



Title: TOS Protocol and Procedure: BET – Ground Beetle Sampling		Date: 02/22/2022
NEON Doc. #: NEON.DOC.014050	Author: K. LeVan	Revision: N

## TOS PROTOCOL AND PROCEDURE: BET - GROUND BEETLE SAMPLING

PREPARED BY	ORGANIZATION	DATE
Katherine LeVan	SCI	01/10/2022
Laura Nagel	Field Science	01/10/2022
Natalie Robinson	SCI	12/05/2014
David Hoekman	SCI	12/13/2013
Kali Blevins	SCI	12/13/2013

APPROVALS	ORGANIZATION	APPROVAL DATE
Kate Thibault	SCI	02/22/2022

RELEASED BY	ORGANIZATION	RELEASE DATE
Tanisha Waters	CM	02/22/2022

See configuration management system for approval history.

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## 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 Substantial revision, including: <ul style="list-style-type: none"> <li>• Clarification to address JIRA tickets and provide more detailed instruction (throughout)</li> <li>• Moved information to appendices and supplementing the information (e.g., Appendix G)</li> <li>• Exported information to (e.g., shipping instructions), and imported information from (e.g., pinning/pointing instructions) to ease the use of multiple protocols for technicians.</li> <li>• Added tolerances for placement of pitfall traps in plots</li> <li>• Added information for dealing with disturbed and/or empty traps</li> <li>• Added information on handling vertebrate bycatch and morphospecies</li> </ul>
G	03/17/2015	ECO-02749	<ul style="list-style-type: none"> <li>• Re-imported shipping information in SOP I</li> <li>• Added corresponding info and tables (e.g., table 9) throughout</li> </ul>
H	05/04/2015	ECO-02890	<ul style="list-style-type: none"> <li>• Language requiring that gloves be worn by anyone touching vertebrate bycatch with their hands</li> <li>• Also, technicians performing cervical dislocation must have received prior training</li> <li>• Fixing an error in the figures in response to FOPS-2034</li> </ul>
J	11/23/2015	ECO-03399	<ul style="list-style-type: none"> <li>• Fixing workflow compatibility with the WebUI</li> <li>• Updated species lists</li> </ul>



			<ul style="list-style-type: none"> <li>Fixed sample timing section; added site specific estimated start dates</li> <li>Added in language/equipment concerning isoflurane</li> <li>Updated label generation section for consistency</li> <li>Updated workflow to remove 'other carabid'</li> </ul>
K	01/31/2017	ECO-04363	<ul style="list-style-type: none"> <li>Updated plot relocation criteria</li> <li>Updated text to reflect new data entry application</li> <li>Revised site specific estimated start dates</li> </ul>
L	01/26/2018	ECO-05253	<ul style="list-style-type: none"> <li>Add language about barcodes</li> <li>Pinning modification to pin all beetles if less than 10 would be stored together</li> <li>Mobile data entry updates</li> <li>Site specific trap deactivation at STER (due to carrion beetle activity)</li> <li>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</li> </ul>
M	04/11/2019	ECO-05966	<ul style="list-style-type: none"> <li>Remove all reference to North trap</li> <li>Clarified when barcodes are needed</li> <li>Minor updates to conform with fulcrum app changes</li> <li>Update Appendix D dates based on latest MODIS data</li> <li>Update Appendix E and F on 2017 expert taxonomy data, where available</li> <li>Update site specific deviations to document D20 modifications</li> </ul>
N	02/22/2022	ECO-06757	<p>Updated to new template (NEON.DOC.050006 Rev K) Significant updates to sample processing workflow, including:</p> <ul style="list-style-type: none"> <li>Contingency decision table: clarified reporting requirements for sampling deviations, discrepancies between budgeted taxonomy and pinned specimen availability (Table 3)</li> <li>Clarified plot reallocation process with regard to IACUC</li> <li>Added missed/Incomplete sampling section; directions on use of Sampling Impractical field</li> <li>Filtering only occurs in the lab (no longer filtering PG in the field)</li> <li>Site specific modifications: documented IACUC waiver of isoflurane euthanasia requirement for D04 (Puerto Rico)</li> <li>Clarified text around requirements for vertebrate bycatch reporting</li> </ul>



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			<ul style="list-style-type: none"><li>• Updated processing to streamline carabid identification (removed 'easy/difficult/abundant/rare' framework)</li><li>• Updated selection criteria for which pinned specimens to send for expert taxonomy</li><li>• Removed fumigant requirement for pinned specimens. Converting to periodical freezing method.</li><li>• Updated Appendices to include species checklists per site with identification references</li></ul>
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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 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 Scientific Data Products Catalog (RD[03]).

### **1.3 Acknowledgments**

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



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## 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 Data Products Catalog
RD[04]	NEON.DOC.001271	AOS/TOS Protocol and Procedure: Data Management
RD[05]	NEON.DOC.001580	Datasheets for TOS Protocol and Procedure: Ground Beetle Sampling
RD[06]	NEON.DOC.003282	NEON Protocol and Procedure: Site Management and Disturbance Data Collection
RD[07]	NEON.DOC.005247	AOS/TOS Standard Operating Procedure: NEON Aquatic and Terrestrial Site Navigation
RD[08]	NEON.DOC.005224	NEON Protocol and Procedure: Shipping Ecological Samples and Equipment
RD[09]	Available via download of data from NEON portal	NEON Raw Data Ingest Workbook for TOS Ground Beetle Abundance and Diversity
RD[10]	NEON.DOC.001953	Pitfall trap spacers assembly instructions
RD[11]	NEON.DOC.001818	Pitfall trap cover assembly instructions
RD[12]	NEON.DOC.001816	Filter cloth assembly instructions
RD[13]	NEON.DOC.001814	Filter cup assembly instructions

### 2.3 Acronyms

Acronym	Definition
IACUC	Institutional Animal Care and Use Committee
RTE	Rare, Threatened and Endangered

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



## 2.4 Definitions

**Fulcrum:** Software tool used to create NEON electronic data entry applications.

**ServiceNow:** Software tool used for problem/incident tracking and resolution.



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### 3 METHOD

Ground beetle sampling involves: preparation for sampling (**SOPA**); pitfall trap deployment (**SOPB**) and the collection of ground beetles (**SOPC**); sample processing in the lab to sort target taxa (ground beetles) from invertebrate and vertebrate bycatch (**SOPC, 0**); identification of individual ground beetles to species and proper specimen preservation (**SOPF, SOPG, SOPH**); data entry and verification (**SOP I**); and instructions for shipping specimens to a taxonomist (**SOP J**). 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 the Site-Specific Protocol Modifications.

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 place 150 or 250 mL of preservative into each trap on an every-other-week 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).

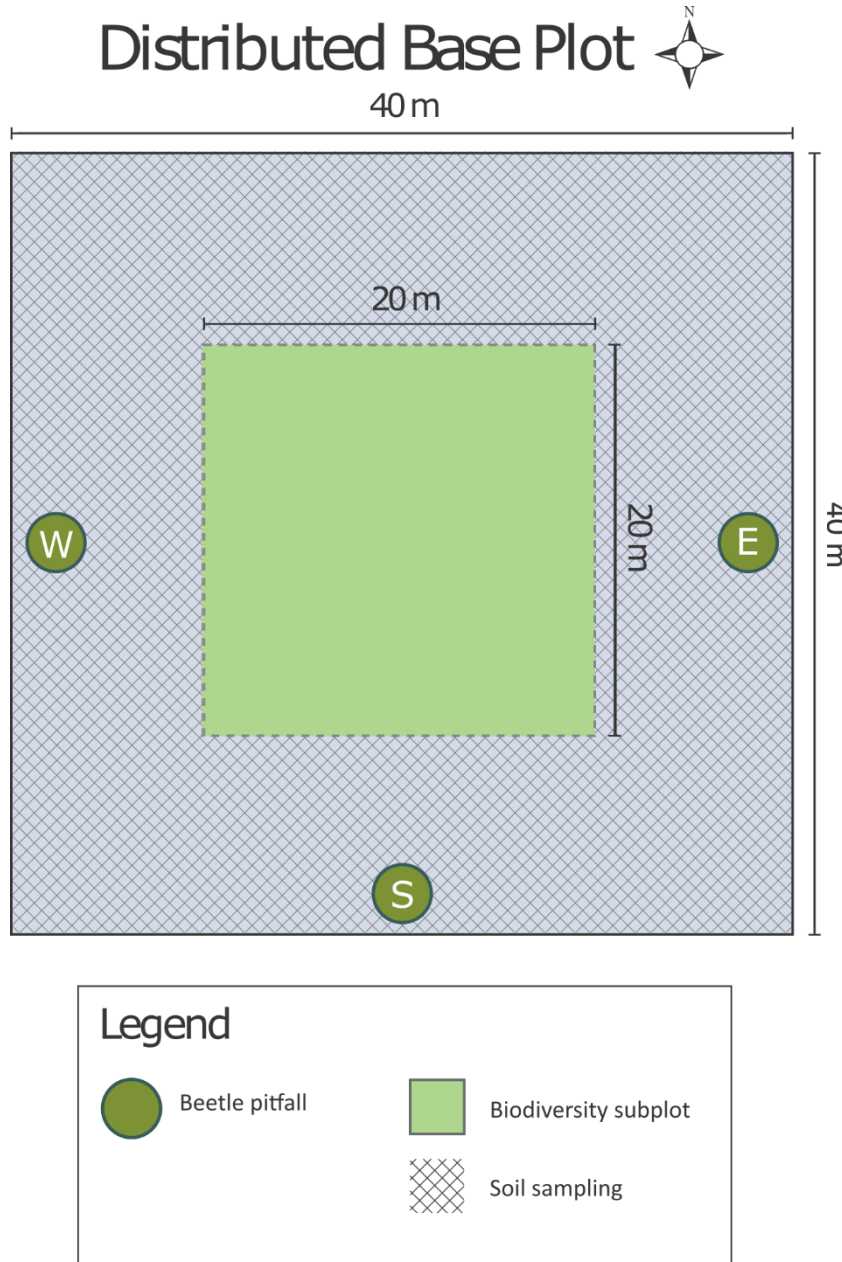
Pitfall traps are placed on the interior edges of the 40m x 40m Distributed Plots (well outside of the 20m x 20m plot interior, where multiple plant protocols are implemented). Ten Distributed Plots each have three pitfall traps (30 traps total per site) located as close as possible to the center of 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 are identified by Science.

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 is 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 biodiversity subplot (exclusion zone) indicated in green/solid fill.





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## 4 SAMPLING SCHEDULE

### 4.1 Sampling Frequency and Timing

Pitfall traps are 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 sampling interval). 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.

**Table 1.** Sampling frequency for Ground Beetle Sampling procedures on a per SOP per plot type basis.

SOP	Plot Type	Plot Number	Bout Duration	Bouts Per Year	Bout Interval	Yearly Interval	Remarks
SOP C	Distributed	10	14 days	See Appendix C	Consecutive	Annual	Minimum temperatures must exceed 4°C over the prior ten-day period to initiate field season.

### 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 C**. 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 initiate and conclude sampling based on the dates listed in **Appendix C**.

Although staff are permitted to begin sampling any time after the estimated start date, NEON staff must perform the specified number of bouts for each site in **Appendix C**. Scheduled initial deployment may be delayed if there are persistent cold temperatures; defined as an average ten-day high below 4 °C. Likewise, sampling may be concluded earlier than the estimated end date if persistent cold temperatures are observed before the end date occurs or when the expected number of bouts has been completed.

NEON Science anticipates performing the number of collections (i.e., ‘**Bouts Expected**’) listed in **Appendix C** during the date ranges provided for each site. Field Operations staff are not expected to sample substantially outside the date ranges listed in **Appendix C**, and should schedule bouts according to the listed dates. If persistent cold temperatures occur (as are described in the paragraph above), the NEON Science staff will work with the Domain manager to shift scheduled dates such that the number of expected bouts are still performed, if temperatures and budget allow. If temperatures are significantly



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warmer outside the window described in **Appendix C** (i.e., the temperature minimums are above 4 °C prior to the start date or after the end date), no additional collections are required above the number of expected bouts.

### 4.3 Timing for Laboratory Processing and Analysis

- Within 24 hours of field sampling:
  - o pitfall trap samples must be rinsed in ethanol (**SOPC**)
  - o remove and identify vertebrates (**SOPC**)
- Throughout field season:
  - o sort invertebrate bycatch from the trap (**0**)
  - o begin identifying carabids (**SOPF**)
  - o begin pinning carabids (**SOPG**)
  - o pool specimens, as needed (**SOPH**); only after QC on sorting data is complete
- Within 4 months of field season end:
  - o complete identifying carabids **SOP F**)
  - o complete pinning carabids (**SOPG**)
  - o complete specimen pooling, as needed (see **SOP H**)

**Table 2.** Storage needs for each sample type.

Sample Type	Field Storage	Post-processing Lab Storage	Domain Hold Time
Pitfall trap samples from the field	Cooler with ice packs	Refrigerator prior to vertebrate bycatch removal	Up to 24 hours post-collection
Pitfall trap subsamples of sorted and unsorted vertebrate bycatch, invertebrate bycatch, and carabids (from a single trap or pooled from a plot) stored in ethanol	--	Room temperature, in a flammable-safe cabinet; DO NOT store at colder temperatures	Samples do not expire but should be shipped by February 1 the year following the field season
Pinned/pointed carabid specimens	--	Room temperature, stored in an insect cabinet; DO NOT store at colder temperatures	Samples do not expire but should be shipped by February 1 the year following the field season; do not leave pinned specimens in temporary containers (e.g., Schmitt boxes) for longer than 2 weeks

### 4.4 Sampling Timing Contingencies

When unexpected field conditions require deviations from the field protocols outlined in this document, contingent decisions, outlined in **Table 3** below, should be followed in the interest of maintaining data



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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 0). For details on the use of Sampling Impractical records to report missed and incomplete sampling, refer to section 4.5 (Missed or Incomplete Sampling).

**Table 3.** Contingency decisions for Ground Beetle Protocol.

Delay/Situation	Action(s)	Outcome for Data Products
<p>Cancellation of initial trap deployment for any reason.</p> <p>Examples: persistent cold temperatures, lack of permits</p>	<p>Create a schedule change request and Sampling Impractical records for all 30 traps for each missed bout.</p> <p>Do not schedule any additional late-season bouts unless approved by Science.</p>	<p>Changes to the sampling window could impact diversity and abundance data products</p>
<p>Cannot collect or reset traps because of missing materials or access.</p> <p>Examples: insufficient quantities of PG, replacement cups, or whirlpaks. Road closures or other unsafe access issues.</p>	<p>Collect and reset as many traps as possible with available materials. Create Sampling Impractical records for each trap that could not be serviced or reset.</p> <p>Create an incident ticket to document the cause of the incomplete sampling.</p>	<p>Data not comparable to standard collection events.</p> <p>Cannot directly calculate comparable diversity indices on an intra-annual basis if trap collections are not comparable.</p>
<p>Collection delayed by 1 or more day(s)<sup>1</sup></p>	<p>Collect traps asap. Do not reset any traps collected late until the next scheduled bout; note duration &amp; cause</p> <p>All traps that are picked up late (and are not reset) need Sampling Impractical records for the bout that they are not deployed.</p>	<p>Data not comparable to standard collection events; integrity of specimen DNA becomes compromised</p> <p>May not be readily able to obtain DNA barcodes; affects Abundance &amp; Diversity measurements.</p>
<p>Catastrophic event that would damage samples</p> <p>Examples: tilling through the plot, burning directly in the plot, hurricane at the site</p>	<p>If event will damage samples <i>in between</i> 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</p>	<p>Data not comparable to standard collection events</p>



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Delay/Situation	Action(s)	Outcome for Data Products
	the disturbance; resume standardized sampling per the schedule as soon as event has ceased. Report the disturbance using the Site Management Application.	Cannot calculate diversity indices if trap collections are not comparable.
Trap location under standing water or snow	If safe to collect, retrieve sample(s) from standing water or snow for lab processing. Do not redeploy traps into standing water or snow; pitfall trapping is not designed to function in those conditions.  All traps that are not reset need Sampling Impractical records for the bout that they are not deployed.	Reduced specimen collection may impact diversity and abundance data products.
Sample Shipping delayed >45 days	Notify ^List-CLA	Delayed data availability

<sup>1</sup>Example: beetle collection normally occur every other Tuesday. During the 3<sup>rd</sup> bout, field crews only recover and reset 20 of the traps. The next day (Wednesday), they return to the site and collect the catch from the remaining 10 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.

#### 4.5 Missed or Incomplete Sampling

Sampling according to the schedule is not always possible, and multiple factors may impede work in the field at one or more plots or sampling locations in a given bout. For example:

- Logistics – e.g., insufficient staff or equipment
- Environment – e.g., deep snow, flooding, inclement weather, or
- Management activities – e.g., controlled burns, pesticide application

Instances such as those listed above must be documented for scheduling, tracking long-term plot suitability, and informing end users of NEON data availability. Some types of missed sampling are due to events that should be recorded in the Site Management App; refer to the Site Management and Disturbance Protocol for more detail (RD[06]).

#### Missed or Incomplete Sampling Terms

Terms that inform Missed or Incomplete Sampling include:

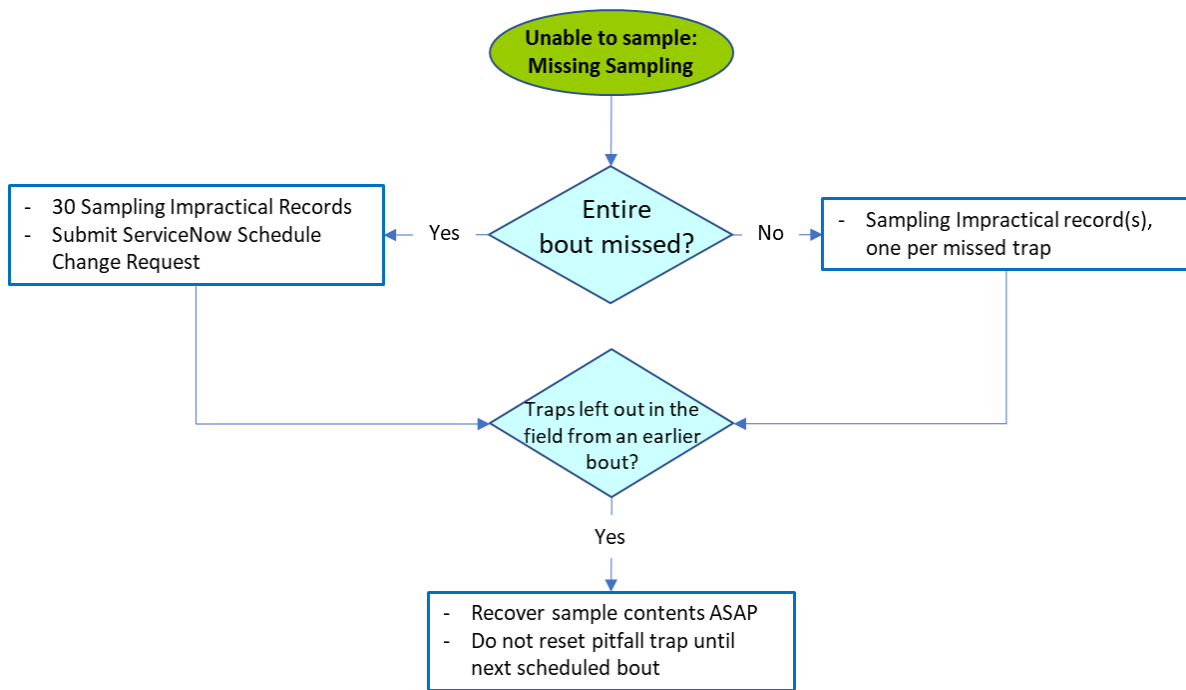
- **Protocol Sampling Dates:** Acceptable ground beetle sampling dates (**Appendix C**).



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- **Scheduled Sampling Dates:** Ground beetle sampling dates scheduled by Field Science and approved by Science. These dates coincide with or are a subset of the Protocol Sampling Dates.
- **Missed Sampling:** Incidence of *scheduled sampling* that did not occur. Missed Sampling is recorded at the same resolution as data that are ordinarily recorded.
- **Incomplete Sampling:** Incidence of *scheduled sampling* that partially occurred. Incomplete sampling is recorded at the same resolution as data that are ordinarily recorded.
- **Sampling Impractical:** The field name associated with a controlled list of values that is included in the data product to explain a Missed Sampling event – i.e., why sampling did not occur.
- **Rescheduled:** Missed Sampling is rescheduled for another time according to one of the scenarios documented in **Figure 2**, resulting in no change to the total number of sampling events per year.

The documentation that must accompany missed sampling depends on the timing, subsequent action, and the audience appropriate for numerous scenarios (**Figure 2**).



**Figure 2.** The documentation to account for a Missed Sampling event depends on the situation for each sampling unit (trap) not sampled per bout that is not sampled. Diamonds represent decision points and boxes describe the required action. Required actions may include: a) Submitting a ServiceNow incident, b) creating a Sampling Impractical record, c) creating a Site Management record, or some combination of (a) – (c).

**To Report Missed or Incomplete Sampling:**

All sampling bouts are assigned an EventID (Site.Year.Week) in the mobile data entry application. The EventID is generated based on the *setDate* assigned to the collection. Example: SJER bout deployed



2021-05-21 has an eventID SJER.2021.20 All traps deployed on this date will have the same eventID identifier even if collection delays occur.

For every date that a trap is set, a record will be created in the Field Collection application. If all traps are set normally, no additional information is required at the time of deployment. If one or more traps are not reset on the scheduled sampling date, additional sampling impractical information is required for each trap to document the occurrence and cause for the missing resets.

Some Sampling Impractical events cause traps to be deployed >14 days at a time. When a trap is deployed for an extended duration, a sampling impractical record is still required for each of the extra bouts that the trap is not reset on schedule.

1. Missed Sampling where an entire bout was not sampled as scheduled must be communicated to Science via Service Now.
  - a. To Reschedule Missed Sampling, approval by Science and Operations is required (**Figure 2**); use a Schedule Change Request Form in Service Now. Depending on available staff, funding and seasonal temperatures, missed bouts may be rescheduled to a later time in the season.
  - b. Use **Figure 2** to determine required actions if scheduled activities are canceled.
2. Missed sampling Fulcrum records must be created for each trap that is not reset according to the planned schedule. Since a bout begins at the moment the trap is set/reset, sampling impractical records are generated off of the set date. (See **Table 4** for data driven examples).
  - a. For each Missed Sampling record, the **Sampling Impractical** field must be populated in the mobile collection device (**Table 5**). The setDate and collectDate for these records will be the scheduled set and collection dates (**Figure 3**).
  - b. A new field collection record must be made for each trap not set, if an entire bout is missed (no traps are reset) then 30 records need to be entered.
  - c. Data in downstream applications (e.g., Lab apps) are not recorded. For example, if samples were not collected at all, no entries would be made in the ethanol rinse or sorting invertebrate bycatch fields in the app.
3. Missed Sampling may cause some traps deployed in the field in a prior bout to have an extended trapping duration (>14 days). Collect the samples from these deployed traps as soon as possible.
  - a. Do not reset traps with new preservative until the next scheduled bout.
  - b. Complete the Fulcrum records that correspond to the delayed trap's set date. These records should already exist in Fulcrum.
  - c. When a cup is deployed for an extended duration, a new Sampling Impractical record is still required for each of the scheduled trap reset events that are missed (**Figure 3**). The Sampling Impractical reason associated with the record (**Table 5**) should be the reason that the cup wasn't reset on schedule during that interval.



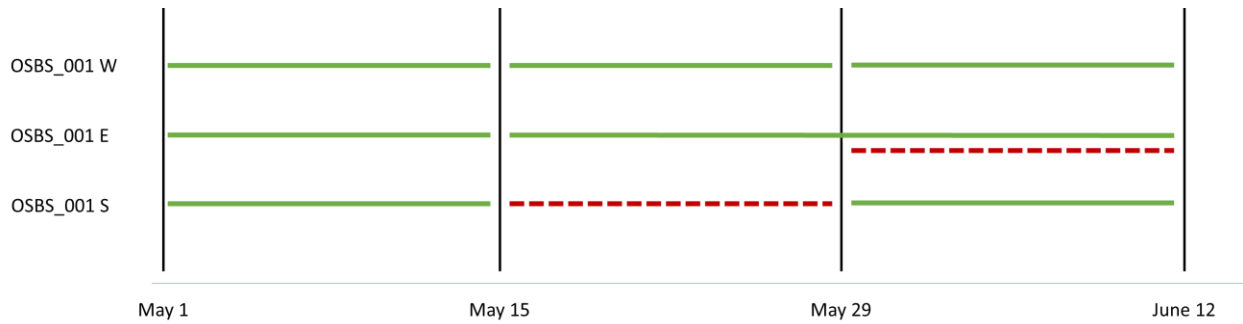
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**Table 4.** Examples of missed and incomplete sampling and the data actions required to document the occurrence. For each scenario, all traps are initially set on June 1. June 15 is the subsequent scheduled sampling event.

Scenario	Data Actions
<p>Collection delayed 2 days (June 17).</p> <p>Traps are not reset until next scheduled field event (June 29)</p>	<p><u>June 1:</u> Create 10 plot records; set date = June 1.</p> <p><u>June 15:</u> Create 10 plot/30 trap records indicating Sampling Impractical reason. Set date = June 15; Collect date = June 29.</p> <p><u>June 17:</u> Update 10 plots records (created on June 1) with the collection information for all 30 traps. Sampling Impractical = OK for all traps. Do not create new plot records.</p> <p><u>June 29:</u> Create 10 plots records; set date = June 29. If an individual trap is NOT set, add a trap-level record and indicate the Sampling Impractical reason.</p>
<p>Collection delayed until next scheduled collection event (June 29).</p> <p>On June 29, traps are collected and reset (on schedule).</p>	<p><u>June 1:</u> Create 10 plots records; set date = June 1.</p> <p><u>June 15:</u> Create 10 plots/30 trap records indicating Sampling Impractical reason. Set date = June 15; Collect date = June 29.</p> <p><u>June 29:</u> Update 10 plot records (created on June 1) with the collection information for all 30 traps. Sampling Impractical = OK for all traps.</p> <p>Create 10 new plots records; Set date = June 29. If an individual trap is NOT set, add a trap-level record and indicate the Sampling Impractical reason.</p>
<p>Partial collection delayed 2 days (June 17).</p> <p>Delayed traps are not reset until the next scheduled collection (June 29).</p> <p>NOTE: This scenario applies to both partial plot-level delayed sampling and partial trap-level delayed sampling.</p>	<p><u>June 1:</u> Create 10 plots records; set date = June 1.</p> <p><u>June 15:</u></p> <ul style="list-style-type: none"> <li>• <u>For the collected/reset traps:</u> Update corresponding plot records with the collection information for the serviced traps. Sampling Impractical = OK for the collected traps. Create new plot records for the plots in which traps are reset; set date = June 15.</li> <li>• <u>For the non-collected/non-reset traps:</u> Create corresponding plot records and indicate the Sampling Impractical reason for each trap. Set date = June 15; Collect date = June 29.</li> </ul> <p><u>June 17:</u> Update the remaining plot records (created on June 1) with the collection information for the serviced traps. Sampling Impractical = OK.</p> <p><u>June 29:</u> Update plot records (created on June 15) with the appropriate collection information for the serviced traps. Sampling Impractical = OK.</p> <p>Create 10 new plot records; set date = June 29. If an individual trap is NOT set, add a trap-level record and indicate the Sampling Impractical reason.</p>



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**Figure 3.** Use of Sampling Impractical records at a single plot, depicting a trap with an extended bout duration. Dashed red lines represent Sampling Impractical records; solid green lines represent sampling records where traps were deployed. In this example, the east trap at plot OSBS\_001 could not be collected on time (May 29) and its data record for the May 15 set has an extended duration. A sampling impractical record is still required for east trap for the missed May 29 reset.

**Table 5.** Protocol-specific Sampling Impractical reasons entered in the Fulcrum application. In the event that more than one is applicable, choose the **dominant** reason sampling was missed.

Sampling Impractical reason	Description
OK	No known issue (default value)
Extreme weather	Events (e.g., thunderstorms, hurricanes) that compromise safety and access; may require site management and disturbance record
Location burned	Location cannot be sampled because of fire; requires a site management and disturbance record
Location flooded	Standing or flowing water too deep to complete sampling
Location snow covered	Location snow covered
Logistical	Site or plot access compromised, staffing issues, errors (e.g., equipment not available in the field)
Management	Management activities such as controlled burn, pesticide applications, etc.
Temperature low	Ambient temperature lower than requirements specified in protocol
Other	Sampling location inaccessible due to other ecological reason described in the remarks

#### 4.6 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





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submitted. Please note that if sampling at particular locations requires significantly more time than expected, Science may propose to move these sampling 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.

**Table 6.** Estimated staff and labor hours required for implementation of the Ground Beetle Sampling protocol.

SOP	Estimated time	Suggested staff	Total person hours
SOP A: Preparing for sampling	1 hr/bout	2	2 hrs/bout for each site
SOP B: Initial Deployment	0.75 – 1.5 hrs/plot	2	8 – 16 hrs/site
SOP C: Field Sampling	1 – 2 hrs/plot	2	2 – 4 hrs/plot
SOP D: Laboratory processing – Sorting Vertebrate Bycatch	3 - 6 hrs/bout	2	3 - 6 hrs/bout
SOP E: Laboratory processing – Sorting Invertebrate Bycatch	0.25 - 1 hr/trap	4	10 - 30 hrs/bout
SOP F: Laboratory processing – Identifying Ground Beetles	0.5 - 2 hrs/trap	2 - 3	10 – 24 hrs/bout
SOP G: Laboratory processing - Pinning	5 mins/beetle ( <i>not including drying time</i> )	2	8 – 40 hrs/site per year
SOP H: Laboratory processing - Pooling	10 mins/plot	3	1.5 – 3 hrs/bout
SOP I: Data entry and verification	1 - 2 hrs/bout	1	1 - 2 hrs/bout
SOP 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)



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## 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**

### **6.1 Training Requirements**

All technicians must complete required safety training as defined in the NEON Training Plan (AD[04]). Additionally, technicians must complete protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[05]).

Training for field techs will include practice performing data entry at various levels. 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.

### **6.2 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.



## 7 STANDARD OPERATING PROCEDURES

### SOP Overview

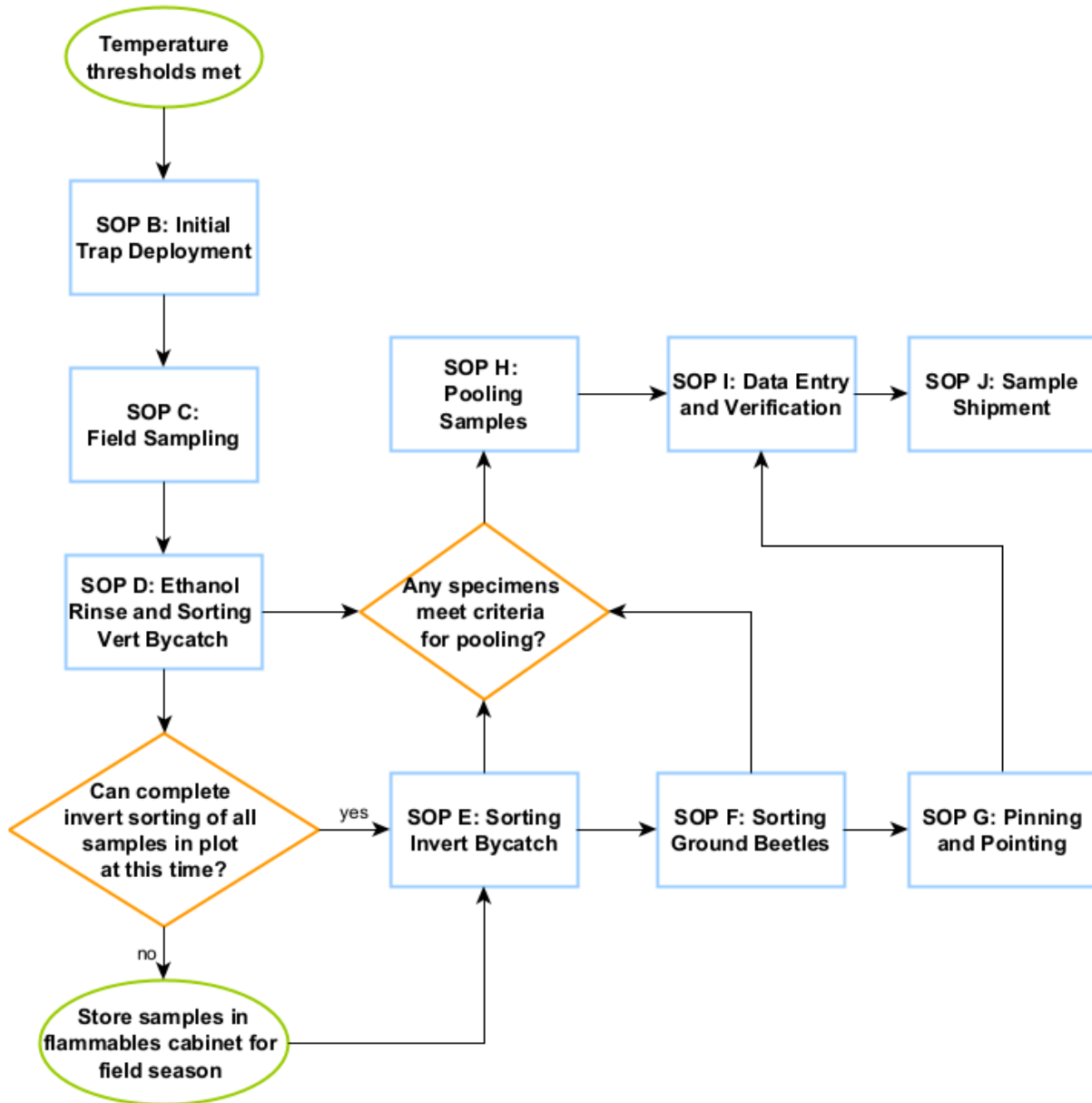


Figure 4. A high level workflow diagram that visually shows how the separate SOPs are sequentially connected.



## **SOP A     Preparing for Sampling**

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

Mobile applications are the preferred mechanism for data entry. Mobile devices should be fully charged and synced at the beginning of each field day, whenever possible.

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.

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. Cut/drill holes in bottom (exterior) containers (16 oz. deli cup) and draw 150 mL and 250 mL fill lines on upper (interior) containers.
4. Prepare sample collection and processing containers by affixing Type I adhesive barcode labels to required containers (i.e. final sample vials and collection Whirl-Paks).
5. Generate and pre-print locality labels. Labels should be cut into strips of the same date/location and inserted into the appropriate Whirl-Pak ahead of sample collection. See SOP A.3 for further instructions regarding Labels and Identifiers.
6. Locate other trap materials including pitfall trap spacers and washers (if necessary) to hold up cover.
7. Review/prepare checklist of materials needed for pitfall sampling.

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

### **A.2        Preparations prior to each sampling bout**

1. Plan and save sampling routes for field teams using standard site navigation procedures. Route planning enhances sampling efficiency and helps avoid accidental foot traffic within NEON plots.
4. Prepare bench and fume hood space in the lab for the preparation of field materials (e.g., PG). Ensure that space in refrigerator or flammable materials cabinet is available for samples. Coordinate with potentially conflicting activities (e.g. soil sieving).



5. Assemble field equipment at least one day prior to field sampling.
6. 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 bottom 1/3 of each marked Whirl-Pak or use a Whirl-Pak bag which has already been labelled with a barcode.
7. 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.
8. 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.
9. Prepare locality labels (see 0).
10. Charge all electronic equipment (e.g., GPS unit, rangefinder, mobile data entry device).
11. Prepare and clearly label one liter bottles of propylene glycol: water solution (500 mL PG:500 mL water, either distilled or deionized). A minimum of 5 L of this solution is required to fill 30 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 (requires 8 L).
  - a. Bottle labels should include: Contents (e.g., “50% propylene glycol”), date, and initials.
12. Prepare additional DI water for the field. Each team member will need one wash/squirt bottle for rinsing and an additional 1L wide mouth bottle for refilling.

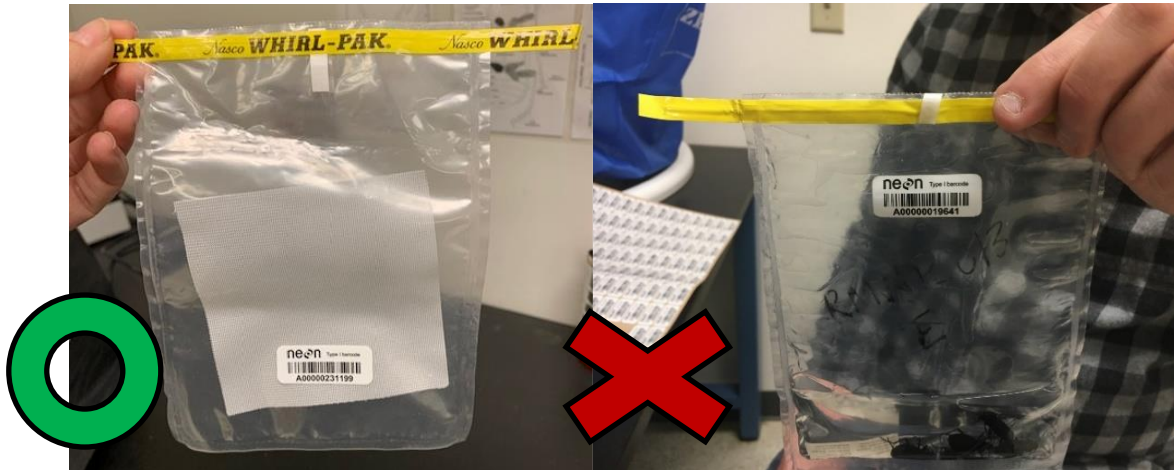
### A.3 About Labels and Identifiers

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). Type I (prefix A, plus 11 numbers) are for all field samples and any non-cryo applications; they have a tolerance from 4C to 105C.

- For vials, barcode labels should be oriented vertically, not horizontally wrapping around the vial; the scanner will not work on a curved surface.
- For Whirl-Pak bags, the barcode should be affixed to the outside on the bottom third of the bag and whirled such that barcode label is not enclosed in the whirled section. **If the barcode label is bent or enclosed, the barcode is more likely to fall off.**



- 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 5.** (left) Correct placement of Type I barcode on Whirl-Pak. (right) Barcode placement in the **wrong** part of the Whirl-Pak; this will tend to make the barcode peel after unrolling.



**Figure 6.** An example of a Type I barcode. These large-size, field-tolerant barcodes have a prefix of 'A' followed by 11 numbers.

**About Barcode Uses and Placement**

This protocol generates mixed samples from the field that may include target species (e.g., carabids) and non-target bycatch (e.g., vertebrates, non-carabid invertebrates). All bycatch is archived in 95% ethanol. Depending on quantities and species composition, carabids may also be curated in 95% ethanol or archived as a pinned voucher specimen. Non-target bycatch and carabids may be pooled with other subsamples of the same type, bout, and plot of collection to save on space when archiving.

Although it is always acceptable to use barcodes, in some cases barcodes are absolutely required. **Table 7** 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, vial samples whose final disposition is the NEON Biorepository or an external laboratory must have a barcode affixed to assist in shipping and receipt.



**Table 7.** Barcode requirements for sample types generated by the Ground Beetle Sampling protocol.

Sample Type	Description	Example Identifier	Fulcrum App	Container Type	Barcode Required?
Trap level Field samples	Unsorted samples; may contain vertebrates, carabids and non-carabid invertebrates	CPER_001.E.20180904 ( <i>plotID.trapID.collectDate</i> )	BET: FIELD SAMPLING	Whirl-Pak	<b>Always Required</b>
Trap level Vertebrate subsamples	Vertebrates sorted out of mixed field samples	CPER_001.E.20180904.PEMA.01 ( <i>plotID.trapID.collectDate.taxonID.tubeNumber</i> )	BET: LAB PROCESSING	50 mL vial	Required if final sample is not pooled
Trap level Carabid subsamples	Carabids sorted out of mixed field samples	CPER_001.E.20180904.PASELO.01 ( <i>plotID.trapID.collectDate.taxonID.tubeNumber</i> )	BET: LAB PROCESSING	50 mL vial	Required if final sample is not pooled
Trap level Invertebrate subsamples	Non-carabid inverts sorted out of mixed field samples	CPER_001.E.20180904.IB.01 ( <i>plotID.trapID.collectDate.taxonID.tubeNumber</i> )	BET: LAB PROCESSING	50 mL vial	Required if final sample is not pooled
Pinned Beetles	Beetles pinned from sorted Carabid subsamples	NEON.BET.D10.000120 ( <i>NEON.BET.domainID.sixDigits</i> )	BET: LAB PROCESSING	Pinned specimen	Not required
Pooled Vertebrate subsamples	Vertebrates sorted out of mixed field samples	CPER_001.20180904.PEMA.01 ( <i>plotID.trapID.collectDate.taxonID.tubeNumber</i> )	BET: LAB PROCESSING	50 mL vial	<b>Always Required</b>
Pooled Carabid subsamples	Carabids sorted out of mixed field samples	CPER_001.20180904.PASELO.01 ( <i>plotID.collectDate.taxonID.tubeNumber</i> )	BET: LAB PROCESSING	50 mL vial	<b>Always Required</b>
Pooled Invertebrate subsamples	Non-carabid inverts sorted out of mixed field samples	CPER_001.20180904.IB.01 ( <i>plotID.collectDate.taxonID.tubeNumber</i> )	BET: LAB PROCESSING	50 mL vial	<b>Always Required</b>



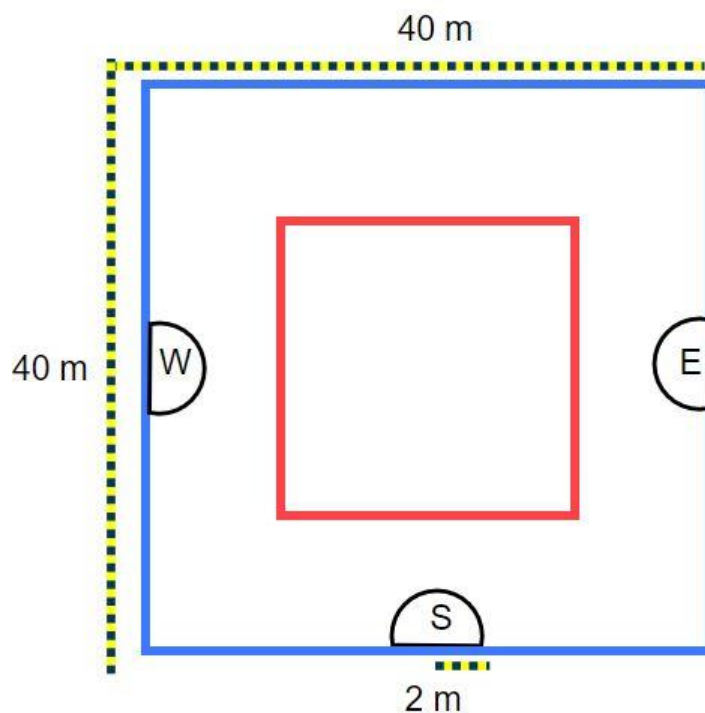


## SOP B Initial Deployment of Pitfall Traps

Pitfall traps are deployed at ground level with the lip of the container flush with the ground. Do not deploy traps into a location that is currently flooded, snow covered, or is otherwise inaccessible.

See Appendix A and Appendix B 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 installation of each trap, you may deploy the trap within 2 m 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.



**Figure 7.** Standard beetle plot layout with the target trap locations at each cardinal (S, E, and W) indicated. This 2-meter radius only applies to area within the plot.

3. Dig a small hole with a soil knife (**Figure 8**). Start smaller than the diameter of the pitfall trap cup; it is much easier to achieve a tight fit by enlarging a hole that is too small than backfilling a space that is too large. 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, pre-bore holes for the spikes with a hammer, using the traplid as a template.
5. The pitfall cup comprises two nested plastic containers: the bottom cup pre-drilled with holes, the top cup without holes. Push the bottom container into the hole in the ground. Slide the top container into bottom container. **Ensure that the lip of the top container is flush with the ground so the cup lip is not sticking up above the ground surface (Figure 10).** If the trap is not flush at ground level, insects will walk around the trap rather than fall inside. Make sure there is no gap between the containers and the ground. The containers should fit snugly into the hole.
6. Use the pre-drawn line to fill the top container with 150 mL of diluted propylene glycol (PG) solution.



*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 8.** Digging a hole for the pitfall trap.

7. Position cover with four plastic spikes and PVC spacers so that it is visually level and 1.5 cm above the surface of the containers (**Figure 9**).



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- a. Label the top of the cover with the name of the plot (e.g., OSBS\_001) and trap (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) If soils are loose (waterlogged or high sand content), PVC spacers may not adequately maintain the cover at 1.5cm above the ground. As an optional modification, a non-metal washer can be placed beneath PVC spacers between the soil and the ground. This provides more surface area and prevents the cover from collapsing on the pitfall cup.
8. 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.
9. **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.
- 
10. Once one trap in one of the cardinal directions has been set, work along the edge of the 40 m<sup>2</sup> 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).
11. For each trap, record data in the mobile data entry device (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.





**Figure 9.** Positioning cover over pitfall trap. Note the pitfall is not sufficiently dug into the ground in this image. The lip should not stick up above the soil surface.



**Figure 10.** 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 11.** Fastening down pitfall cover. Note the spacer below the cover.



### SOP C Field Sampling

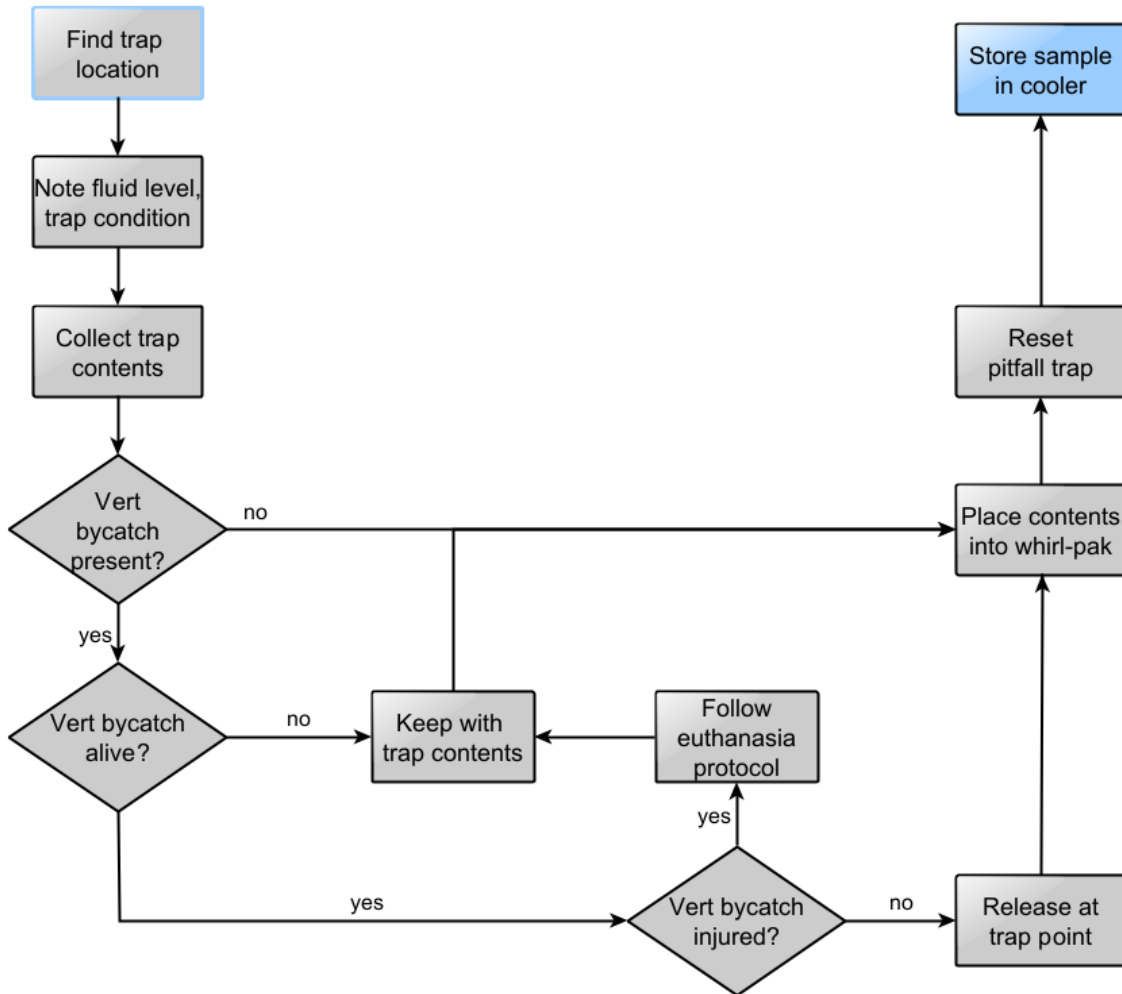


Figure 12. An expanded diagram of the workflow for a single field sampling SOP.

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

Upon arrival at the trap:

1. Record data in the mobile data entry device or (if inoperative) 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. Select the appropriate setting record. Ensure all traps that have been set previously have a Sampling Impractical field indicated as “OK”.
3. Note the condition of the trap using the **lidStatus**, **cupStatus**, and **fluidLevel** fields.



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- a. Lid Status: Used to indicate if the 20 x 20 cm trap cover is still in place.
  - “OK” indicates the lid is in place at the appropriate height above the cup.
  - “Missing” indicates the lid is not present.
  - “Disturbed” indicates that the cover is still present but no longer placed 1.5 cm above the trap.
- b. Cup Status: Used to indicate if the upper cup is flush with the ground and sample is likely to be well preserved.
  - “OK” indicates cup appears level with the soil surface with no apparent problems.
  - “Missing” indicates that the upper cup is not present.
  - “Disturbed” indicates that the cup is present, but is no longer flush with the ground.
  - “Disturbed, cup previously flooded” indicates (in addition to any other disturbance) that the cup was also likely subject to heavy water flow. This may be determined qualitatively by the appearance of water up to the brim of the cup, large quantities of standing water nearby or large amounts of silt in the cup from run off or heavy rains. In borderline situations, it is always acceptable to use “Disturbed” over “Disturbed, cup previously flooded”.
- c. Fluid Level: Used to indicate if preservative is present in the trap and at what level.
  - “OK” indicates that between 150 and 250 mL of liquid are present in the cup.
  - “High” indicates a volume above 250 mL.
  - “Low” indicates a volume less than 150 mL.
  - “None” indicates no fluid present.
4. Record the date of collection and whether a sample is present in the trap. NOTE: the Sampling Impractical will not change even when a sample is not present (i.e disturbed).
5. If the upper pitfall cup is missing and the bottom container does not obviously contain beetles or fluid, skip to **SOP C.2**. Otherwise continue to the next steps to collect a sample regardless of fluid level (e.g., no preservative, flooded conditions, etc) and even if the trap appears empty.
6. [OPTIONAL] Put on disposable gloves to protect your hands from the materials used. Gloves can be reused.
7. Pick up the upper cup containing the sample, leaving the lower container in the ground.
8. 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. It is not necessary to remove all debris at this time.



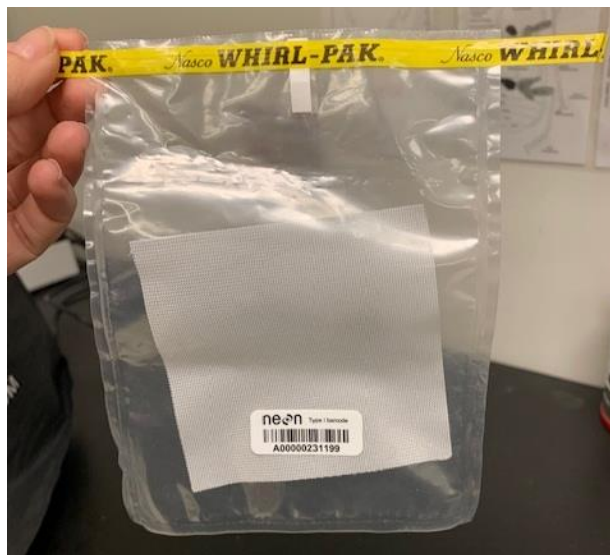
- In the unlikely event that you find live vertebrate bycatch in the trap, attempt to remove and release the animal. Watch the animal for two minutes to verify its ability to move of its own volition. If the vertebrate cannot move and appears injured, euthanize the animal with isoflurane and perform 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. At least one technician per field team must be trained in the administration of isoflurane and performance of cervical dislocation prior to implementing this protocol.

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





**Figure 14.** (Above) A packed field sample. (Below) A post-processing sample. Note the whirl of the pack needs to be tight and corner tabs tightly folded to reduce leaks. Storing samples upright in hard-sided containers can also reduce spills.

12. Place the PG solution from the pitfall trap directly into the Whirl-Pak container and seal tightly. 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. If samples are present and there is no preservative in the cup, use a small amount of DI water in a squirt bottle to rinse the sample off the cup and into the Whirl-Pak.

After each sample is collected, the PG solution needs to be removed and replaced with ethanol within 24 hours (**SOP A**).

13. Tightly roll or whirl the Whirl-Pak bag shut and close the bag's twist ties to prevent leaks.
14. Store the Whirl-Pak samples in the shade (e.g., in a backpack) until you return to the field vehicle. To protect samples further, consider storing samples in hard-sided container before transporting in field backpack.



**NOTE:** It is extremely important to ensure that the Whirl-Pak is tightly sealed by either whirling or rolling the top. Fold the tabs over tightly and/or twist together to secure. Always store Whirl-pak bags upright to reduce the likelihood leaks. Loosely or haphazardly rolling the whirl-pak will result in the potential loss of sample and damaged specimens for downstream lab analysis.



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15. 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).
- a. Sample Condition: Use this field to indicate problems with sample condition not otherwise captured by the cup status, lid status, and fluid status quality flags.
- By default, assumed to be ‘OK’.
  - “Handling error” indicates the sample has not been handled directly according to the protocol, however the sample is not necessarily compromised to the extent that downstream analysis is affected (e.g., sample is not chilled with ice packs in the field, ethanol from a whirl-pak leaks leaving specimens only partially saturated).
  - “Damaged, analysis affected” indicates the sample has serious/severe damage associated with it (e.g., carrion beetle damage to sample, preservative levels so low that sample is now crusty and dried out, etc).
  - “Sample incomplete” indicates a partial sample (most common situation for this flag is if the cup gets dropped in the field and only part of the sample is recovered).
  - “other” is a catchall for any other issue with sample condition.
- b. Sample Fate: By default, assumed to be ‘active’; indicates that the sample was generated and exists (is only entered when ‘sample collected?’ = ‘yes’); use sample fate of “lost” to indicate that the sample went missing between the field and final processing.

## C.2 Resetting the Pitfall Trap

After each sample is collected, the pitfall trap is reset with new preservative so that it can collect new specimens. Do not reset traps in locations that are currently flooded or snow covered, as pitfall traps will not perform as designed in those conditions. Use the Sampling Impractical workflow (**Section 4.5**) to document missing sampling events for instances where traps cannot be reset on schedule.

1. Using new solution prepared in the lab, refill the PG mixture in the container to 150 mL line. If less than 150 mL of PG solution was present 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.



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4. Create a new data record with the set date for the newly reset pitfall trap.
5. Repeat this procedure until the contents of all 30 traps have been collected and each trap has been reset.

### C.3 Sample preservation

1. Before leaving the plot, 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). 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.
4. In the lab, samples must be stored in the fridge (4°C) prior to removal of vertebrate bycatch. Do not use a freezer, as the Type I barcodes may fall off at colder temperatures.
5. Filter sample with ethanol **within 24 hours** of collection and remove vertebrate bycatch (**SOPA**).
6. After vertebrate bycatch are removed, samples containing the remaining invertebrates must be maintained at room temperature in flammable safe storage.

### C.4 Collecting the final sample of the field season

1. Remove all trap components from the field and return them to the lab. Flagging material may be left at the trap location if site host and permitting allow.
2. Backfill holes with local substrate. These same holes will be excavated in following years for pitfall trapping.



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**SOP D Laboratory Processing – Ethanol Rinse and Vertebrate Bycatch**

**D.1 Timing of Sample Processing**

Propylene glycol must be removed from each sample **within 1 day of collection** ( $\leq 24$  hours). At this stage, all vertebrate bycatch must also be removed from the sample and curated in ethanol.

For samples collected at sites requiring overnight travel or without access to a laboratory area, this SOP may be performed at the time of trap collection or directly after collection as long as all samples are converted to ethanol and vertebrates are removed within 24 hours.

- The NEON ground beetle sampling protocol relies on timely data entry related to vertebrate bycatch; it is a requirement of the IACUC and permitting authorities that the quantity and identity of vertebrate bycatch be documented without delay.
- Identification of vertebrate bycatch to species-level (where possible) should be conducted during or soon after the ethanol rinse stage.
- If time and personnel allows, invertebrate bycatch can be removed from the sample during the ethanol rinse (see **0** for details).

Following the rinse, all specimens are stored in ethanol at room temperature. Final specimen processing (vertebrate bycatch, invertebrate bycatch and carabid beetles) must be completed within 4 months of the end of the field season.

**Reporting requirements for vertebrate bycatch**

Most reporting of vertebrate bycatch is satisfied by normal data entry in the Fulcrum application, but additional documentation in Service Now is required in 3 instances: **(Figure 15)**.

1. **If  $\geq 15$  of a species is captured in a plot:** NEON’s IACUC requires notification when 15 or more individuals *of any given vertebrate species* have been collected, cumulatively, within a single plot **each** field season.
  - a. If the above criteria are met, NEON staff must create a Incident ticket to document this event. 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).
  - b. NEON Science will deliver a recommendation to the Battelle IACUC, which will make a determination as to mitigation measure(s) to implement, if any.
2. **Captured quantities exceed permit:** NEON collection permits contain lists of allowable quantities per species that NEON is authorized to collect each field season; if NEON collections exceed the threshold for any species in a permit for a given location, NEON staff must create a



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problem ticket to document this issue so that the NEON Permitting department can address the issue with the relevant regulatory body.

3. **Any RTE species is captured**: The bycatch of rare, threatened, or endangered (RTE) species will involve specialized handling that depends on the permitting at each site.
  - a. Should any RTE species be caught, consult site-specific permits and notify both NEON Science and NEON Permitting of the RTE species that was captured via an Incident ticket in Service Now.
  - b. Captures of RTE species may result in limited action, such as the temporary suspension of sampling at the plot in which that species was captured, or extensive remediation (e.g., deactivation of a whole site for the remainder of the field season). Any response will necessarily be site- and species-specific.

See below for example scenarios that do, and do not, require the creation of an Incident ticket:

*Example 1:* Fifteen leopard frogs are caught in HARV\_001 during bout 1. An Incident ticket must be created 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 10 more are caught in HARV\_001 during sampling bout 7. An Incident ticket is required prior to the next sampling bout.

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

*Example 4:* Eight leopard frogs and eight shrews are caught in HARV\_001 pitfall traps over the whole season. No Incident ticket is required.

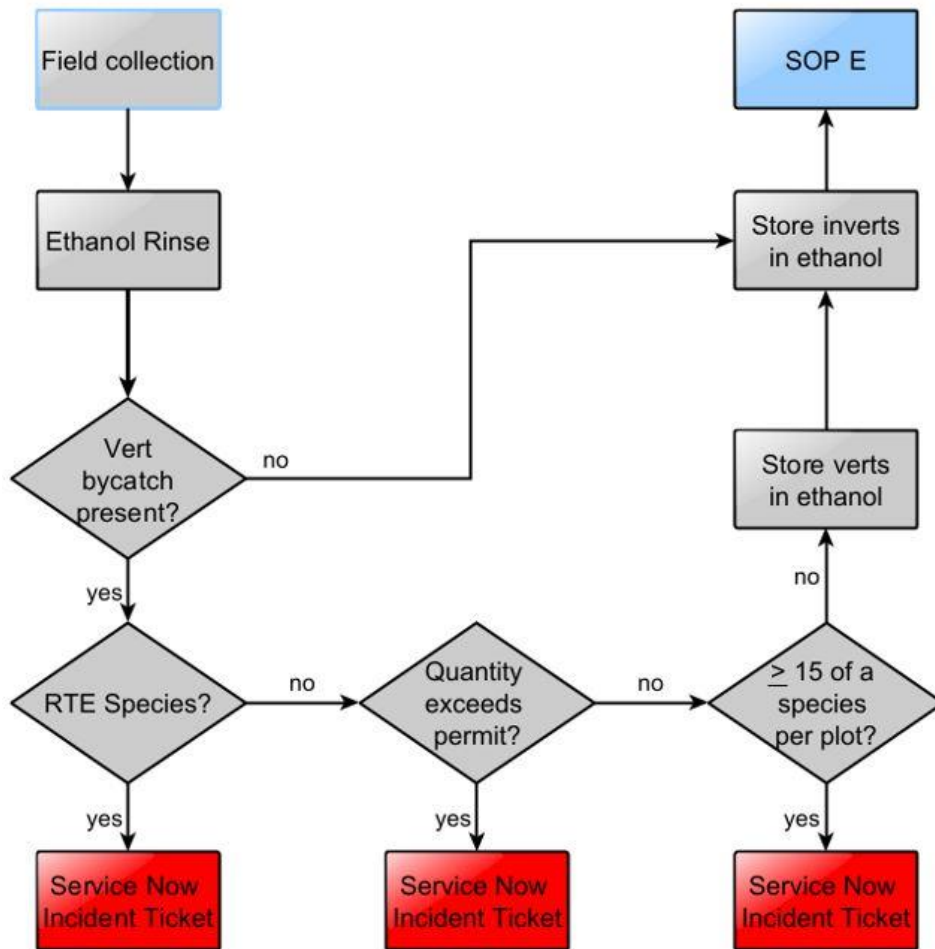


Figure 15. Decision workflow for creating a problem ticket to document vertebrate bycatch each field season.

## 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: Lab Processing [PROD]’ (or, if unavailable, on the paper datasheet for specimen identification). In addition, all vertebrate bycatch must be removed from the pitfall sample at this time (with taxonomy and quantities specified in the data), so that large, soft-bodied animals do not degrade the quality of the etOH in the pitfall sample.

If vertebrates are found and removed during the filtering process, refer to **SOPD.3** for processing and storage instructions.

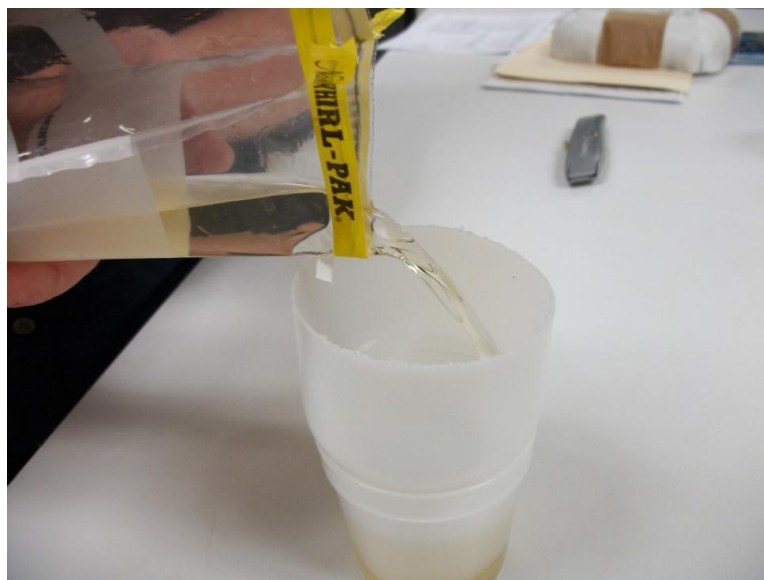
1. [Recommended but optional step] Put on nitrile gloves.
2. Prepare the filter assembly apparatus by attaching a new mesh filter cloth to the top of a modified Nalgene (Figure 16).





**Figure 16.** Mesh filter cloth fitted to the top of a modified Nalgene bottle.

3. For each trap, pour the sample (including all specimens and diluted propylene glycol) from the field Whirl-Pak through a clean filter assembly and into an ethanol waste container (**Figure 17**). Rinse specimens thoroughly with 95% ethanol over the filter cloth until all specimens have been transferred from the Whirl-Pak (**Figure 18**). Use additional 95% ethanol to rinse down the sides of the Whirl-Pak if specimens are stuck to the bag.
4. Remove all vertebrate bycatch from the trap, identify specimens to species-level, where possible, and process according to **SOP D.3**.



**Figure 17.** Filtering diluted ethanol off sample into a specimen cup (a temporary waste container).



**Figure 18.** Pouring sample through filter directly into larger waste container.

5. (OPTIONAL) 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.
6. Place the filter cloth, locality labels and any invertebrates (including carabid beetles) back into the original Whirl-Pak, keeping the filter with the sample in case small beetles and bycatch are stuck to it.
7. Pour fresh 95% ethanol into each Whirl-Pak bag, ensuring that all of the insects are submerged.
8. Confirm that the appropriate labels are still in the Whirl-Pak bag.
9. Tightly seal the bag, leaving as little airspace as possible. Failure to tightly whirl/roll the whirl-pak will result in ethanol leaking from the bag over time.
10. Keep all traps from within a plot together in individual resealable bags. Store all Whirl-Pak bags from the same sampling date upright in a labeled airtight plastic container (or similar) (**Figure 19**) until further processing (sorting, pinning, etc., detailed in the following steps).
11. Store processed invertebrate samples at room temperature in a flammables cabinet.







**Figure 19.** Samples from one sampling bout stored in a sealed container. Label containers using lab tape and permanent markers.

### D.3 Processing Vertebrate Bycatch

All vertebrate bycatch is recorded such that each *taxon* from each trap has its own child record which accurately reports:

1. The sampleID of the trap from which it originated.
2. The subsampleID and barcode of the destination tube.
3. The number of vertebrates of that taxon transferred from that pitfall trap *to the destination tube*.

In general, this means that at least 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 in a given tube.

If the paper datasheet is used, each unique subsample receives its own line in the datasheet.

#### Processing Vertebrate Bycatch Procedure

1. Separate individuals by taxon and place each taxon into the minimum number of 50 mL tubes required to hold them. Do not combine individuals from other traps. Wrap the top of the tube threads clockwise with Teflon tape. This creates a seal between the vial and the lid to prevent ethanol leakage.
2. 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

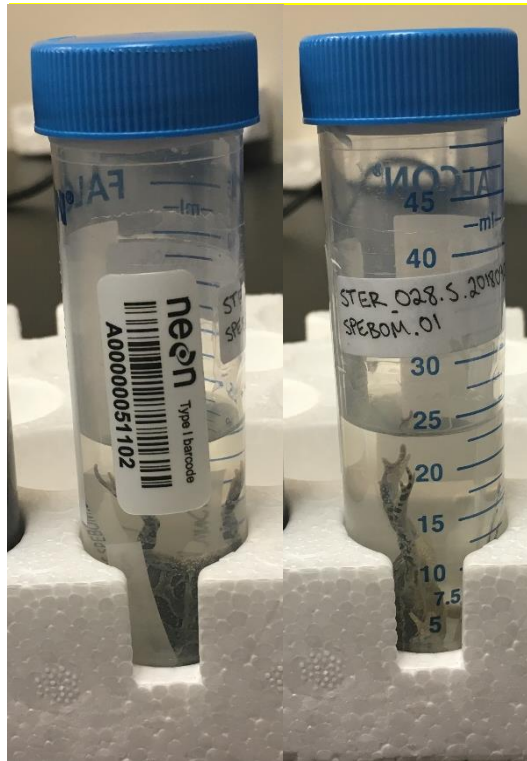


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the level of the specimens, split the specimens into multiple tubes to ensure that there is 1/2 inch or more of ethanol above the specimens in each tube.

3. Include the following labels inside each tube (**0**): a locality label (a label with the location where the specimen was collected) and a determination label (e.g., label with the taxonomic ID of the specimen).
4. Record data about vertebrates directly into the data entry application or (if unavailable) on the paper datasheet (RD [05]). Local and/or state permits should be consulted for reporting requirements of vertebrate bycatch (**Figure 15**). Refer to the ‘Manual for Fulcrum Application: TOS Ground Beetle Sampling [PROD]’ for full details on appropriate electronic data entry. For each tube generated, NEON staff record:
  - a. The source sample (e.g., the barcode on the field sample) and identifier for the tube the vertebrates are placed into. Where multiple tubes are generated from the same trap and contain varying quantities of the same taxon, tube numbers (.01, .02) are used to differentiate subsamples.
  - b. Taxonomic information, which includes:
    - 1) The sample type (mammal or herptile bycatch)
    - 2) The taxon ID, or a morphospecies ID (if applicable; see **section SOP F.4** for instructions on morphospecies naming conventions)
    - 3) Date of identification, and
    - 4) Identification references.
  - c. Quantity: the number of individuals of that taxon that were in that trap and placed in the subsample tube.
5. Unless this sample is pooled with other specimens from the same plot (see **SOP H** for directions on pooling), then this tube will be the **final archive vial** for the specimen. All final archive containers must have both a barcode and an exterior archive label (**Figure 20**). Scan the barcode label of the vial into which the vertebrates have been placed to associate it with the data record. Take care to make sure that the physical label and electronic record match.

*Example:* If barcode ‘A0000000001’ 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’ and *not* ‘CPER\_001.W.20171031.PEMA.**02**’ or ‘CPER\_001.**E**.20171031.PEMA.01’.
6. Store processed vertebrate subsamples at room temperature in the flammables cabinets.



**Figure 20.** Barcode and subsampleID labels are placed onto SPEBOM herpetile subsample. If this sample is NOT pooled (i.e., this is the final container for this specimen), the tube requires a barcode.

### About sample storage and pooling within plots

NEON always reports vertebrate quantities and taxonomy at the trap level. After completing SOP D for a single plot, 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) should *only* be done when:

- The specimens are identified to species level
- The small mammal lead or beetle lead has confirmed the taxonomic ID of the vertebrates.
- Pooling would *reduce* the number of vials used to archive the sample. Otherwise, samples should only contain individuals from a single taxon and single traps.



#### D.4 Details about species identifications

The NEON taxon list of vertebrate species codes can be found in Fulcrum (herptileTaxa\_allDomains, mammalTaxa\_allDomains). 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 a carabid.



## 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. Ethanol waste storage.
  - b. Sink for washing materials.
  - c. At least one dissecting microscope
  - d. Storage space for samples (e.g., flammable storage for tubes of individuals)
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 (compared to vertebrate species) and these taxa 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, are stored separately as invertebrate bycatch (taxonomic aids to separate these groups are provided in **Appendix D**). 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 a dissecting 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.

All invertebrate bycatch will be recorded from each trap such that material from each trap has its own child record which accurately reports:

1. The **sampleID** of the trap it originated from.
2. The **subsampleID** and barcode of the destination tube.



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In general, this means that at least one subsample tube will be needed for each taxon/trap combination. However, *extra* child records are required if specimens from a single trap are split between two vials.

If the paper datasheet is used, each unique subsample receives its own line in the datasheet.

### About sample storage and pooling within plots

This SOP describes the process of separating invertebrate bycatch from carabid beetles, placing non-carabid invertebrates into one or more tubes, and generating data about invertebrate bycatch within each cup trap. NEON always reports invertebrate bycatch at the trap level. After completing SOP E for a single plot, if invertebrate bycatch is found in multiple traps from the same plot and bout (e.g., invert bycatch present in both CPER\_001 West trap and in CPER\_001 East trap collected 2018-09-03) – pooling is allowed to save on archival costs. Pooling (see **SOP H**) should *only* be done when:

- Invertebrate bycatch is completely devoid of carabid beetles
- Pooling would *reduce* the number of vials used to archive the sample. Otherwise, samples should only contain invertebrates from a single trap.

### Sorting Invertebrates Procedure

Steps for sorting invertebrate bycatch are detailed in **Figure 21**, with instructions in the following text.

1. Sort each sample individually, from one trap at a time.
2. Scan the field sample barcode to find the lab processing record for the sample being sorted.
3. 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.
4. 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).
5. Remove the filter cloth from the Whirl-Pak and use a dissecting microscope to 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.
6. 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.

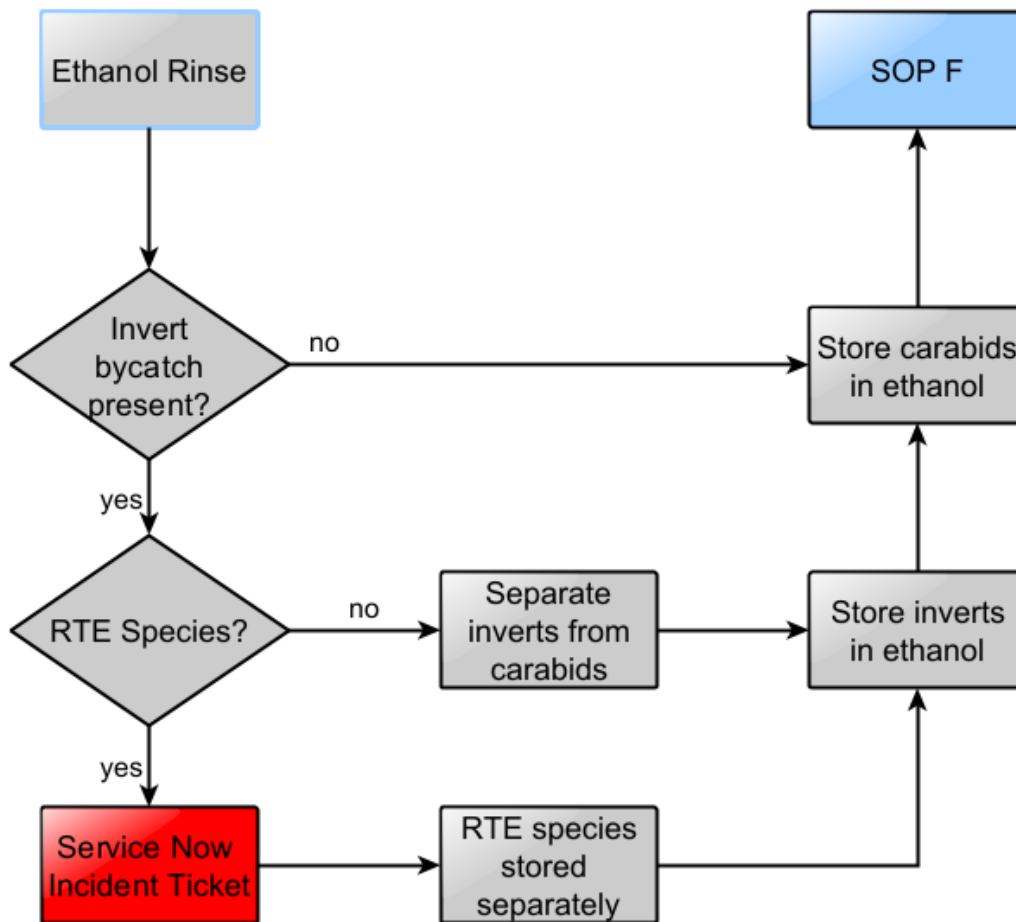


Figure 21. Workflow for processing invertebrate bycatch.

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



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10. Depending on quantity, each plot may produce:
- 1 vial with all bycatch from one trap,
  - Multiple vials with bycatch from a single trap, or
  - A pooled vial containing bycatch from multiple traps with the same plot and date.
11. When the sorted invertebrate bycatch consists of specimens from a single trap (situations 1 and 2 above), then:
- 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.
  - 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.
  - Include a locality label (e.g., label with the location where the specimen was collected; **0**) 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 (SJER.001.E.20210514.01, .02, etc.).  
  
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. Because these vials are to remain trap-level (not-pooled), a scanned barcode is required in the sorting menu for each subsample created. Take care to make sure that the physical labels and electronic records match.

Example: If barcode 'A0000000001' 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.

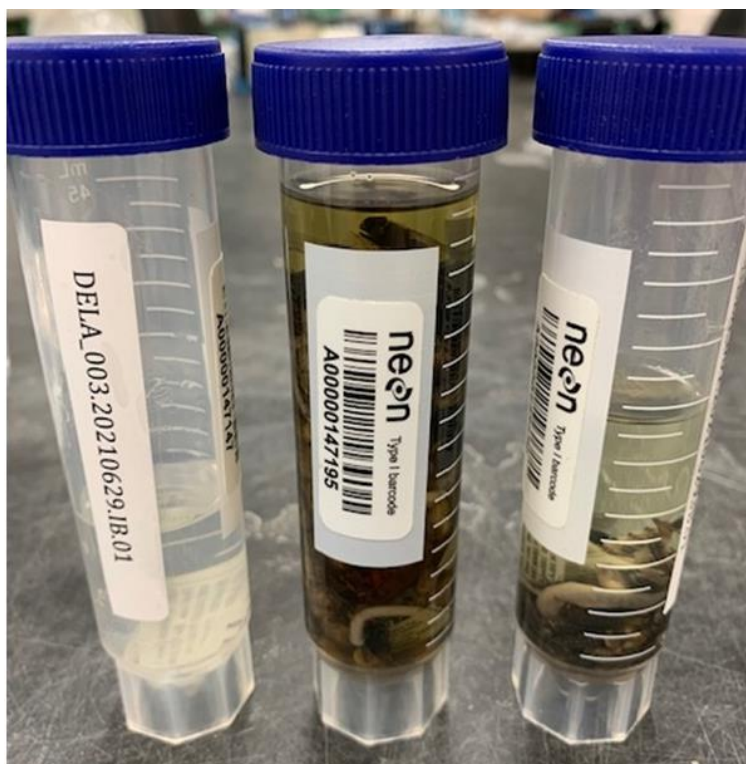
Example: Two tubes are required to hold invertebrate bycatch for a trap E. SampleIDs for these tubes are plotID.E.collectDate.IB.01 and plotID.E.collectDate.IB.02.

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





**Figure 22.** Bycatch samples stored in a 50 mL centrifuge tubes, the amount of ethanol in a bycatch sample will vary by sample size.



**Figure 23.** Sorted invertebrate bycatch. Samples have a barcode and external sample identifier.



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12. When invertebrate bycatch from the sorted subsample will be pooled with invert bycatch from other traps in the same plot (situation 3 above), then: The sorted subsample may be held in a temporary container until other subsamples from the same plot and date are complete, and then pooled immediately (**SOPH**).

**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 menu, it will be scanned later in the Pooling menu.

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.

13. Wrap the threads clockwise on the top of the tube with Teflon tape. This creates a robust seal between the vial and the lid to prevent ethanol leakage.
14. Record data directly into the data entry application 'BET: Lab Processing [PROD]' or (if unavailable) on the identification datasheet (RD [05]). *Neither counts nor taxonomic identifications are expected for invertebrate bycatch specimens.*
- a. Refer to the 'Manual for Fulcrum Application: TOS Ground Beetle Sampling [PROD]' for details on appropriate electronic data entry.
15. Store processed samples at room temperature in the flammable storage cabinets.
16. Transfer carabids (if any) from the temporary container marked 'carabids' and at least one locality label back into the original Whirl-Pak bag from the field. Submerge the carabids in ethanol and tightly seal the whirl-pak.

OPTIONAL: Carabids can be transferred to a labelled 50mL vial instead of back into the field whirl-pak. This storage method may be preferred where additional storage space is available. Special care should be taken to either preserve the field whirl-pak barcode or to carefully ensure the correct records are updated during carabid identification.

*Note: Any 50 mL tubes that were used as temporary storage may be rinsed and reused for temporary storage, assuming they are still in good condition. Reused vials that will contain final archival samples should be thoroughly cleaned with soap and water before reuse.*



## SOP F Laboratory Processing - Identifying Ground beetles

### F.1 Timing of carabid identification

After field collection, vertebrate bycatch are removed within 24 hours (discussed in **SOP A**). Invertebrate bycatch (discussed in **0**) can be separated from unidentified carabids anytime during the field season, as staff time and resources allow. After all bycatch are removed, carabid beetles are identified to species and either pinned/pointed (instructions in **SOP G**) or stored in 95% ethanol. Select pinned specimens are archived within the domain teaching collection; remaining specimens (both dry pinned and ethanol samples) are curated at the NEON Biorepository. This SOP provides instructions for beetle processing up to the pinning or pointing stage.

Carabid identifications, morphospecies, and counts are input after lab processing. Species-level sorting occurs on a trap-by-trap basis so that specimens from individual traps are kept separate through the entire sorting process. 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 and any pinning completed can groups of the same species be pooled by plot and collection date (**SOP H**).

NEON intends to provide species-level determinations for collected carabid specimens. While an inexperienced parataxonomist will require more time to perform a carabid identification compared to a very experienced identifier, it is expected that experienced staff should spend no more than an hour identifying beetles of a given taxonomic grouping within a trap (e.g., if there are 4 taxa or 'morphs' in a given trap, do not spend more than 4 hours making determinations for those individuals).

To stay within these timelines, NEON staff are encouraged to either:

- Apply genus or subgenus identifications with morphospecies groupings for particularly difficult taxonomic groups (e.g., *Amara*, certain *Harpalus*, etc)
- Use slash species designations for difficult species complexes or difficult sister taxa.

In all cases, NEON staff should use the dichotomous keys, species checklists, and resources from **Appendix D** to perform their identifications. Specimens that receive determinations coarser than species level (e.g., genus-level, slash taxa) will be prioritized for review by an expert taxonomist. All identifications need to be completed by February 1 following the field season.

### F.2 Identifying carabids to species

1. Sort each sample individually, from one trap at a time.
2. Find the lab processing record for the sample being identified by scanning the field sample barcode into the data entry application.



3. Separate 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. Use taxonomic keys and other resources as described in Appendix D.
4. When all carabids from the trap are grouped by species, they are ready to be placed into a prepared vial. Use the minimum number of vials necessary to hold carabids of a single species from a single trap. Do **not** pool samples until all pinned carabids have been allocated; pinned specimens require known trap-level information.
  - a. Place all carabids from the same trap of the *same species or morphospecies*, into as few 50 mL tubes as possible.
  - b. Wrap the top of the tube threads clockwise with Teflon tape. This creates a seal between the vial and the lid to prevent ethanol leakage.
  - c. 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.
  - d. Include a locality label (e.g., label with the location where the specimen was collected; 0) and a determination label (e.g., label with the taxonomic ID of the specimen; see 0) in each tube.
5. Record data about carabids directly into the data entry application or (if unavailable) on the paper datasheet (RD [05]). Refer to the ‘Manual for Fulcrum Application: TOS Ground Beetle Sampling’ for full details on appropriate electronic data entry. For each tube generated, record:
  - a. The field sample barcode and tube number for the tube the vertebrates are placed into. Where multiple tubes are generated from the same trap and contain varying quantities of the same taxon, tube numbers (.01, .02) are used to differentiate subsamples.
  - b. Taxonomic information: includes the sample type (carabid), the taxon, a morphospecies ID (if applicable; see **SOP F.4** for instructions on morphospecies naming conventions), date of identification, and identification references.
  - c. Quantity: the number of individuals of that taxon that were in that trap and placed in the subsample tube.
6. 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*



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*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.

7. 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 **SOPG** 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 1<sup>st</sup> of April 2016.

8. If this sample will not be pooled (see **SOPH** 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 the physical labels match the electronic records.

Example: If barcode ‘A000000001’ 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 pooled vial into the subsample barcode field in the sorting menu; only enter a barcode in the subsample barcode field of the sorting menu 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.

9. Store processed samples at room temperature in the flammable cabinets.



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### F.3 Equipment maintenance, cleaning, and storage

After all samples have been sorted:

- Empty, wash, and dry all waste receptacles (e.g. ethanol and PG containers).
- Return all flammables to the appropriate cabinets.
- 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 and reused for temporary storage, assuming they are still in good condition. Reused vials that will contain final archival samples should be thoroughly cleaned with soap and water before reuse.*

### F.4 Handling uncertainty in species identifications

All specimens must have an identification 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 8**).
- 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.





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- An inappropriate usage of the qualifier occurs when the level of the selected identification qualifier does not match the given identification of the specimen.
  - Example: If the scientific name of a specimen is *Harpalus* sp., then the genus is known and 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.

- 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”.
- The qualifier ‘cf’ roughly equals “not sure,” whereas ‘aff’ roughly equals “similar to, but is not”.

**Table 8.** 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

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



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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 co-occur (or might co-occur) at a site. NEON adds 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 makes only species thought to be present in a domain 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.5 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





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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 27<sup>th</sup> morphospecies ID should include two letters at the end (e.g., the 27<sup>th</sup> 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 54<sup>th</sup> 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 27<sup>th</sup> morphospecies at the Utah facility will be MorphZAA.*

When identifying carabids or vertebrate bycatch, morphospeciesIDs and descriptions can be entered directly into the 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

The objective of pinning is three-fold.

1. Pinned beetles are sent to contracted taxonomic experts for identification. Specimens in ethanol are generally not eligible for expert review, but exceptions may be made for limited quantities of extremely small, delicate specimens.

Create an ‘request’ ticket in Service Now to request permission to send very delicate specimens in ethanol with all labels. If approved, the taxonomist can point the individual or maintain the specimen in ethanol, as needed.

2. Pinned beetles are a source of additional specimens for the teaching collection at each domain support facility (the reference guide of known specimens derived from field operations collections). A subset of specimens confirmed by the taxonomist are sent back to the domains each year to supplement each domain’s teaching collection.
3. Pinned individuals are available as DNA barcoding candidates.

A properly mounted insect specimen can be stored for centuries in an archive and can be used over and over for research.

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

Pinning involves mounting larger specimens directly onto a pin; pointing involves mounting small specimens onto a paper triangle on a pin (called a ‘point’) as in **Figure 24**. When the mounting process is complete, each pinned or pointed specimen is curated with labels that describe the place, date and manner of collection, the taxonomic determination of the specimen by domain staff, and the specimen identifier (see **Figure 25**). The pin of a mounted specimen always includes some part of the pin that is above the upper surface of the pinned insect or the point to allow a person to grip the pin to handle the specimen without damaging it.



**Figure 24.** Pinned (left) and pointed (right) beetle specimens.

Pinned specimens are centered over their labels as in **Figure 25** and the pin runs through the center of all labels; the head of a pinned beetle is always oriented toward the left edge of the label. Pointed



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specimens are always centered over their label (as in **Figure 24**) with the head of the beetle pointing toward the top of the locality label.

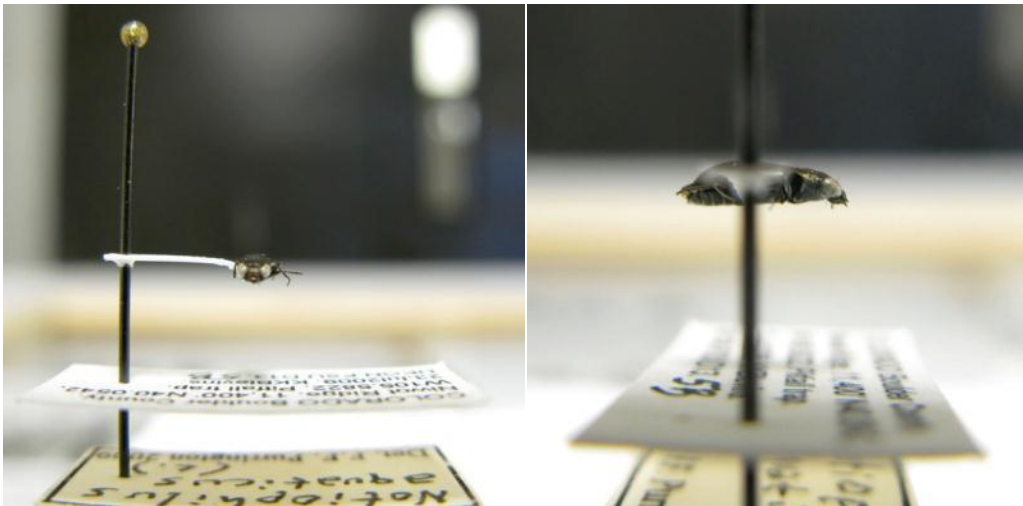
For a pointed specimen, the labels and beetle are centered over each other, but the pin is offset of center (**Figure 26**). Specimens 5 mm and greater are usually pinned and specimens smaller than 5 mm are pointed (**Table 9**). However, pointing is a good default option for any specimen you are concerned will be destroyed by pinning. If you cannot put a No. 2 archival quality pin through the beetle safely, then the beetle should be pointed rather than pinned.

**Table 9.** 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 25.** View of a pinned specimen from above (left) and the side (right). The order of material on a pin is always: specimen at the top, the locality label, the NEON staff determination label, and lastly the individual ID of the specimen. The pictured specimen has been returned to the domain teaching collection after review by an expert taxonomist, so an extra determination label is at the bottom of the pin.



**Figure 26.** A pointed beetle specimen, with associated labels. The pin should be at least a centimeter from the right edge of the label; a pin that is too close to the label edge could rip.

## G.2 Specimen selection

There are substantial differences between the long-term storage costs for individuals stored in ethanol and a pinned specimen. Due to this cost disparity, if fewer than 10 carabids would be slated for long-term storage in a single vial of ethanol, it is more cost effective to archive pinned individuals than to archive those carabids in ethanol.

All individuals that do not qualify for pooling (**SOP H**) are to be pinned or pointed.

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. Because more than 10 are present in one subsample and all 15 fit into one vial, these specimens can be maintained in ethanol at the trap level.

*Example 2:* 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 the same plot and date. Because more than 10 individuals will be present in one archiveVialID, these specimens will be pooled according to SOP H into a single vial and maintained in ethanol.

*Example 3:* 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.



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*Example 4:* Four *Pasimachus elongatus* are captured in each trap of plot HARV\_001 (12 individuals total) with the same collection date. However, only 5 *Pasimachus elongatus* fit into a vial. Because they cannot be consolidated into a single vial of ethanol that would have more than 10 individuals in it, all 12 individuals will be pinned.

*Example 5:* 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.

Data for each pinned or pointed specimen should be recorded directly into the data entry application ‘BET: Lab Processing [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.

### G.3 Prepare labels for all specimens

See 0 for instructions on creating the following labels required for pinning.

1. Locality labels. These are created for all mounted beetle specimens during field preparation and will be placed on the pin below each specimen.
2. 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.
  - a. About Quantities: determination labels can be pre-printed in bulk at the start of the season with the name of each determiner for the domain and the year of collection, sufficient to create 20 labels of every taxon from each site’s species checklist. These labels can be cut to size and stored in an organizational box with the pinning supplies. Additional labels can be made during the season on an as needed basis. When all pinning of a field season’s material is complete for all sites in the domain, any unused pre-printed labels must be discarded.
3. 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. Individual IDs are unique to each beetle specimen and must never be repeated.
  - a. About Quantities: individual ID labels can be printed in bulk at the start of the season, cut to size, and stored in a small box with all pinning supplies. Make a note on the box of the last identifier used. When this stockpile of individual IDs is close to running out, additional labels can be printed. Update the note on the box to reflect the new ‘last’ identifier printed.



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.4 Preparation for mounting specimens

When first transitioning beetles from ethanol storage to a pin, the beetles are very wet and contain a lot of moisture. Specimens need to be given an opportunity to dry into final position in a safe location before labels are applied (the ‘drying box’). A dried, positioned beetle results in a compact specimen which takes up less archival space during final curation (**Figure 27**); this keeps the specimens from being damaged during handling.



**Figure 27.** Examples of pinned specimens of *Carabus goryi*. In the upper panel, pinned specimens have their legs and antennae tucked in close to the specimen and had a drying step. In the lower panel, there was no drying step, so legs and antennae are spread out.

**Prepare drying boxes prior to mounting pinned specimens** (a typical domain might need 6 total).

1. Acquire a Schmitt Box. These boxes are wooden, have a foam pinning bottom and a tight-fitting lid.



2. Cut a piece of 1-inch thick Styrofoam to size so that it exactly fits inside the Schmitt box. This depth is needed so that drying specimens can have the drying beetle body directly touching a solid surface.
3. Place a clean piece of standard copy paper (archival quality is not needed) over the Styrofoam and secure with No. 3 archival pins. The paper surface is useful for making annotations about specimens (e.g., these beetles belong to the same grouping) and keeps the beetle from sticking to the Styrofoam.
4. Drying boxes can be stored closed while not in use.

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

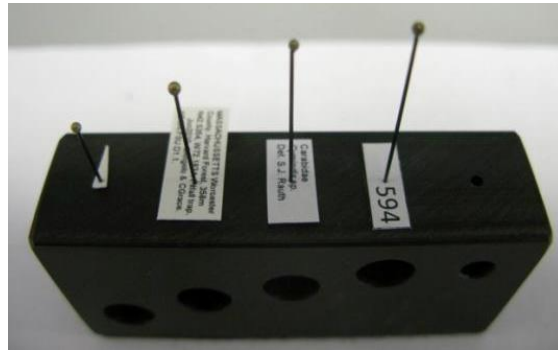
1. Using 100% rag cotton paper (Bristol or equivalent), prepare triangles using the point punch. Large quantities of paper points can be created ahead of time and stored for future use in a small box.
2. On the day that pointing will occur, points can be attached to pins so that many individuals can be pinned in sequence. Only attach as many as you plan to use that day. To attach each paper point, press a No. 3 archival quality pin into the point at the center of the point's base (see the "X" in **Figure 28**) over a balsa wood surface. Leave some space between the pin and the edge of the point base, as there is a risk of tearing if the pin is too close to the edge. Ensure that the point is at a perpendicular, 90-degree angle from the pin (not angled up nor down).



**Figure 28.** Materials for pointing including a schematic of a point showing where to place pin.

3. Use a pinning block (**Figure 29**) to push the point up the pin until it is 15 mm from the head 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.



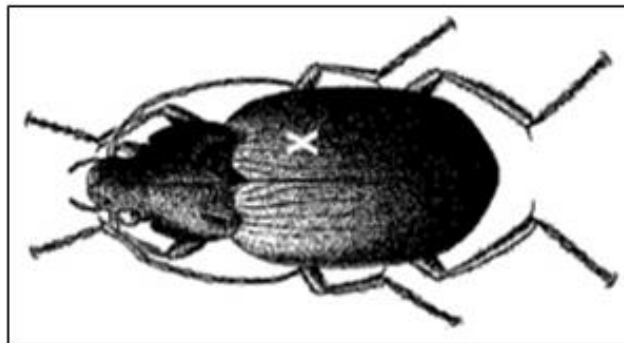


**Figure 29.** A pinning block with a point and the various labels that will be affixed to a pinned or pointed specimen. This shows the order and heights at which points and various labels should be placed on a pin.

### G.5 Mounting Specimens

Once all ground beetle identification is complete for a site (**SOPF**), specimen mounting can be performed. Note: if you are new to pinning, start with larger species as they are easier. Keep specimens in ethanol until ready to pin.

1. Prepare all required labels, set up a drying box, and (if needed) create paper points (as described in this SOP).
2. Working through one subsample at a time, remove the number of individuals that are to be pinned or pointed.



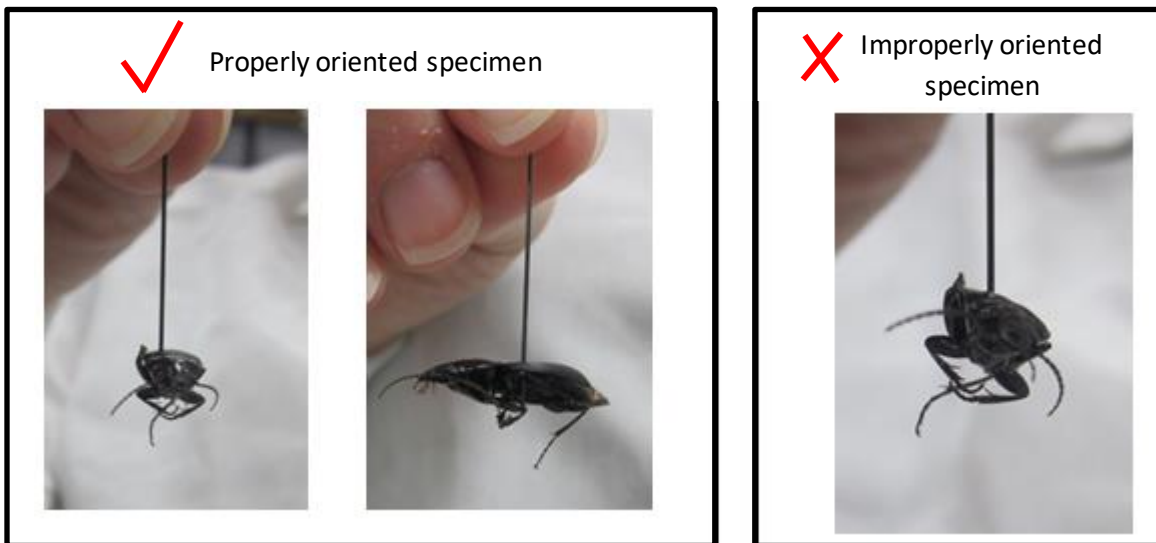
**Figure 30.** Ground beetle pinning schematic showing where to place the pin (marked by an “X”).

3. For pinned specimens:
  - a. Most pinned specimens can be mounted using No. 3 archival quality pins (No. 2 or No. 4 pins may also be appropriate depending on the beetle). Pins finer than a No. 2 are too flexible and vibrate in the specimen, destroying them from within. Pins thicker than a No. 4 will damage specimens as they are inserted. If the specimen is too delicate for a No. 2 pin, point the specimen instead.



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- b. Place the specimen directly onto a pinning surface (the prepared drying box or a piece of Plastazote foam work well for this), holding it to the pinning surface with a finger.
- c. Insert the pin vertically through the top layer of the specimen's body 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 30**). It is key that the beetle be pinned in this exact location as it allows researchers to see the most important features of a beetle unimpeded.
- d. Gently slide the pin through the body of the specimen. Push the pin slowly and with constant pressure. Ensure that the pin is at a right angle to the beetle's body and stop when the pin is about one centimeter through the body of the beetle. This step ensures the pin is firmly inside the beetle. See **Figure 31** for examples of well-pinned and poorly-pinned specimens.



**Figure 31.** Correctly and incorrectly pinned beetle specimens.

- e. Next, use a pinning block to stage the specimen at the correct height on the pin. To do this, remove the pinned specimen from the foam surface (the pin will still be inside the specimen about about a centimeter in). Place the specimen's pin into the pinning block directly over the deepest hole (this is the leftmost position in **Figure 29**). Hold the beetle in position it so that it is straight in your fingers and the legs are facing downward.
- f. Ensure that the pin is at a right angle to the beetle's body and then 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.
- g. Place the pinned specimen (now at the correct height on the pin) into the drying box so that the lower surface of the beetle body is directly touching the paper work surface of the box.



Using an extra pin or forceps, tuck the legs underneath the beetle body and move antennae back toward the main body of the beetle (**Figure 32**). Once all appendages are compactly positioned, brace pins can be used to hold legs or antennae in place.

- h. Just above each drying beetle, place all the labels that will accompany the pinned beetle onto a second placeholder pin (individual ID, determination label and locality label). After the beetle is dry, these labels will be transferred from the placeholder pin to the specimen's pin. Use the paper surface of the drying box to indicate specimen groupings as needed (e.g., beetles from the same bouts, taxa, etc).

**Note:** Now is a great time to double check that the label information is correct

- i. Let specimens dry in the drying box. Note that it takes time for beetles to dry and airflow accelerates the process. If the drying box is closed in a cabinet, specimens could be dry in 5 – 7 days. If the drying box lid is ajar, specimens could be dry in 2 - 3 days. Position the drying box (either closed or ajar) so that staff are available to continue specimen processing when the specimens are dry. Depending on the humidity of the lab space, more time may be needed to dry the specimen.



**Figure 32.** Pinned specimens drying in position within a drying box. These beetles had their legs and antennae tucked into position; brace pins are used to keep the appendages in place during drying.

4. 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 taxon, pitfall trap, and 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.

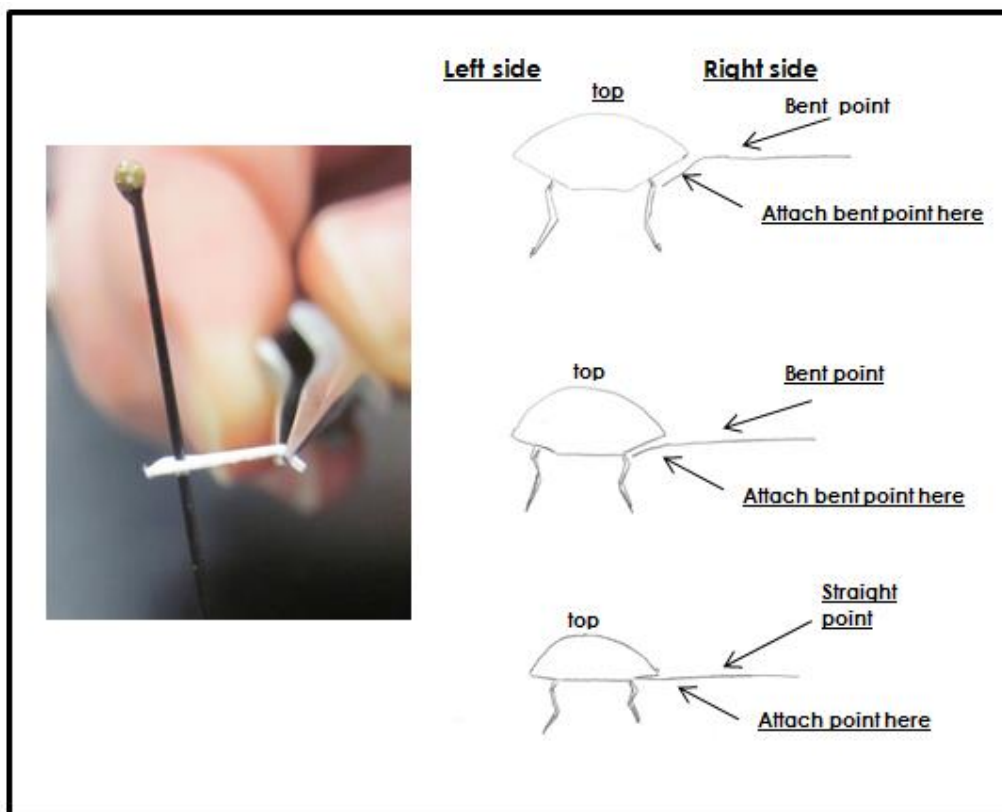


- c. Use forceps to put the beetle specimen on its left side (**Figure 33**). Examine the slope of the ground beetle's thorax to determine if the tip of the point needs to be bent to ensure that the specimen will lie flat on the point. Use forceps to bend the point if needed (**Figure 34**).

**About point angle:** The sides of a beetle's thorax can slope inward to differing degrees, therefore, the tip of the point is 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 35** for examples of well-pointed and poorly-pointed specimens, respectively.



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



**Figure 34.** Examples of bending a point tip to maintain a level body position of the specimen.

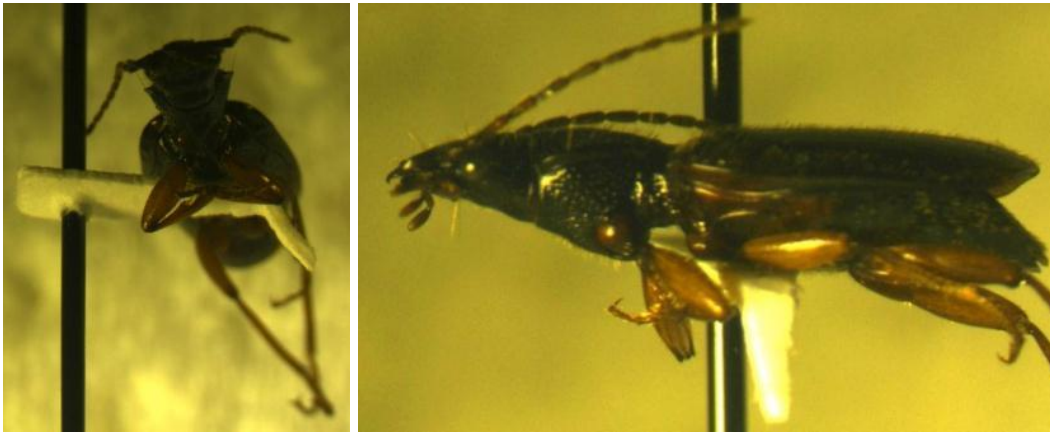


Figure 35. A poorly pinned beetle specimen.

- d. 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 36). Use the minimum amount of glue needed in order to attach the specimen firmly to the point. The glue should be a little tacky, not very runny or dry, so that a firm connection is made (Figure 37).



Figure 36. Preparing to glue a specimen to a point by dipping the tip of the point in glue.

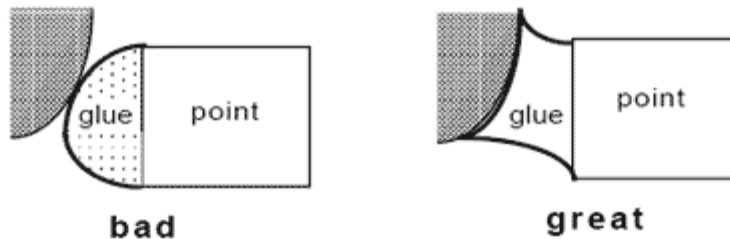
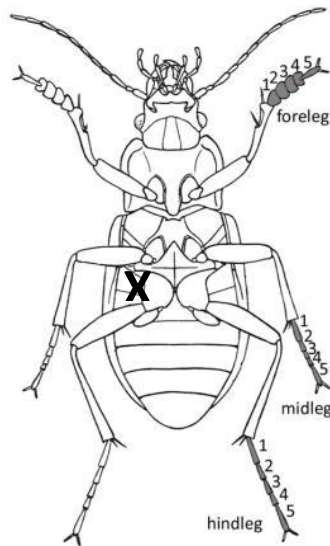


Figure 37. The glue should make good contact between the specimen and the point; the example on the left is barely attached to the glue, the one on the right has good coverage. Image credit: [entmuseum.ucr.edu/specimen\\_preparation/](http://entmuseum.ucr.edu/specimen_preparation/)



- e. 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.

**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.



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



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

- f. 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** and **Figure 39**.









**Figure 41.** Attaching a locality label below a pointed beetle.

7. Attach the determination label (instructions in **0**) 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.
8. Attach the insect individual ID label (instructions in **0**) below the determination label using the fourth-deepest (shallowest) hole. Orient the label in the same manner as the locality label.
9. When all of the specimens are mounted and labeled, examine any pointed insects 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.

Data for each pinned or pointed specimen should be recorded directly into the data entry application 'BET: Lab Processing [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.

10. Check specimen for sign of any pests (usually indicated by seeing chewed up body parts in the drying box). If pests are present, you may need to decontaminate the box using the freezing procedure for pinned beetles (SOP **G.7**). If there's no sign of pest damage, place this fully labelled specimen into a unit tray within the Cornell cabinet.
11. Replace the paper in the drying box with fresh copy paper.

## **G.6 Loss of Body Parts**

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 42**). To do this:

1. Hold the part being reattached with forceps.
2. Touch the top of the body part to a bit of glue.
3. Place the body part back into correct location and orientation and hold until the glue has set.



**Figure 42.** A leg being inserted back into its socket, using forceps and a dab of glue .

### G.7 Sample Preservation

Pinned and pointed ground beetle specimens are to be stored in airtight Schmitt boxes or in unit trays in Cornell cabinets. Annually, the entire collection of pinned beetles held at each domain for long term storage should be put in a -80 °C Ultralow freezer for 48 hours to kill any insect pests that may damage the specimens. This process is particularly important for beetles stored in more humid environments.

DO NOT freeze recently pinned specimens until they have dried completely (>7 days).

In addition to annually freezing the entire collection, specimens should be routinely checked (minimum every 6 months) for evidence of dermestid beetle damage. Typical signs include debris under a specimen or larval skins of dermestid beetles are present. **If any dermestid or other pest damage is perceived at any time, immediately freeze the entire drawer/box for 48 hours .**

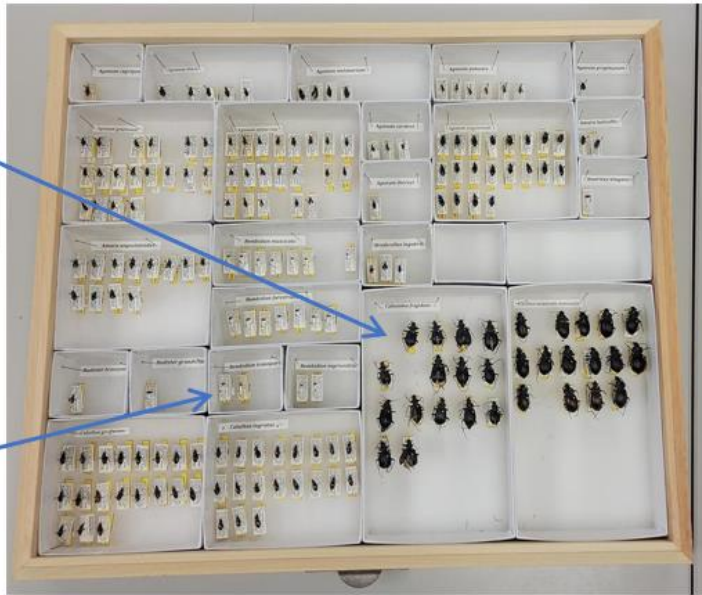
1. After a specimen has been pinned or pointed, and any glue has dried, it should be placed in a Schmitt box or unit tray so that locality labels are oriented in the same direction as those of the other specimens in the box or tray (**Figure 43**).
2. Specimens should be grouped into unit trays within 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.
3. 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 (**Figure 43**).



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



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



**Figure 43.** Proper orientation of mounted specimens in unit trays within a Cornell drawer. Also note entire Cornell box is filled with unit trays to prevent movement.

4. Specimen storage containers and workspaces should be kept clean to prevent pests.
5. Periodically check for dermestid damage to specimens. If damage is perceived, place the entire drawer/box in a tightly-sealed garbage bag and place in a  $-80^{\circ}\text{C}$  Ultralow freezer for 48 hours. (**Figure 44**).



**Figure 44.** Cornell drawer properly prepared for decontamination and placed in the Ultralow freezer. Ensure the trash bag is tightly sealed to keep moisture and condensation away from the box.



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6. After the 48-hour freeze, clean up any damage/debris from under the specimens. Allow the drawer to thaw, wiping away any moisture from the glass lid if it appears. Return the drawer once all specimens have reached room temperature.
7. All specimens in a collection must be frozen in this way on an annual basis to preserve specimen integrity.
8. Never leave boxes or drawers sitting out, put them away when you are not working on them.
9. Keep counters clean, always wipe down with ethanol before putting a box or drawer on a countertop.
10. Minimize amount of time lids are off drawers; never put drawers or lids on the floors.
11. 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.



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## 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 a plot **may not be conducted** until all lab processing data has been entered at the trap level for all subsamples to be pooled.

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 only if the identification is to the species or subspecies level.

Carabids are preferentially pooled by plot and collection date when more than 10 individuals of a species are present when combined from each trap and all 10 individuals can fit into a single 50ml vial.

If invertebrate bycatch from one trap will not fill a vial, it can be pooled with other traps from the same plot and collection date. Samples should only be pooled such that the least number of vials is needed while maintaining the lowest possible geospatial resolution possible. If it is not possible to reduce the number of ethanol vials by pooling samples, then it is *desirable* to maintain tubes filled with a single trap's contents.

Example: MOAB\_002 west trap fills an entire 50 ml vial and only a small portion of a second vial. Both the east and south traps fit into one vial without additional space for the remaining west trap contents. The west trap will have 2 non-pooled vials because pooling does not result in fewer archival vials. And the east and south traps will be pooled into 1 archival vial.

If subsample vials are to be pooled, record associated data directly into the data entry application 'BET: Lab Processing [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 datasheet).
  - a. 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.



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- b. Enter the processing date (date of pooling).
- c. 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.
- d. Specify the archiveVialID tube number (1, 2, 3, etc)

**NOTE:** Each pooled tube requires its own record. Always begin with .01 for the first vial. 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 specimens within

- e. Apply a barcode to the archiveVialID. Scan the barcode into the mobile data entry application.
- f. On the paper datasheet (to be used only when the unusual circumstance where the mobile application is not available):
  - 1) Enter plotID and collection date
  - 2) Circle the trapIDs of the tubes to be pooled
  - 3) The taxonID in the 'taxonID' field
  - 4) The tube numbers (.01, .02, etc) for each subsampleID (corresponding to trapID on the same line)
  - 5) 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. Fill each tube with enough 95% ethanol that there is at least 1/2 inch of ethanol above the fully submerged specimen(s).

**NOTE:** If there would be too many specimens to allow for 1/2 inch of ethanol above the level of the specimens, do not add these specimens to the pooled vial. Instead, leave these at the trap level and enter data according to **0**.

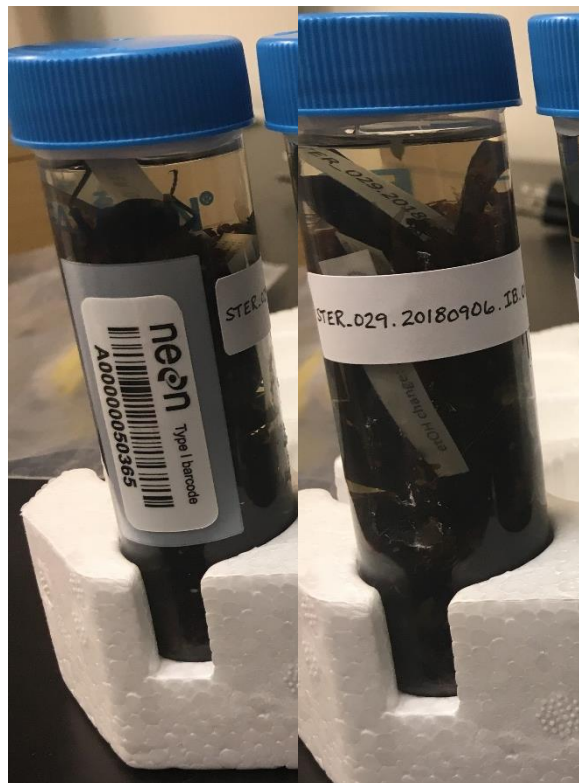
- 4. For pooled invertebrate bycatch, internally include a locality label for each of the subsamples that were included in the pool. Externally write the archivalVialID (autogenerated in the data entry application) in the format **plotID.collectDate.IB.tubeNumber** using archival ink on an ethanol-safe label.





5. For pooled vertebrate bycatch and carabids, internally include a determination label and a locality label for each of the subsamples that were included in the pool. Externally write the archiveVialID (autogenerated in the data entry application) in the format **plotID.collectDate.taxonID.tubeNumber** using archival ink on an ethanol-safe label.
6. OPTIONAL: Place a piece of scotch tape over the external label to improve long-term adhesion and reduce smudges.
7. Ensure the physical label matches the archiveVialID generated by the data entry application.

Example: If barcode 'A0000000001' 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



**Figure 45.** 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.





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## 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 be processed anytime between collection and the end of the field season; digital data transcription of *invertebrate* bycatch is therefore not required until 14 days after sorting of invertebrates occurs.

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

### I.1 Documenting the use of Paper Datasheets

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 AOS/TOS Protocol and Procedure: Data Management (RD[04]).
1. Scan datasheets and save in PDF file format.
2. 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. See detailed instructions for recording missed and incomplete sampling (Section 4.5).



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## I.2 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[04]) and included in the QAQC Checklist available in the Sampling Support Library.

## I.3 Sample Labels and 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 to use 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.**

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. Mobile devices should be synced at the end of each field day,



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

#### I.4 Document Incomplete Sampling Within a Site

Ground Beetle sampling is scheduled to occur at all prescribed sampling locations according to the frequency and timing described in **Appendix C**. Ideally, sampling will occur at these sampling locations for the lifetime of the Observatory (core sites) or the duration of the site’s affiliation with the NEON project (gradient sites). However, sampling may be shifted from one location to another when sampling is compromised. In general, a sampling location is compromised when sampling becomes so limited that data quality is significantly reduced.

There are three main pathways by which sampling can be compromised and plot relocation is warranted:

1. Ecology of the plot is no longer suited to answer meaningful biological questions (i.e., a terrestrial sampling plot becomes permanently aquatic).
2. Logistics and access prevent regular sampling. (e.g., distance to travel, safety considerations). For the ground beetle sampling program, a given plot **must** be sampled at least 50% of the bouts expected for the site (see **Appendix C** 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.
3. Vertebrate Impact. NEON Science reviews vertebrate bycatch impacts on an annual basis for its Institutional Animal Care and Use Committee (IACUC) members. The NEON Principal Investigator for the ground beetle program evaluates and reports the role of plot location as it relates to annual vertebrate bycatch quantities, the composition of vertebrate bycatch (highlighting captures of any RTE, IUCN red list or other sensitive status species), and value of that plot location to the ground beetle sampling data product. NEON will consult with the IACUC membership to determine what reallocation of plots may be needed on an annual basis. NEON Field Science staff do not need to issue separate problem tickets to trigger this review.

A problem ticket should be submitted by Field Science staff if they recognize that a sampling location is compromised (as defined above).

To document locations that may need replacement:



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1. Review Fulcrum records to determine which locations were scheduled for sampling but were not sampled.
2. Create an incident with the following naming convention to document the missed sampling: ‘TOS Plot Relocation Needed: BET – [Root Cause Description]’
  - a. Example: ‘TOS Plot Relocation Needed: BET – Could not access plot due to permanently closed road’
3. Staff scientists review incident tickets periodically to determine replacement sampling locations if a sampling location is compromised.



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## SOP J Sample Shipment

This protocol requires shipment of samples to external facilities. Refer to ‘NEON Protocol and Procedure: Shipping Ecological Samples and Equipment’ (RD[08]) for detailed instructions on proper sample shipment.

### J.1 Choosing pinned specimens for shipment

All samples stored in ethanol are sent to the NEON Biorepository for archive. A subset of pinned specimens are sent for secondary identification by a taxonomist; all remaining pinned or pointed individuals are sent directly to the NEON Biorepository. Specimens sent for taxonomic confirmation must be intact, as a badly damaged specimen (e.g., one with a missing head) is not identifiable, even by experts.

In a typical year, this process will result in sufficient material from all terrestrial sites to send a total of 9400 specimens for taxonomic confirmation. NEON staff must complete pinning for all sites processed at their support facility and complete all data QC before shipping specimens for taxonomic review. Quantities in **Table 10** reflect the expected number of pinned beetles per site that experts will annually review.

- To account for annual variation *at the site level*, Domain Support Facilities will ship up to the maximum quantity specified *for their domain*.
- EXCEPTION: For Support Facilities that process samples across multiple domains, the maximum quantity is generated by the sum of the site-level expected quantities for the sites they process.

When pinned beetle quantities at a domain are lower than the caps set in **Table 10**, NEON staff will ship **all** pinned beetles to the expert taxonomist according to the master schedule (see Shipping Protocol, RD[08]).

If a domain has more beetles pinned than reflected in **Table 10**, NEON staff will ship the total quantity allowed for their domain using the following criteria for selecting specimens and using the site-level expected quantities as a guide. Do Not Exceed Domain-Level Caps.

1. Send up to 20 specimens *per site per taxon* for all fine-scale identifications. These are specimens that are identified to species or subspecies level. If fewer than 20 specimens of a taxon are available from a site, then **all** individuals from that site will be sent.
2. Send 100 specimens *per site per taxon* for all coarse-scale identifications. These are specimens that were *not* identified to species. This includes any specimens identified to a species group (‘slash taxa’), subgenus, genus, tribe or subfamily within the family Carabidae.
3. If additional space remains to fill domain-level maximum, send all remaining coarse-scale identifications (if available) followed by more fine-scale identifications focusing on cryptic



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species, individuals with identification qualifiers, or commonly misidentified species until capacity is reached.

Annually, NEON Science will review unused capacity from sites with lower-than-expected quantities to ensure that a minimum of 7600 specimens are sent for review to each taxonomic lab. If this minimum is not projected to be met, domains with greater-than-expected quantities may be instructed to send a second smaller shipment of additional pinned individuals using the criteria above. After this review, all excess pinned specimens will be sent to the NEON Biorepository no later than April 15.

**Table 10.** Number of pinned specimens to be sent for taxonomic review per domain. Site-level expected quantities are based on data from taxonomic experts in 2019. Domain-level shipment caps are to be strictly adhered to.

Domain	Site	Site-level Expected	Domain-level Maximum
D01	BART	250	470
	HARV	220	
D02	BLAN	350	1100
	SCBI	300	
	SERC	450	
D03	DSNY	135	350
	JERC	90	
	OSBS	125	
D04	GUAN	25	110
	LAJA	85	
D05	STEI	250	660
	TREE	210	
	UNDE	200	
D06	KONA	350	840
	KONZ	240	
	UKFS	250	
D07	GRSM	300	900
	MLBS	300	
	ORNL	300	
D08	DELA	180	490
	LENO	160	
	TALL	150	
D09	DCFS	250	900



Domain	Site	Site-level Expected	Domain-level Maximum
	NOGP	300	
	WOOD	350	
D10/13	CPER	300	1030
	RMNP	110	
	STER	450	
	NIWO	170	
D11	CLBJ	125	260
	OAES	135	
D12	YELL	300	300
D14	JORN	70	370
	SRER	300	
D15/13	ONAQ	100	215
	MOAB	115	
D16	ABBY	230	370
	WREF	140	
D17	SJER	85	385
	SOAP	210	
	TEAK	90	
D18/19	BARR	100	460
	TOOL	160	
	BONA	55	
	DEJU	70	
	HEAL	75	
D20	PUUM	190	190





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**8 REFERENCES**

K. Didan. 2015. MOD13Q1 MODIS/Terra Vegetation Indices 16-Day L3 Global 250m SIN Grid V006. NASA EOSDIS Land Processes DAAC. <https://doi.org/10.5067/MODIS/MOD13Q1.006>



## APPENDIX A QUICK REFERENCES

### 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. Lip of *top container* is flush with the ground



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

#### 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



#### QUICK REFERENCE: COLLECTING INSECTS FROM TRAP

---

**STEP 1** – Record collect date, plotID, and trapID of sample being collected

**STEP 2** – Remove top container

**STEP 3** – Remove large sticks or debris from sample

**STEP 4** – Verify absence of live vertebrates in trap

**STEP 5** – Transfer **ALL** organisms and locality labels to Whirl-Pak bag. **Seal tightly.**

**STEP 6** – Record all metadata and any irregularities on datasheet.

**STEP 7** – Scan the barcode on the Whirl-Pak into the electronic record

**STEP 8** – Reset the trap (if applicable).

#### QUICK REFERENCE: INITIAL PROCESSING AND VERTEBRATE 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.

**STEP 2** – Remove vertebrate bycatch, record relevant information, including the associated barcode for each subsampleID, in the mobile data entry application (or if unavailable, on paper 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** – Transfer remaining trap contents (non-vertebrate specimens, labels, mesh filter) back into original Whirl-Pak bag.

**STEP 4** – Fill Whirl-Pak bag with enough 95% ethanol to completely cover its contents, and tightly seal the bag (leaving as little air space as possible).

**STEP 5** – Store: 1) 50 mL tubes and 2) Whirl-Pak bags from the same sampling bout together.

#### QUICK REFERENCE: 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.



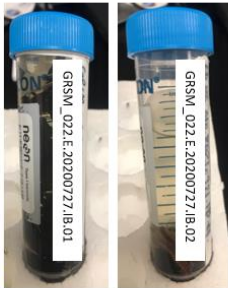



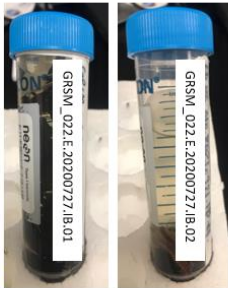



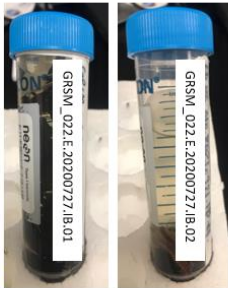





**STEP 3** – Sort specimens into two temporary containers, one marked ‘invertebrate bycatch’ and the other marked ‘carabids’. Make sure each container contains locality labels and enough 95% ethanol to keep its contents submerged.

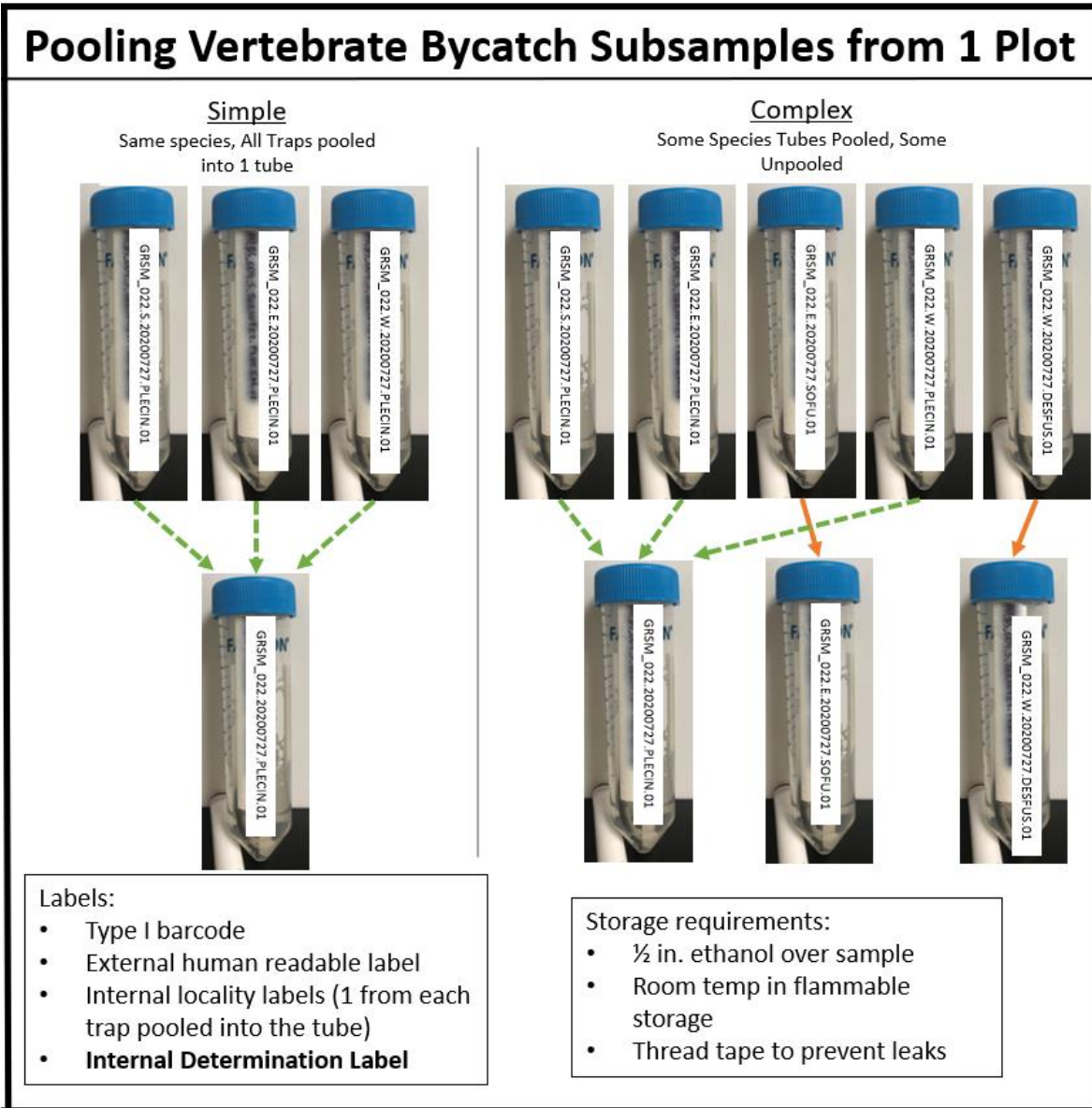
**STEP 4** – Place invertebrate bycatch in labeled 50 mL tubes, with locality labels (Be sure to associate a barcode with each subsample ID by scanning it into the record).

**STEP 5** – Return carabid beetles to the original Whirl-Pak bag or a labelled 50ml vial until ready for sorting, identification, and pinning.

**STEP 6** – Store subsamples at room temperature in flammable safe storage.

Bycatch Sorting of Field Collected Sample									
Field Whirlpak Samples	Subsamples generated from Bycatch Sorting from 1 trap Whirlpak								
<p>1 Plot, 3 Whirlpaks (E, S, and W)</p>   <p>*Samples are post EtOH rinse</p>	<table border="0"> <tr> <td> <p><b>Invertebrate Bycatch</b></p>  </td> <td> <p>One or Multiple tubes Labels:</p> <ul style="list-style-type: none"> <li>Type I barcode</li> <li>External human readable label</li> <li>Internal locality label</li> </ul> <p>½ in. ethanol over sample Store at room temp in flammable storage Thread tape to prevent leaks</p> </td> <td> <p><b>Vertebrate Bycatch</b></p>  </td> <td> <p>Labels:</p> <ul style="list-style-type: none"> <li>Type I barcode</li> <li>External human readable label</li> <li>Internal locality label</li> <li>Internal Determination label</li> </ul> <p>½ in. ethanol over sample Store at room temp in flammable storage Thread tape to prevent leaks</p> </td> </tr> <tr> <td> <p><b>Carabids</b></p>  </td> <td> <p><b>OR</b></p>  </td> <td colspan="2"> <p><b>Stored Unsorted in a tube OR in field Whirlpak</b></p> <p><u>Tube Labels:</u></p> <ul style="list-style-type: none"> <li>Optional: Type I barcode</li> <li>External human readable label</li> <li>Internal locality label(s)</li> </ul> <p>½ in. ethanol over sample Store at room temp in flammable storage</p> <p><u>Whirlpak Labels:</u></p> <ul style="list-style-type: none"> <li>Type I Barcode (from field collection efforts)</li> <li>Internal locality label</li> </ul> <p>Ethanol covering sample</p> </td> </tr> </table>	<p><b>Invertebrate Bycatch</b></p> 	<p>One or Multiple tubes Labels:</p> <ul style="list-style-type: none"> <li>Type I barcode</li> <li>External human readable label</li> <li>Internal locality label</li> </ul> <p>½ in. ethanol over sample Store at room temp in flammable storage Thread tape to prevent leaks</p>	<p><b>Vertebrate Bycatch</b></p> 	<p>Labels:</p> <ul style="list-style-type: none"> <li>Type I barcode</li> <li>External human readable label</li> <li>Internal locality label</li> <li>Internal Determination label</li> </ul> <p>½ in. ethanol over sample Store at room temp in flammable storage Thread tape to prevent leaks</p>	<p><b>Carabids</b></p> 	<p><b>OR</b></p> 	<p><b>Stored Unsorted in a tube OR in field Whirlpak</b></p> <p><u>Tube Labels:</u></p> <ul style="list-style-type: none"> <li>Optional: Type I barcode</li> <li>External human readable label</li> <li>Internal locality label(s)</li> </ul> <p>½ in. ethanol over sample Store at room temp in flammable storage</p> <p><u>Whirlpak Labels:</u></p> <ul style="list-style-type: none"> <li>Type I Barcode (from field collection efforts)</li> <li>Internal locality label</li> </ul> <p>Ethanol covering sample</p>	
<p><b>Invertebrate Bycatch</b></p> 	<p>One or Multiple tubes Labels:</p> <ul style="list-style-type: none"> <li>Type I barcode</li> <li>External human readable label</li> <li>Internal locality label</li> </ul> <p>½ in. ethanol over sample Store at room temp in flammable storage Thread tape to prevent leaks</p>	<p><b>Vertebrate Bycatch</b></p> 	<p>Labels:</p> <ul style="list-style-type: none"> <li>Type I barcode</li> <li>External human readable label</li> <li>Internal locality label</li> <li>Internal Determination label</li> </ul> <p>½ in. ethanol over sample Store at room temp in flammable storage Thread tape to prevent leaks</p>						
<p><b>Carabids</b></p> 	<p><b>OR</b></p> 	<p><b>Stored Unsorted in a tube OR in field Whirlpak</b></p> <p><u>Tube Labels:</u></p> <ul style="list-style-type: none"> <li>Optional: Type I barcode</li> <li>External human readable label</li> <li>Internal locality label(s)</li> </ul> <p>½ in. ethanol over sample Store at room temp in flammable storage</p> <p><u>Whirlpak Labels:</u></p> <ul style="list-style-type: none"> <li>Type I Barcode (from field collection efforts)</li> <li>Internal locality label</li> </ul> <p>Ethanol covering sample</p>							

**Figure 46.** Required labelling and storage requirements for all possible subsample types generated from sorting a single field whirlpak.



**Figure 47.** Required labelling and storage requirements for pooling vertebrate bycatch. Includes a simple and complex pooling example for a single plot and collection date.

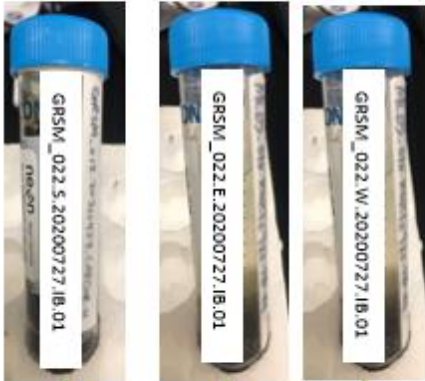




## Pooling Invertebrate Bycatch Subsamples from 1 Plot

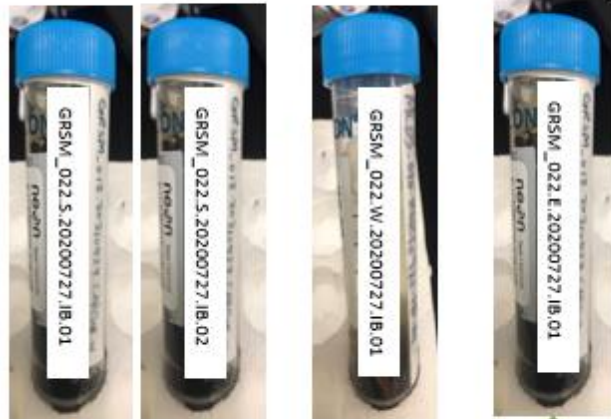
### Simple

All Traps pooled into 1 tube



### Complex

Some Trap Tubes left Unpooled



#### Labels:

- Type I barcode
- External human readable label
- Internal locality labels (1 from each trap pooled into the tube)

#### Storage requirements:

- ½ in. ethanol over sample
- Room temp in flammable storage
- Thread tape to prevent leaks

**Figure 48.** Required labelling and storage requirements for pooling invertebrate bycatch. Includes a simple and complex pooling example for a single plot and collection date.



## APPENDIX B REMINDERS

### CHECKLIST: PREPARING FOR FIELD SAMPLING

---

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 DI 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...

- Double check that your actual location matches the one on the locality label
- plotID & trapID are written on the trap cover
- Remove twigs, leaves and debris from trap
- Rinse the cup with DI water to ensure that all beetles and bycatch are transferred to the Whirl-Pak bag
- Check Whirl-Pak bag for locality labels
- Whirl/roll the Whirl-pak tightly. Always store samples upright.
- Record all metadata (plotID, date, field staff, barcode etc.) on the datasheet or 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.

#### 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 it cannot tip over during driving
- Samples are transferred promptly to the laboratory upon return from the field





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### 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 paper datasheet or mobile data entry application
- Locality labels are with specimens (in Whirl-Pak bag)
- Whirl-paks are tightly sealed, stored upright 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



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**APPENDIX C ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING**

The dates in **Table 11** are based on the most recently available decade (2005-2014) of MODIS (Moderate Resolution Imaging Spectroradiometer) EVI phenology data from NASA (Didan 2015). 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 estimates 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 11.** 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



Domain	Site	Start	End	Bouts expected
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*	14-Apr	13-Oct	13

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



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**Site-Specific Protocol Modifications**

**C.1 DOMAIN 04**

At all terrestrial sites in Puerto Rico, a modification has been implemented in response to site-specific conditions. However, unless detailed in this paragraph, sampling will occur in Puerto Rico in a way that is identical to sampling conducted at any other site. At these sites, NEON staff have received a waiver from the Battelle IACUC allowing staff members to perform this protocol without carrying isoflurane into the field. If live, moribund vertebrates are encountered at a trap, staff will use manual cervical dislocation alone. This technique (without the supplementation of isoflurane anesthetic) is acceptable when performed by individuals with a demonstrated high degree of technical proficiency. NEON implemented a training program on this method; only staff that have completed this training program may execute the field sampling portions of the ground beetle protocol. Those responsible for the use of this method must ensure that all personnel performing cervical dislocation have been properly trained and consistently apply it humanely and effectively.

**C.2 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.

**C.3 DOMAIN 10**

At Sterling (gradient 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.

**C.4 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 30 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).

### C.5 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



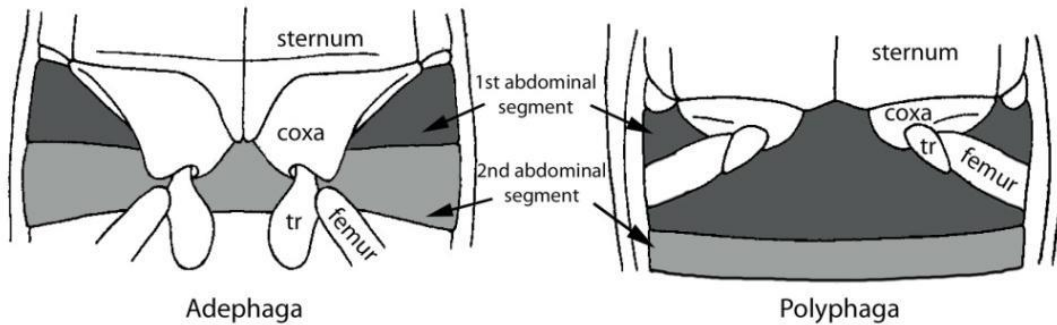
Figure 49. Hardware cloth enclosures are placed outside traps at certain PUUM plot locations.



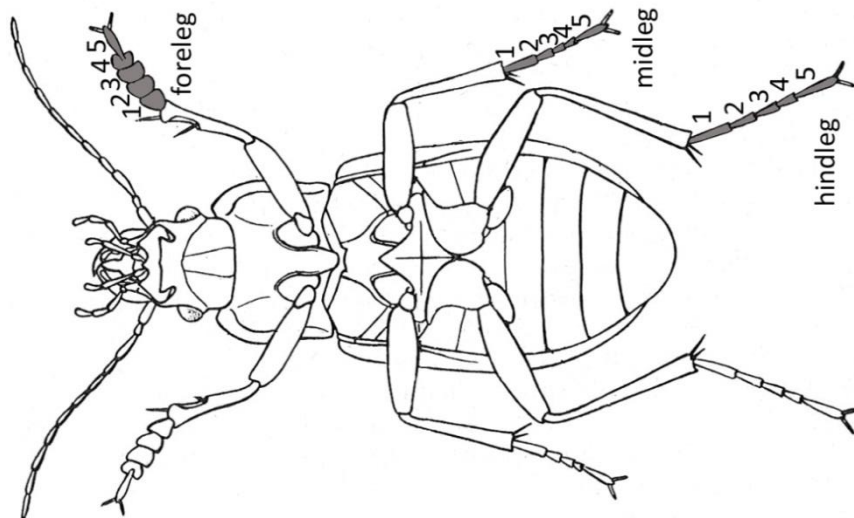
## APPENDIX D INSECT TAXONOMIC IDENTIFICATION AIDS

### D.1 Distinguishing Carabidae from other invertebrate taxa

The suborder Adephaga includes ground beetles (Carabidae) 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 50**), as well as the tarsal formula (5-5-5; **Figure 51**). 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 50.** Hind leg articulation in Adephaga (which includes Carabidae) and Polyphaga (invertebrate bycatch).



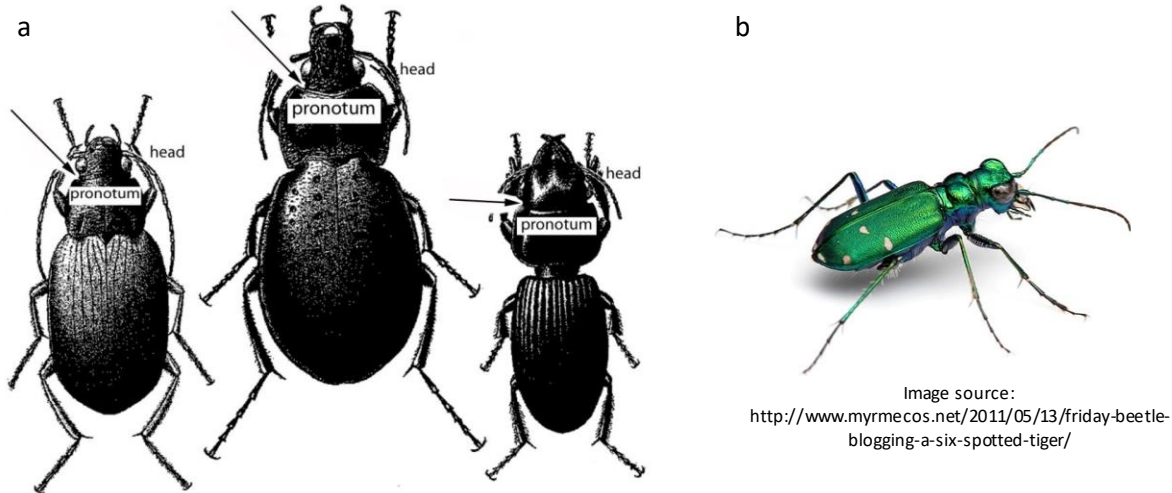
**Figure 51.** Tarsal formula of adephagan beetles.





## Distinguishing Carabid Adephagan Beetles

Adephagan beetles of the family Carabidae are *often* black and shiny, although they may also be metallically colored, and have large eyes, powerful legs, and large mandibles that fit their predatory lifestyles (**Figure 52**).



**Figure 52.** 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.

## Distinguishing Non-carabid Adephagan Beetles

Non-carabid adephagans (= invertebrate bycatch) can be readily recognized as belonging to other insect families using the following morphological features (**Figure 53 - Figure 58**), which will allow for rapid sorting of invertebrate bycatch.

- **Family Gyrinidae:** Gyrinid beetles are most easily recognized for their divided compound eyes (**Figure 53**). 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 54**).



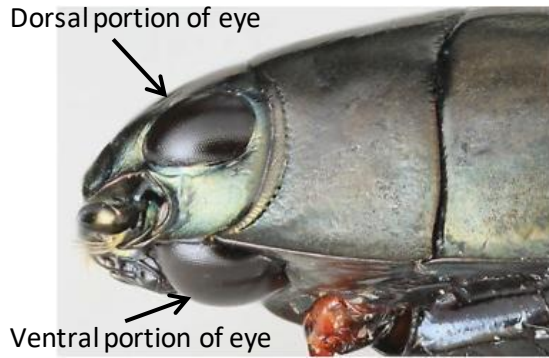


Image source:  
<http://www.studyblue.com/notes/note/n/holometabola-coleoptera-dav-1-w-dynastinae/deck/1547765>

**Figure 53.** 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 54.** 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 55**), by which their hind legs become oar-like structures for movement through water.

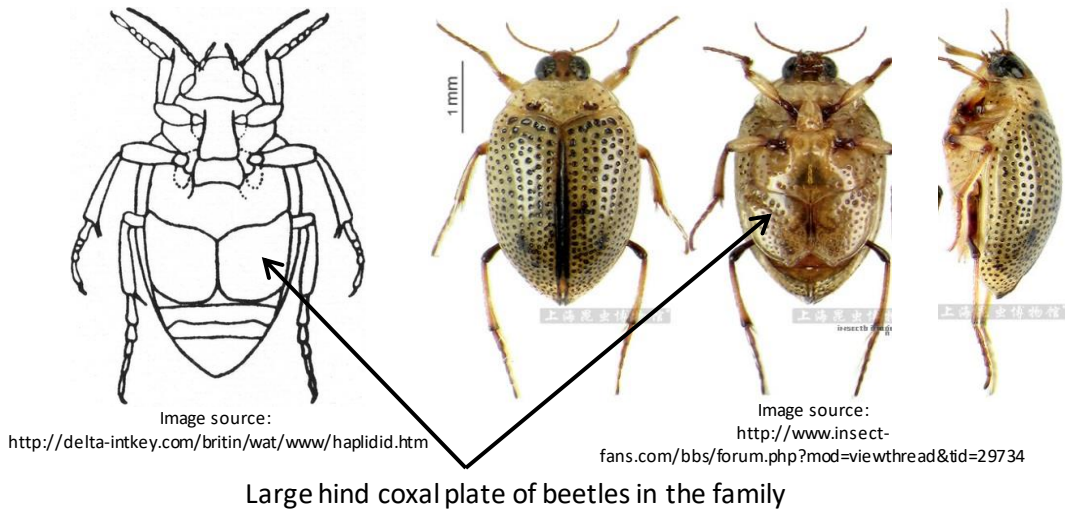


Image source:  
<http://flickrhivemind.net/Tags/taxonomy:family=dytiscidae/Interesting>

**Figure 55.** Dorsal view of a beetle in the family Dytiscidae, showing the oval shaped body and long hairs on the hind legs.

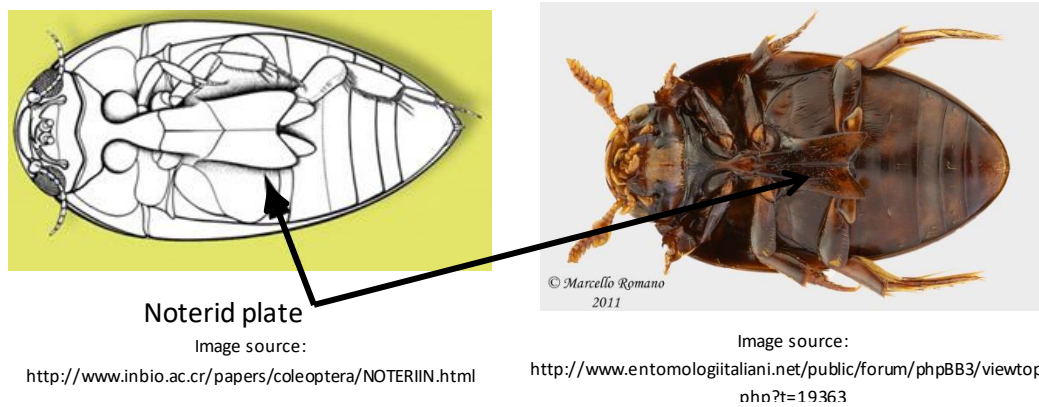


- **Family Haliplidae:** Haliplid beetles are most easily recognized by their unusually large hind coxal plates (Figure 56), which cover most of the ventral side of the abdomen (and actually obstruct the view of the aedephan-specific hind leg articulation described above).



**Figure 56.** 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 57).



**Figure 57.** Diagram and photo of a noterid beetle, showing the noterid plate between the second and third pairs of legs, on the ventral side.



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- **Family Rhysodidae:** Rhysodid beetles have elongated bodies, beadlike antennae, pronota that narrow to a ‘neck’ before joining with the head, and heads, pronota, and elytra bearing deep longitudinal grooves (this being their most distinguishing feature; **Figure 58**).



Image source:  
<http://www.entomology.umn.edu/museum/links/coursefiles/Coleop%20characters.html>



Image source:  
[http://www.biodiversity.ubc.ca/entomology\\_pictures/Coleoptera/Rhysodidae/Clinidium%20calcaratum%20\(1dorsal\).jpg](http://www.biodiversity.ubc.ca/entomology_pictures/Coleoptera/Rhysodidae/Clinidium%20calcaratum%20(1dorsal).jpg)

**Figure 58.** 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.

## D.2 Identifying specimens of Carabidae to species

NEON uses dichotomous keys to identify ground beetles to the species level. Ground beetles have been the subject of taxonomic treatment since the mid-1800s and numerous resources are available to support their identification, but no single modern taxonomic reference comprehensively covers the full scope of the NEON program. As such, NEON staff are directed to start by using the key outlined for their domain to attain coarse identifications for their taxa (**Table 12**); additional keys will be used to make a species-level determination, where possible. All materials are posted in the TOS – Sampling Support Library (i.e., ‘SSL’; see the “Identification Resources (Beetle Wiki)” link). This wiki includes photo-annotation for select keys in “American Beetles”, species checklists per domain and site, details on keys useful for each domain and notes on what taxonomic groups are treated in each key. Guides to the species of each domain (created by NEON staff members), definitions of entomological terms, and techniques and best practices are also provided in the wiki. Lists of endangered invertebrates and keys are posted in the SSL under “RTE Species Resources (Species Names and Keys)”.



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**Table 12.** Initial taxonomic key to be used for carabid identification (required to achieve tribe, genus or subgenus determination; for certain groups these keys may resolve specimens to species-level). Domains must start coarse-level grouping using the keys specified below.

Domain	Identification Key
D01, D02, D03, D04, D05, D06, D07, D08, D09, D10, D11, D12, D13, D14, D15, D16, D17	Ball and Bouquet 2000 key to Carabidae Found in “Arnett Jr., R. H., and M. C. Thomas. 2001. American Beetles Archosemata, Myxophaga, Adepaga, Polyphaga: Staphyliniformia. 1st Vol. (C. Press, Ed.). 1st Ed. Boca Raton, FL.”
D18, D19	Lindroth, C. H. 1969. The ground-beetles (Carabidae, excl. Cicindelinae) of Canada and Alaska, parts 1-6. Opuscula E. Entomologiska Sallskapet.
D20	Liebherr, J. K. and E. C. Zimmerman. 2000. Insects of Hawaii. Volume 16. Hawaiian Carabidae (Coleoptera), Part 1: Introduction and Tribe Platynini. University of Hawai'i Press, Honolulu.

*DOMAIN 01 – Keys to species*

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## Label formatting for samples and specimens

### D.3 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 59**; 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
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**Figure 59.** Example label for pitfall trap sample. Size shown is larger than size of actual label.

### D.4 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 (**Figure 60**).

<i>Cratacanthus dubius</i> det. J. S. Smith 2015	D10.2015.MorphA det. J. S. Smith 2015
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**Figure 60.** Example determination and morphospecies ID labels; size shown is larger than size of actual labels.

### D.5 Individual identifiers for pinned specimens

Individual ID 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.BET.DXX.#####, where XX is the domain number, and the number contains 6-digits (**Figure 61**).



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**NEON.BET.D10.000139**

**Figure 61.** Individual ID label for a beetle specimen.

## D.6 Creating Labels

The label generator (available via The Aviary) is a web application that automatically generates locality labels, determination labels, or individual IDs based on user inputs.

1. Select the siteID, the first date of collection and last date of collection.
2. 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.
3. Click the download button to receive a PDF with labels pre-printed for each trap and date specified.
4. Print the labels on ethanol-safe archival paper (e.g., Bioquip 1223RA).
5. 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.
6. 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.

In the event that the label generator is down, additional documentation to manually generate all three required labels is provided in the Sharepoint Sampling Support Library.

## APPENDIX E EQUIPMENT

The following equipment 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 13.** Equipment list – Preparation for field sampling all plots.

Supplier/Item No.	Exact Brand	Description	Purpose	Quantity
<b>Durable Items</b>				
Fisher Thomas	N	Bottle, 1 L wide-mouth HDPE	Prepare DI water (2), PG/DI (2), Ethanol (2)	6
Fisher	N	Jug, 1 gal narrow mouth	Prepare PG/DI	2
Fisher	N	Unitary wash bottle	Prepare DI water (2), Ethanol (2)	4
<b>Consumable Items</b>				
Grainger	N	All weather copypaper	Print datasheets	
		Distilled or deionized water	Prepare PG:DI solution, prepare water rinse bottle	4 L
Fisher Thomas	N	Ethanol, 190 proof, 95%, 55 gal. 5 gallons (D05)	Prepare Ethanol bottle	2 L
Bioquip 1223RA	Y	Label paper, ethanol-safe	Print locality and determination labels	
G2Solutions	N	Propylene Glycol. Clear, 95% Virgin Uninhibited	Prepare PG:DI solution	5 L





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**Table 14.** Equipment list – Initial deployment of pitfall traps.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
<b>Durable Items</b>				
Amazon	N	Deli container, 16 oz.	Pitfall trap container (half with pre-drilled holes for drainage)	60
HQ	Y	Lexan square with pre-drilled holes	Pitfall trap cover	30
Ben Meadows Forestry Suppliers	N	Measuring tape, 100 m	Locate trap installation points	1
Amazon B&H Photo	N	Laser Rangefinder, 0.5yd accuracy, 1500m range	Locate trap installation points	1
Grainger	N	Plastic spike	Secure pitfall trap covers	120
	N	PVC, ½" schedule 40, 1.75 cm length	Pitfall trap spacers	120
Amazon	N	Fender washers (diameter of inner hole 0.5 inches; diameter of outer edge 2 inches)	Optional; to place between pitfall trap spacers and the ground in loose soils	120
	N	Trowel or soil knife	Dig holes for traps	2
Amazon Grainger	N	Hand mirror or compass with mirror	Used to check that traps are flush with the ground	1 per person
<b>Consumable items</b>				
Fisher	N	Bottle with 50:50 PG:DI solution, 1 L wide-mouth HDPE	Preserve samples	6
Grainger	N	Flagging tape	Flag location of trap	1 roll
<b>Resources</b>				
RD[05]	Y	Field datasheet	Record data	



**Table 15.** Equipment list – Sampling beetles at one site for one sampling day.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
<b>Durable Items</b>				
	N	Hand mirror or compass with mirror	Used to check that traps are flush with the ground	1 per person
	N	Cooler	Chill perishable samples in field	1
Amazon	N	Deli container, 16 oz.	Spare pitfall trap container	10
VWR	N	Ice pack, 0°C	Chill perishable samples in field	2
Fisher	N	Jug, 4 L, narrow-mouth	Store Propylene Glycol	2
		Lexan square with pre-drilled holes	Spare pitfall trap cover	10
Grainger	N	Plastic spike	Spare spikes used to secure pitfall trap cover	40
		PVC, ½" schedule 40, 1.75 cm length	Spare pitfall trap spacer	40
		Scissors	Separate locality labels	2
		Tote bag, bucket organizer or other field pack	Carry gear	2
		Trowel or soil knife	Dig holes for traps	2
		Tupperware container	Organize samples	10
Fisher	N	Unitary wash bottle	Rinse deli cup	4
		Forceps	Remove large debris	1
Grainger	N	Amber bottle, 30 ml with dropper	Administer isoflurane	2
Amazon	N	Tea infuser spoon	Administer isoflurane	2
<b>Consumable items</b>				
		Bottle with 50:50 PG:DI solution, 1 L narrow-mouth HDPE	Preserve samples	6



Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
Fisher	N	Wash bottle with DI water	Rinse deli cup	2
Grainger	N	Flagging tape	Reflag location of trap	1 roll
		Nitrile gloves, powderless	Protect hands; must be used during cervical dislocation and/or isoflurane administration	1 pair
Bioquip 1154F	N	Permanent marker, archival ethanol-safe	Label whirl-paks	4
	N	Permanent marker, fine tip	Record plotID and date on resealable plastic bag	4
Grainger	N	Resealable plastic bag, 1 gal	Contain Whirl-Pak bags from each plot	12
Thomas Fisher	N	Whirl-Pak bags, sterile, 13 oz., 24oz. or larger as needed	Contain samples	30
MWI Veterinary 502017	N	Isoflurane	For administration of anesthesia, in case of moribund vertebrate bycatch	25 mL
Fisher	N	Cotton balls	To administer isoflurane	150
		Adhesive barcode labels (Type I)	Labeling sample containers with barcode-readable labels	1 sheet
<b>Resources</b>				
RD[05]	Y	Field datasheet from trap deployment	Record data	10
		Locality label	Label samples; 3 per trap	90



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**Table 16.** Equipment list – Post-field sampling (<24 hour) ethanol rinse for one sampling event.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
<b>Durable Items</b>				
Fisher Thomas	N	Bottle, 1 L wide-mouth HDPE	Propylene glycol/Ethanol waste storage	2
Fisher	N	Modified Nalgene top	Filter samples	3
Thomas	N	Jug, 4 L, narrow-mouth	Store ethanol	2
Fisher	N	Specimen cup	Chemical waste containment during field rinse	3
		Tupperware container	Organize samples	10
Fisher	N	Unitary wash bottle	Rinse samples	4
Amazon Fisher	N	Forceps	Push filter into Whirl-Pak bag during rinse	1
Amazon	N	Funnel	To help balance filter assembly on top of waste container (optional)	1 per team
<b>Consumable items</b>				
Fisher	N	Wash bottle with ethanol	Rinse samples	2
Grainger	N	Flagging tape	Reflag location of trap	1 roll
Fisher	N	Jug with ethanol, 4 L, narrow-mouth	Store/transport extra ethanol	2 L
HQ	Y	Mesh filter cloth	Filter samples	30
	N	Nitrile gloves, powderless	Protect hands; optional	1 pair
	N	Permanent marker, fine tip	Record plotID and date on resealable plastic bag	4
Grainger	N	Resealable plastic bag, 1 gal	Contain Whirl-Pak bags from each plot	12



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Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
<b>Resources</b>				
RD[05]	Y	Field datasheet from trap deployment	Record data	10
		Locality label	Label samples; 3 per trap	90

**Table 17.** Equipment list – Laboratory sorting of bycatch from carabids.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
<b>Durable Items</b>				
Fisher	N	Centrifuge tube rack	Organize 15 mL and 50 mL tubes	Variable
Grainger	N	Chemical waste drum (15 gallon)	Store Ethanol and PG waste	1
Lab Essentials	N	Dual gooseneck light	Illuminate specimens under microscope	3
		Forceps	Manipulate insects	3
Fisher	N	Microscope	Aid in species identification	2
Fisher	N	Petri dish, plastic	Sort specimens under microscope	2
		Secondary containment bin	Spill containment	1
Fisher	N	Specimen cup	Chemical waste containment during rinse	1
		Filter assembly	Rinse samples	1
		Tupperware container	Organize each bout of Whirl-Pak bags	10
		Unitary wash bottle	Rinse samples	1



Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
<b>Consumable items</b>				
		Adhesive label	Label sample tubes externally	As needed
Fisher Thomas	N	Ethanol, 190 proof, 95%, 55 gallon 5 gallons (D05)	Preserve samples	
		Filter cloth	Rinse samples	Variable
Thomas	N	Whirl-Pak bags, 13 oz.	Contain samples	Variable
Fisher 05-539-8	N	Tubes, 50 mL	Contain samples	Variable
		Jar, 16 oz. wide mouth jars	Contain samples	50
Bioquip	N	Label paper, ethanol-safe	Create sample ID labels	
		Nitrile gloves, powderless	Protect hands	Variable
		Permanent marker	Label sample containers	5
Bioquip	N	Permanent marker, archival ethanol-safe	Record sample ID number on sample ID labels	5
Amazon	N	Transfer pipette	Remove ethanol from petri dish and Whirl-Pak during transfer	1
		Tube or container	Contain vertebrate bycatch while sorting	
		Tube or container	Contain invertebrate bycatch while sorting	
		Tube or container	Contain carabids while sorting	
		Adhesive barcode labels (Type I)	Labeling sample containers with barcode-readable labels	1 sheet



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Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
<b>Resources</b>				
		Determination label	Label samples	Variable
RD[05]		Sorting datasheet	Record data	

**Table 18.** Equipment list – Laboratory pinning and pointing of carabids.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
<b>Durable Items</b>				
Lab Essentials	N	Dual gooseneck light	Illuminate specimens under microscope	1
		Forceps	Manipulate insects	3
John Robert Rose	N	Insect pinning block	Position point and labels on pin	1
		Microscope	Aid in pinning/pointing specimens	2
Bioquip		Permanent marker, archival ethanol-safe	Record sample information on labels	5
Bioquip		Point punch	Create points	1
<b>Consumable items</b>				
Amazon	N	Balsa wood	Hold specimens or pins/points and attach points/labels to pins	4
		Copy paper, white	Aid in pinning/pointing specimens under microscope	1
Arrow Amazon	N	Large trash bag	Protect specimens from moisture while freezing	25
Bioquip	N	Archival quality insect pins, sizes: No. 2, No. 3, or No. 4	Mount insects	10





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Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
Grainger	N	Multi-purpose glue	Attach specimens to points	2
Bioquip	N	Schmitt box	Store mounted specimens	Variable
Amazon	N	Strathmore paper	Paper for points	1 sheet
Arrow Amazon		Yellow cardstock	Print individual ID labels	
		Large trashbag	freezing pinned specimens	1
<b>Resources</b>				
		Determination label	Label samples	1 per specimen
		Locality label	Label samples	1 per specimen
RD[05]		Pinning datasheet	Record data	1



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**Table 19.** Equipment list – Insect labels.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
<b>Durable items</b>				
Grainger	N	Cutting mat	Cutting apart labels	1
Grainger Arrow	N	Scissors	Cutting apart labels	1
ULINE	N	X-acto knife	Cutting apart labels	2
Grainger	N	Ruler, Metal, 18 inch	Cutting apart labels	1
<b>Consumable items</b>				
Bioquip	N	Label paper, ethanol safe	Printing labels	Variable
Arrow Amazon	N	Yellow cardstock	Printing individual ID labels	Variable