.pdf)
- Insects of Hawaii, Volume 16: *Coleoptera: Carabidae*: Part 1: Introduction and Tribe Platynini

- Liebherr, J.K., and Zimmerman, E.C. 2000. University of Hawai'i Press, Honolulu, HI. Available from: http://www.uhpress.hawaii.edu/p-2012-9780824823566.aspx

**APPENDIX I    Guide to Distinguishing the Family Carabidae**

The suborder Adephaga includes ground beetles and some aquatic groups.  Adephagan beetles are easily identified by the manner in which the last pair of legs articulates with the beetle's underside (**Figure 54**), as well as the tarsal formula (5-5-5; **Figure 55**). The former feature is denoted by the last pair of legs completely separating the first abdominal segment.  The latter feature is denoted by each of the beetle's tarsi (or feet) being comprised of 5 segments. Foretarsus = 5 segments; midtarsus = 5 segments; hindtarsus = 5 segments.
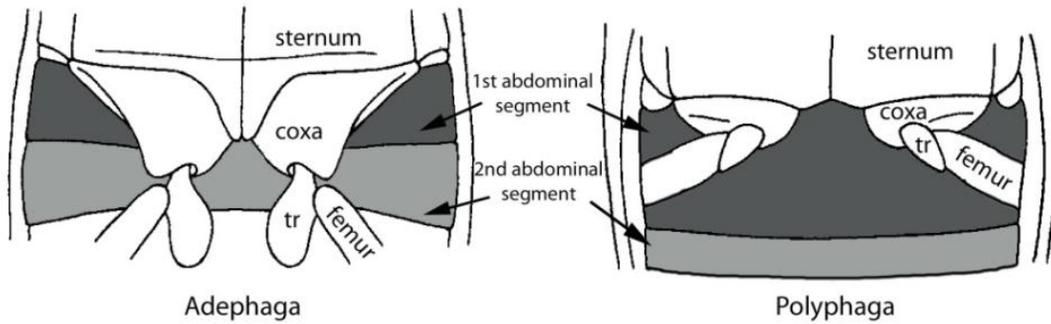


**Figure 54. Hind leg articulation in Adephaga and Polyphaga.**
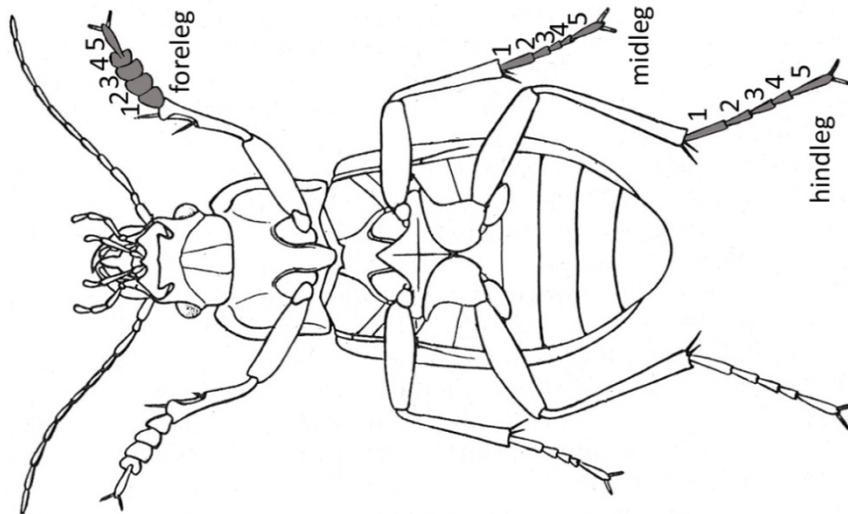


**Figure 55. Tarsal formula of adephagan beetles.**

Adephagan beetles of the family Carabidae are *usually* black and shiny, although they may also be metallically colored, and have large eyes, powerful legs, and large mandibles that fit their predatory life styles (

Figure 56). Otherwise these beetles lack easily recognizable and distinct morphological features. However, several other families of adephagan beetles do have distinct morphological features, by which they can easily be identified as *not* carabids. Below are some descriptions and illustrations of these identifying features (**Figure 57 - Figure 62**), which will allow for the identification of non-carabid adephagans, (a.k.a. invertebrate bycatch).



Image source:
http://www.myrmecos.net/2011/05/13/friday-beetle-blogging-a-six-spotted-tiger/
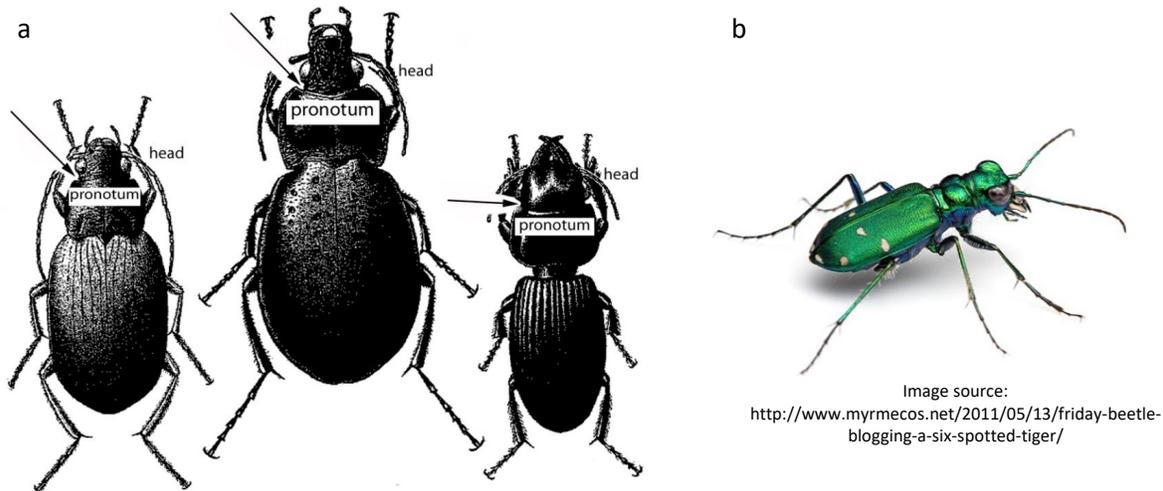
**Figure 56. a) Ground beetle specimens with head and pronotum labeled; arrow shows division between head and pronotum. b) Tiger beetles (a group within the Carabidae family) are frequently metallic in coloration.**

- Family Gyrinidae:
  Gyrinid beetles are most easily recognized for their divided compound eyes (**Figure 57**). Each of these beetles' eyes has a top (or dorsal) and bottom (or ventral) portion, such that their two eyes actually look like four. In addition, gyrinids have short, paddle-like mid and hind legs (**Figure 58**).

Dorsal portion of eye

Ventral portion of eye

Image source:
http://www.studyblue.com/notes/note/n/holometabola-coleoptera-day-1-w-dynastinae/deck/1547765
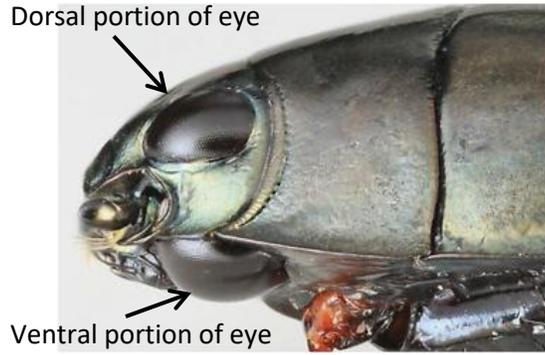
**Figure 57. Lateral view of a beetle in the family Gyrinidae, showing dorsal and ventral portions of the compound eye.**



Image source:
http://www.zin.ru/animalia/coleoptera/rus/g

**Figure 58. Dorsal view of beetle in the family Gyrinidae showing mid and hind legs adapted for swimming**

- Family Dytiscidae:

Dytiscid beetles are recognizable by their oval bodies and the long hairs on their hind tibia (**Figure 59**), by which their hind legs become oar-like structures for movement through water.



Image source:
http://flickrhivemind.net/Tags/taxonomy:family=dytiscidae/Interesting

**Figure 59. Dorsal view of a beetle in the family Dytiscidae, showing the oval shaped body and long hairs on the hind legs.**

- Family Haliplidae:

Haliplid beetles are most easily recognized by their unusually large hind coxal plates(**Figure 60**), which cover most of the ventral side of the abdomen (and actually obstruct the view of the adephagan-specific hind leg articulation described above).

Large hind coxal plate of beetles in the family
Haliplidae
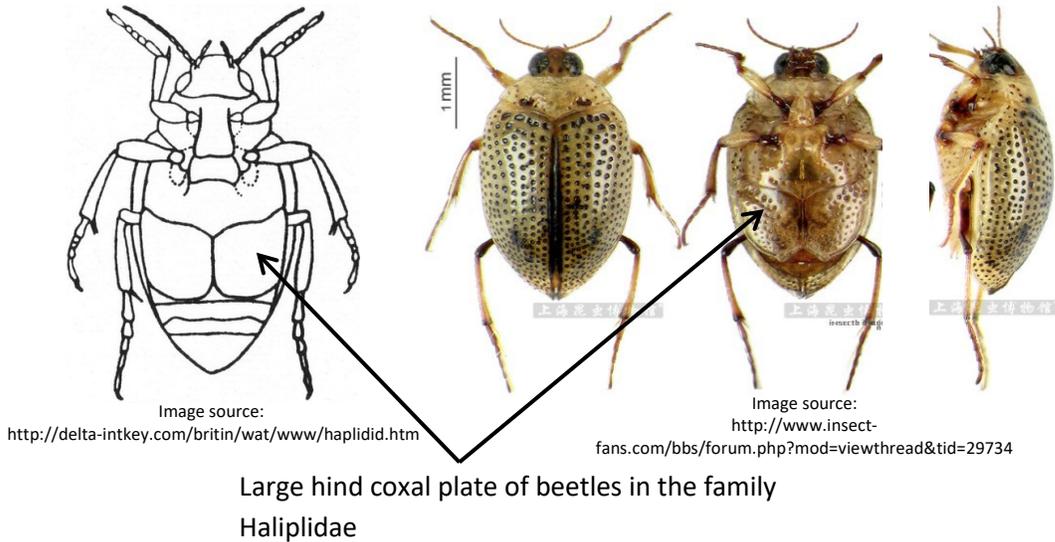
**Figure 60. Diagram and photo of a Haliplid beetle, showing the large coxal plates that are characteristic for this family.**

- Family Noteridae:

Noterid beetles have smooth, oval bodies, and range in color from light brown to dark, reddish brown. Their heads are small, and they have a distinct 'noterid' platform, or plate between the second and third sets of legs on their ventral side (**Figure 61**).
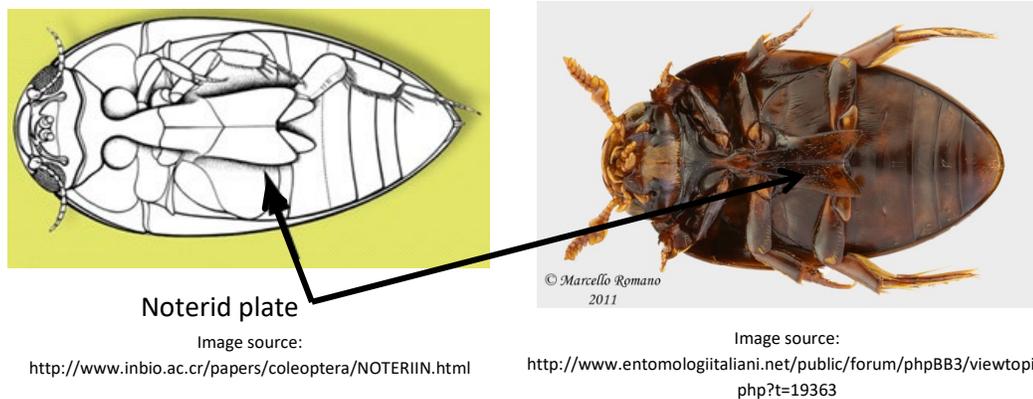


**Figure 61. Diagram and photo of a noterid beetle, showing the noterid plate between the second and third pairs of legs, on the ventral side.**

- Family Rhysodidae:

Rhysodid beetles have elongated bodies, beadlike antennae, protona that narrow to a 'neck' before joining with the head, and heads, protona, and elytra bearing deep longitudinal grooves (this being their most distinguishing feature; **Figure 62**).

antennae
moniliform

pronotum and head
with deep grooves

Rhysodidae

Image source:
http://www.entomology.umn.edu/museum/links/coursefiles/Coleop%20ch aracters.html
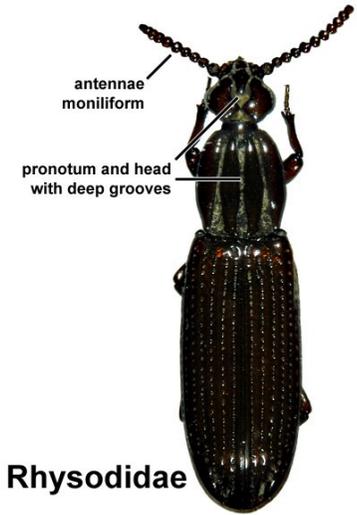
Image source:
http://www.biodiversity.ubc.ca/entomology_pictures/Coleo ptera/Rhysodidae/Clinidium%20calcaratum%20(1dorsal).jpg

**Figure 62. Diagram and photo of a rhysodid beetle, showing the 'neck' where the head and pronotum meet, and the characteristically deeply grooved head, pronotum, and elytra.**

## APPENDIX J    Locality Labels

Locality labels are used to identify the location and date where a sample was collected. They are used in the field to label traps, and in the laboratory to label vials of samples and individually mounted specimens. Locality labels include particular information in order to be consistent with collection records from other global campaigns (see **Figure 62**; details to follow).  At NEON we are using locality labels for samples containing ground beetles, invertebrate bycatch, and vertebrate bycatch.

> USA, COLORADO Weld County.
> Central Plains Expm Rng. 1639m
> N40.8509 W104.7292
> Pitfall trap 4Jul2015. TBaldwin
> NEON CPER_001.N.20150704

**Figure 63. Example label for pitfall trap sample. Size shown is larger than size of actual label.**

## J.1    CREATING LABELS

1. The locality label generator (available via the sampling support library) is a web application that automatically generates labels (up to 240 per page) for all plots and traps given a site and the date range of sampling.

2. Select the siteID, the first date of collection and last date of collection.

3. Determine how many labels are needed for each plot for the selected activity. For field sampling, three labels are generated for each trap in a bout. For sorting and pinning, different quantities of labels may be required. Set the number of labels to be output appropriately.

4. Click the download button to receive a PDF with labels pre-printed for each trap and date specified.

5. Print the labels on ethanol-safe archival paper (e.g., Bioquip 1223RA).

6. Cut labels using a snap-off blade knife and ruler. There should be no obstructions on the cutting mat or the ruler while doing this. Using a snap-off blade is essential to making clean cuts. Using scissors to make a cut of this length is highly prone to error.
   a. Cut labels with as little white space on all sides of the text as possible.

7. Use ethanol-safe pens whenever adding information to labels to ensure longevity of markings.

**APPENDIX K    Determination Labels**

Determination labels are used to label individual specimens (pinned, pointed, or stored individually in tubes or vials) or groups of pooled specimens of the same taxon with a species or morphospecies identification. These labels contain the following three lines of information: 1) The scientific name or morphospecies ID of the individual or group of individuals, 2) the first and middle initials and last name of the person who identified the individual or group of individuals (these following the letters "det."), and 3) the year in which the identification was made (see example in **Figure 64**).

| *Cratacanthus dubius* | D10.2015.MorphA |
|---|---|
| det. J. S. Smith | det. J. S. Smith |
| 2015 | 2015 |

**Figure 64. Example determination and morphospecies ID labels; size shown is larger than size of actual labels.**

**K.1    COMPILE DETERMINATION LABEL DATA**

1.  Compile the label data in a Microsoft Excel spreadsheet.

    a.  Enter label data into a Microsoft Excel spreadsheet (**Figure 65**) with one column for each piece of information required for the determination label, as follows:

        i.   Column Header = "Species", content = scientific name or morphospecies ID for the taxon.

            A.  For scientific names: text needs to be italicized and with the first letter of the genus capitalized

            B.  For morphospeciesIDs: text should not be italicized

        ii.  Column Header = "det.", content =the characters "det." (with the period at the end and without quotes)

        iii. Column Header = "Identifier", content =First and middle initials and last name of the person who identified the individual or group of individuals

            A.  Initials should be capitalized and followed by a period

            B.  Last names should be capitalized

            C.  All initials should be separated by a space, and there should be a space between the middle initial and the last name

iv. Column Header = "Year Identified", content =The year in which the identification was made, in numeric format (e.g., 2015)

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Species | det. | Identifier | Year Identified |
| 2 | *Abaris splendidula* | det. | R. Davidson | 2015 |
| 3 | *Acalathus advena* | det. | R. Davidson | 2015 |
| 4 | *Acupalpus alternans* | det. | R. Davidson | 2015 |
| 5 | *Acupalpus canadensis* | det. | R. Davidson | 2015 |
| 6 | *Aephnidius opaculus* | det. | R. Davidson | 2015 |
| 7 | *Agonoleptus conjunctus* | det. | R. Davidson | 2015 |
| 8 | *Agonoleptus dolosus* | det. | R. Davidson | 2015 |
| 9 | *Agonoleptus parviceps* | det. | R. Davidson | 2015 |
| 10 | *Agonoleptus rotundatus* | det. | R. Davidson | 2015 |
| 11 | *Agonoleptus rotundicollis* | det. | R. Davidson | 2015 |
| 12 | *Agonoleptus thoracicus* | det. | R. Davidson | 2015 |
| 13 | *Agonoleptus unicolor* | det. | R. Davidson | 2015 |
| 14 | *Agonum aeruginosum* | det. | R. Davidson | 2015 |
| 15 | *Agonum affine* | det. | R. Davidson | 2015 |

**Figure 65. Spreadsheet containing information for determination labels**

b. If labels are printed prior to insect sorting, leave the identifier blank so that it can be filled in as sorting occurs

2. Name the sheet using a meaningful name such as 'BetDet2015'. Save and close the file – if you do not close the file, the Mail Merge may not work correctly.

**K.2    EXPORT THE DATA INTO MICROSOFT WORD USING MAIL MERGE**

**NOTE**: It will likely be helpful to review documentation specific to the version of Microsoft Office you are using on creating labels using a Mail Merge. Here we describe the process using Microsoft 2010.

1. Open a Microsoft Word document and under **Mailings**, select **Start Mail Merge** and then **Labels**.

2. Dimensions for these labels are the same as for locality labels, so the correct label may already exist in the list.  If not, in the **Labels** menu select **New Label** and enter information for the dimensions of the label in the "Label Details" box, exactly as in **Figure 66**. Give the template a name that makes it easy to locate when creating a new sheet of labels (e.g. 'insect determination labels').
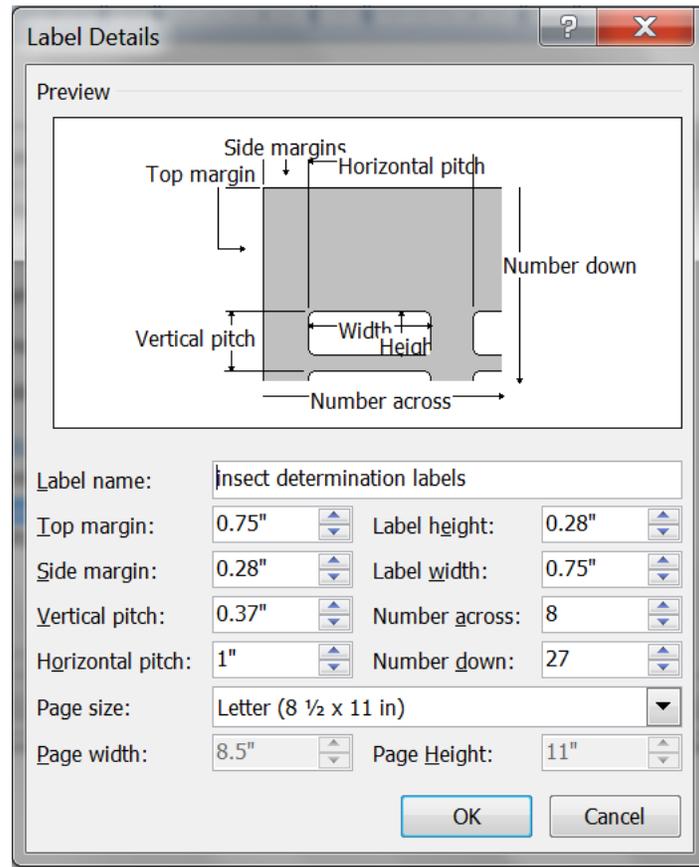
**Figure 66. Dimensions for creating a determination label template in Microsoft Word**

3. When label dimensions have been specified, a blank template document will appear. In the **Mailings** tool bar, click on **Select recipients** and select **Use existing list**. A file navigation window will appear. Navigate to and select the Microsoft Excel spreadsheet created in step (**K.1**).

4. A "Select Table" pop-up window (**Figure 67**) will appear, showing a list of the sheets within the Excel workbook created in step (**K.1**). Select the worksheet containing the determination label information. Make sure to check the box that says 'First row of data contains column headers'.
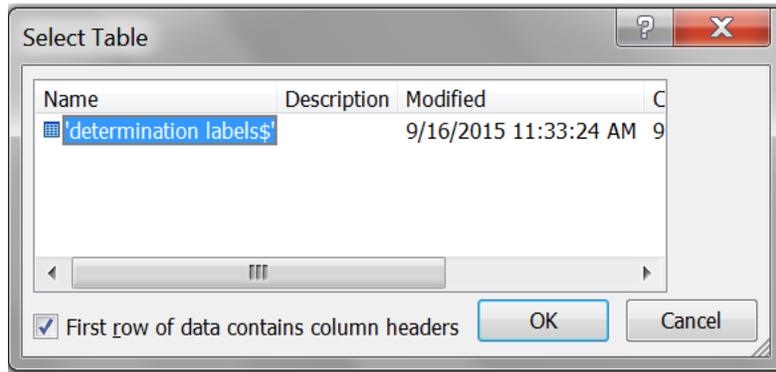
**Figure 67. Selecting recipients from a Microsoft Excel file during a Mail Merge**

### K.3    PERFORM FINAL FORMATTING IN MICROSOFT WORD

1.  Place the cursor in the top left cell of the label sheet.

2.  Populate the labels by clicking on **Insert Merge Field** in the **Mailings** menu. An "Insert Merge Field" drop-down menu will appear, containing the names of the column headings from the Microsoft Excel spreadsheet. Add the headers to the cell as follows:

    a.  Highlight "Species" in the dropdown, then click "Insert"

    b.  Highlight "det." In the dropdown, then click "Insert"

    c.  Highlight "Identifier" in the dropdown, then click "Insert"

    d.  Highlight "Year Identified" in the dropdown, then click "Insert"

    e.  Click "Close" and you will see that the top left cell contains:


    «Species»«det»«Identifier»«Year_Identified»


3.  Insert line breaks and spaces as follows:

    a.  Place the cursor at the end of "«Species»" and hit enter

    b.  Place the cursor at the end of «det» and hit the spacebar

    c.  Place the cursor at the end of «Identifier» and hit enter

    d.  Place the cursor at the end of «Year_Identified» and hit enter

e.  The top left label will now contain:

«Species»
«det» «Identifier»
«Year_Identified»

4.  Highlight the contents of the top left cell and set the font to Arial, font size 3.5.

5.  *Note: Formatting such as italics does not get transferred from Microsoft Excel to Microsoft Word during a Mail Merge; italics will have to be applied to scientific names after the labels have been created*

6.  After finishing the first cell, click **Update Labels** – this will copy formatting into remaining labels.

7.  Preview the labels by clicking on **Preview Results**.

8.  Confirm that the first row in each label has either a scientific name OR morphospeciesID, the second line has 'det.' and an identifier's name, and the third row has the identification year.

9.  In the **Mail Merge** menu, select **Finish & Merge**, then **Edit Individual Documents**. A pop-up menu will appear. Choose the 'All' option. This will create a Microsoft Word document with the labels.

10.  Save the document. Use a meaningful name such as 'BetDet2015'.

11.  In the Microsoft Word document, italicize scientific names (but not morphospeciesIDs).

12.  Copy and paste each label as needed, in order to generate the required number of labels for specimens of each taxon. Group all labels for the same taxon within the same column.

13.  Re-save the document after editing

## K.4     PRINT LABELS

1.  Print the labels on ethanol-safe archival paper (e.g., Bioquip 1223RA).

2.  Cut the labels as detailed in **APPENDIX J**

**APPENDIX L    IndividualID Labels**

individualID labels are used to label individual (pinned, pointed, or stored individually in tubes or vials) specimens from a specific location and sampling period. The label format is: NEON.MOD.DXX.######, where MOD is the module (in this case "BET" for beetles), XX is the domain number, and the number contains 6-digits (see example in **Figure 68**).

## NEON.BET.D10.000139

**Figure 68. IndividualID label for a beetle specimen**

**L.1            INDIVIDUALID SPECIMEN LABELS**

1. Use the Microsoft Excel file titled "Insect Individual ID Labels". Each lab should already have a copy of this file; if you do not have one, make a problem ticket.

    a. "Domains" (first) tab of the file: This tab contains the correctly formatted domainIDs, which are the first component of the individualID labels. Select the domain ID that corresponds to the location in which labels are being printed.

    b. "BET_IndividualID" (second) tab: Each page in this tab contains a total of 329 individualID labels. The column width is 0.91", row height is 0.21", and font is Calibri size 7. All the borders are turned ON around each cell – borders will help keep straight lines when cutting out the labels.

        i. The first 6 pages (through label 001974) have already been created, although the domainID may need to be altered to reflect the correct location. To do this:

            A. Select all labels on all pages in the "BET_IndividualID" tab

            B. In the "Find and Replace" menu, select the "Replace" tool

            C. In the "Find what" box, type "D15" (without quotes)

            D. In the "Replace with" box, type "DXX" (without quotes and where XX is the correct domain number)

            E. Click the "Replace All" button

ii.     When ready to print, be sure to ***only print one*** copy of each page, starting with the first page and not printing the second page until after all labels from the first page have been used.

This ensures that the same individualID is ***NEVER*** used for more than one specimen, regardless of where or when specimens are captured (e.g., through all years of sample collection, individual ID labels are ***never*** to be used more than once per domain).

iii.     Keep careful track of the page of labels, and pay close attention to the last number used during the previous bout of printing individualID labels, even if that bout was in a previous sampling year.

Note: D13 is spread over two domain support facilities. NIWO will use labels from 000001 to 499999; MOAB will use 500000 to 999999.

iv.     More label pages will be added to the "Insect Individual ID Labels" spreadsheet in future years, as requested.

2.  Insert a sheet of yellow cardstock into the manual feed tray of the printer. Select all labels on the page of individualID labels to be printed. Under the print menu, select "Print Selection". Under Printer Properties, set the Paper source to Manual Feed.

3.  Cut the labels as detailed in **APPENDIX J**