

<i>Title:</i> TOS Protocol and Procedure: Ground Beetle Sampling		<i>Date:</i> 11/23/2015
<i>NEON Doc. #:</i> NEON.DOC.014050	<i>Author:</i> K. LeVan	<i>Revision:</i> J

TOS PROTOCOL AND PROCEDURE: GROUND BEETLE SAMPLING

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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		<p>Migration to new protocol template Substantial revision, including:</p> <ul style="list-style-type: none"> - Clarification to address JIRA tickets and provide more detailed instruction (throughout) - Moved information to appendices and supplementing the information (e.g., Appendix G) - Exported information to (e.g., shipping instructions), and imported information from (e.g., pinning/pointing instructions), related document RD[06] to ease the use of multiple protocols for technicians. - Added tolerances for placement of pitfall traps in plots - Added information for dealing with disturbed and/or empty traps - Added information on handling vertebrate bycatch and morphospecies
G	03/17/2015	ECO-02749	Re-imported shipping information in SOP I; added corresponding info and tables (e.g., table 9) throughout
H	05/04/2015	ECO-02890	Language requiring that gloves be worn by anyone

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			touching vertebrate bycatch with their hands. Also, technicians performing cervical dislocation must have received prior training. Fixing an error in the figures in response to FOPS-2034.
J	11/23/2015	ECO-03399	Fixing workflow compatibility with the WebUI Updated species lists Fixed sample timing section; added site specific estimated start dates Added in language/equipment concerning isoflurane Updated label generation section for consistency Updated workflow to remove 'other carabid'

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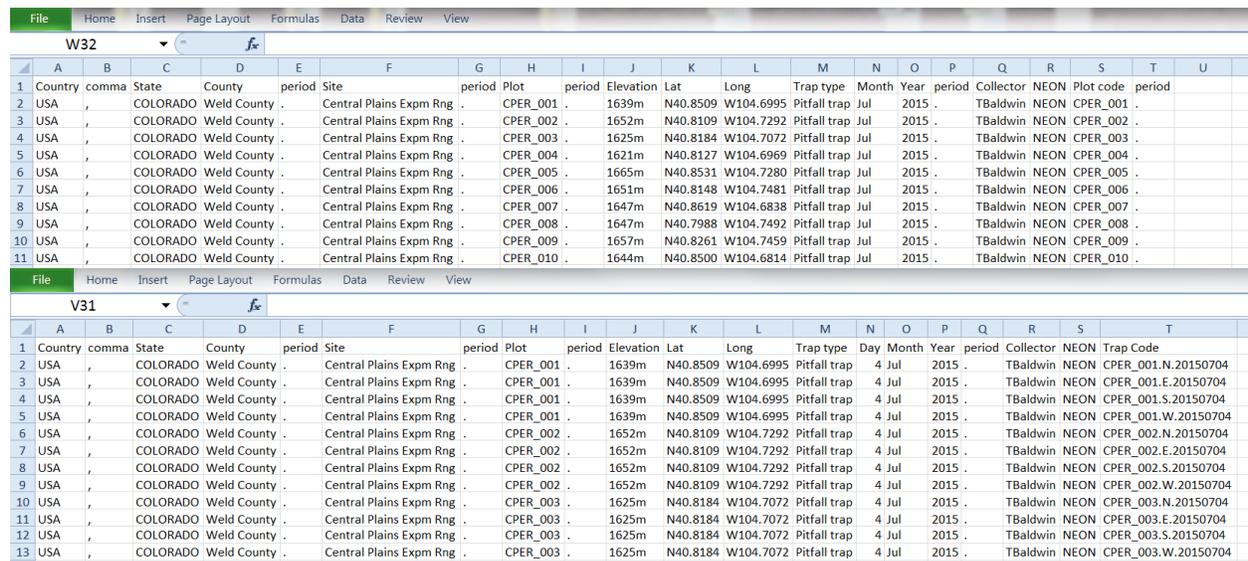


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1 OVERVIEW

1.1 Background

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

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

1.2 Scope

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

1.2.1 NEON Science Requirements and Data Products

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

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

1.3 Acknowledgments

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

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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.000724	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.014051	Field Audit Plan

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Level 1, Level 2 and Level 3 Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.001580	Datasheets for TOS Protocol and Procedure: Ground Beetle Sampling
RD[06]	NEON.DOC.001400	NEON Raw Data Ingest Workbook for TOS Ground Beetle Abundance and Diversity
RD[07]	NEON.DOC.001953	Pitfall trap spacers assembly instructions
RD[08]	NEON.DOC.001818	Pitfall trap cover assembly instructions
RD[09]	NEON.DOC.001816	Filter cloth assembly instructions
RD[10]	NEON.DOC.001814	Filter cup assembly instructions
RD[11]	NEON.DOC.001238	NEON Algorithm Theoretical Basis Document: TOS Ground Beetle Abundance and Diversity - QA/QC of Raw Field and Lab Data

2.3 Acronyms

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

2.4 Definitions

None given.

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3 METHOD

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

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

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

Pitfall traps are placed on the interior edges of the 40m x 40m Distributed Plots (well outside of the 20m x 20m plot interior, where plant sampling will occur). Ten Distributed Plots will each have four pitfall traps (40 traps total per site) located as close as possible to the center of each of the four edges of the plot (20 meters from the center of the plot on the north, 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., 'N' for the north trap). Plots for pitfall sampling will be identified prior to the field season. Standard Operating Procedures (SOPs), in Section 7 of this document, provide detailed step-by-step directions, contingency plans, sampling tips, and best practices for implementing this sampling procedure. To properly collect and process samples, field technicians **must** follow the protocol and associated SOPs. Use NEON's problem reporting system to resolve any field issues associated with implementing this protocol. The value of NEON data hinges on consistent implementation of protocols across all NEON domains. 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.

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The procedures described in this protocol will be audited according to the Field Audit Plan (AD[06]). Additional quality assurance will be performed on data collected via these procedures according to the NEON Algorithm Theoretical Basis Document for TOS Ground Beetle Abundance and Diversity: QA/QC of Raw Field and Lab Data (RD[11]).

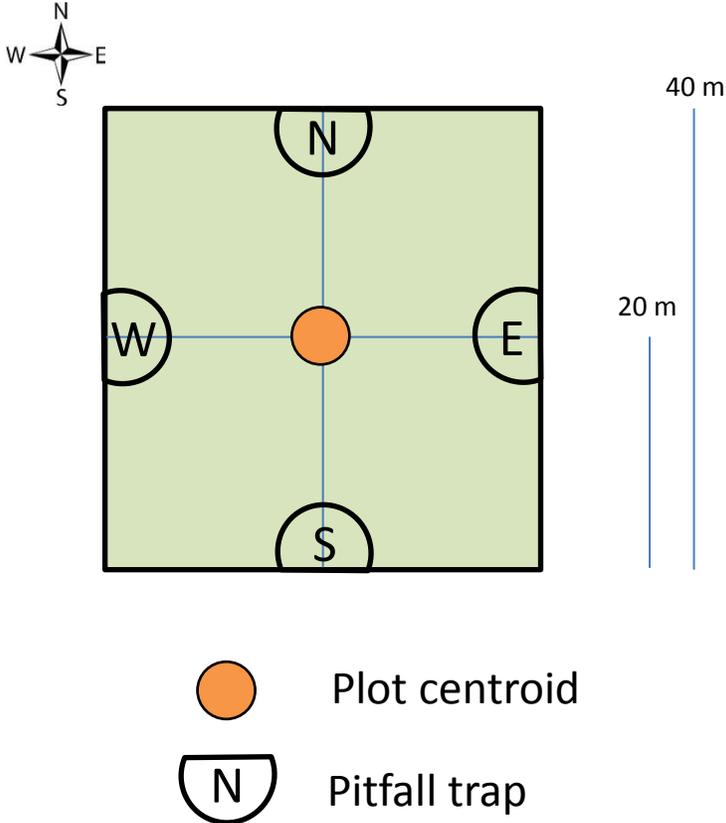


Figure 1. Standard beetle plot layout

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Pitfall traps will be checked, emptied, and reset every 14 days. For example, if a plot is visited on a Thursday morning for the first sampling bout, it must be visited every other Thursday (morning) subsequently, so that the samples from each bout are directly comparable (i.e., beetles are collected over the same time intervals). The selected day of the week is discretionary; however, the sampling should occur consistently every 2 weeks for the entire field season, on the same day of the week, and within 12 hours of the previous time sampled. Sampling schedules that would make a trap collection event fall on a holiday are to be avoided to the extent possible.

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4.2 Criteria for Determining Onset and Cessation of Sampling

Ground beetles should be sampled during the growing season when biological activity is highest (typically in concert with the plant phenology sampling at each site). The start and end of sampling each season will be triggered by biologically relevant thresholds. Sampling should begin within two weeks of the onset of green-up and end within two weeks of dormancy/senescence. In temperate sites with defined growing seasons (i.e., northern latitude domains), green-up is characterized by a combination of rising temperatures (i.e., the minimum low temperature > 4 °C) and the occurrence of breaking leaf/needle buds or presence of initial growth (as determined by domain staff). Sampling concludes at dormancy, which is characterized by falling temperatures (i.e., the minimum low temperature < 4 °C) and the senescence of 95% of the plant growth (as determined via the plant phenology protocol). At sites at lower latitudes (e.g., tropical climates), plant green-up and senescence will inform sampling timing even in the absence of sharp temperature changes.

Estimated dates for onset and cessation of sampling are provided per site in **APPENDIX D**. These dates are based on the average timing of green-up and senescence for each site over the last decade based on satellite data.

4.3 Timing for Laboratory Processing and Analysis

Following the collection of each pitfall trap, its contents must be rinsed in ethanol with any vertebrates removed and identified (see **SOP D**) within 24 hours. Additional processing (e.g., sorting invertebrate bycatch from the trap (see **SOP E**), identifying (**SOP F**), pinning (**SOP G**) and pooling carabids) may be conducted at a later date, when there is sufficient time and personnel for such activities. In general, sorting of bycatch should happen throughout the field season as samples are collected, so that the first stage of sample processing is completed prior to the end of the field season. Identification, pinning, and pooling of carabids occurs at the end of the season when the majority of species have been collected. Although these activities may occur several months after sample collection, all beetle processing must be completed within four months of the end of the field season.

4.4 Sampling Timing Contingencies

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

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Table 1. Contingent decisions

Delay/Situation	Action	Outcome for Data Products
Collection delayed by 1 day ¹	Collect traps asap. Do not reset trap until all traps can be reset simultaneously (e.g., onset of <i>next</i> sampling bout) ² ; note duration & cause; resume standardized sampling (2 weeks)	Data not comparable to standard collection events. Cannot calculate diversity indices if trap collections are not comparable.
Collection delayed by 2 or more days	Collect traps asap. Do not reset trap until all traps can be reset simultaneously (e.g., onset of <i>next</i> sampling bout) ² ; note duration & cause; resume standardized sampling (2 weeks)	Data not comparable to standard collection events; integrity of specimen DNA becomes compromised May not be readily able to obtain DNA barcodes; affects Abundance & Diversity measurements

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

² For example, beetle collection normally occurs every other Tuesday. During the 3rd bout, field crews only recover and reset 30 of the traps. On Wednesday, they return to the site and collect the catch from the rest of the traps. However, they *do not reset* any of the 10 belatedly-collected traps. Thirteen days later (the next regularly scheduled Tuesday collection), the contents of the 30 traps are recovered and all 40 traps are reset with new preservative.

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]).

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6 PERSONNEL RESOURCES

6.1 Equipment

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

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

Table 2. Equipment list - Preparation

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items					
MX100665	R	Bottle, 1 L wide-mouth HDPE	Prepare Ethanol (2), DI water (3), PG/DI (3)	8	N
MX104741	R	Jug, 1 gal narrow mouth	Prepare Ethanol	1	N
Consumable items					
MX103942	R	All weather copy paper	Print datasheets		N
	R	Distilled or deionized water	Prepare PG:DI solution, prepare water rinse bottle	4 L	N
MX100213		Ethanol, 190 proof (95%)	Prepare Ethanol bottle	2 L	Y
MX103249	R	Label paper, ethanol-safe	Print locality and determination labels		N

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Item No.	R/S	Description	Purpose	Quantity*	Special Handling
MX100585	R	Propylene Glycol	Prepare PG:DI solution	3 L	Y

R/S=Required/Suggested

Table 3. Equipment list – Initial deployment of pitfall traps

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items					
MX103582	R	Deli container, 16 oz.	Pitfall trap container (half with pre-drilled holes for drainage)	80	N
EB07710000	R	Lexan square with pre-drilled holes	Pitfall trap cover	40	N
MX100318	R	Measuring tape, 100 m	Locate trap installation points	1	N
MX104742	R	Laser Rangefinder, 0.5yd accuracy, 1500m range	Locate trap installation points	1	N
MX103881	R	Plastic spike	Secure pitfall trap covers	160	N
EG07890000	R	PVC, ½" schedule 40, 1.75 cm length	Pitfall trap spacers	160	N

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Item No.	R/S	Description	Purpose	Quantity*	Special Handling
	R	Trowel or soil knife	Dig holes for traps	2	N
Consumable items					
MX100585	R	Bottle with 50:50 PG:DI solution, 1 L wide-mouth HDPE	Preserve samples	6	N
MX103940	S	Flagging tape	Flag location of trap	1 roll	N
Resources					
RD[05]	R	Field datasheet	Record data		

R/S=Required/Suggested

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

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items					
MX100665	R	Bottle, 1 L wide-mouth HDPE	Ethanol waste storage	2	N
	R	Cooler	Chill perishable samples in field	1	N

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Item No.	R/S	Description	Purpose	Quantity*	Special Handling
MX103582	R	Deli container, 16 oz.	Spare pitfall trap container	10	N
EB07680000	R	Filter assembly	Rinse samples	3	N
MX105087	R	Ice pack, 0°C	Chill perishable samples in field	2	N
MX105571	R	Jug, 4 L wide mouth	Store waste Propylene Glycol	2	N
EB07710000	R	Lexan square with pre-drilled holes	Spare pitfall trap cover	10	N
MX103881	R	Plastic spike	Spare spikes used to secure pitfall trap cover	40	N
EG07890000	R	PVC, ½" schedule 40, 1.75 cm length	Spare pitfall trap spacer	40	N
	R	Scissors	Separate locality labels	2	N
MX104728	R	Specimen cup	Chemical waste containment during field rinse	3	N
	R	Tote bag, bucket organizer or other field pack	Carry gear	2	N
	R	Trowel or soil knife	Dig holes for traps	2	N
	R	Tupperware container	Organize samples	10	N
MX100308	R	Unitary wash bottle	Rinse samples	4	N

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Item No.	R/S	Description	Purpose	Quantity*	Special Handling
	R	Forceps	Push filter into Whirl-Pak bag during rinse	1	N
MX104715	R	Amber bottle, 30 ml with dropper	Administer isoflurane	2	N
MX100729	R	Tea infuser spoon	Administer isoflurane	2	N
Consumable items					
MX100585	R	Bottle with 50:50 PG:DI solution, 1 L wide-mouth HDPE	Preserve samples	6	N
MX100585	R	Bottle with distilled or deionized water, 1 L wide-mouth HDPE	Rinse samples	6	N
MX100665	R	Bottle with ethanol, 1 L wide-mouth HDPE	Rinse samples	2	N
EB07690000	R	Filter cloth	Rinse samples	40	N
MX103940	S	Flagging tape	Reflag location of trap	1 roll	N
MX104741	R	Jug with ethanol, 1 gal narrow mouth	Store/transport extra ethanol	2 L	N
	S	Nitrile gloves, powderless	Protect hands; must be used during cervical dislocation and/or isoflurane administration	1 pair	N

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Item No.	R/S	Description	Purpose	Quantity*	Special Handling
MX104422	R	Permanent marker, archival ethanol-safe	Record trapID and date on locality labels	4	N
	R	Permanent marker, fine tip	Record plotID and date on resealable plastic bag	4	N
MX100592	R	Resealable plastic bag, 1 gal	Contain Whirl-Pak bags from each plot	12	N
MX100686	R	Whirl-Pak bags, 13 oz.	Contain samples	40	N
MX100672	R	Isoflurane	For administration of anesthesia, in case of moribund vertebrate bycatch	25 mL	Y
MX100591	R	Cotton balls	To administer isoflurane	150	N
Resources					
RD[05]	R	Field datasheet from trap deployment	Record data	10	N
	R	Locality label	Label samples; 3 per trap	120	N

R/S=Required/Suggested

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Table 5. Equipment list – Laboratory sorting of bycatch from Carabids

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items					
MX108278	R	Centrifuge tube rack	Organize 15 mL and 50 mL tubes	Variable	N
MX100692	R	Chemical waste drum	Store Ethanol and PG waste	1	N
MX100270	R	Dual gooseneck light	Illuminate specimens under microscope	3	N
	R	Forceps	Manipulate insects	3	N
MX102896	R	Microscope	Aid in species identification	2	N
MX103528	R	Petri dish, plastic	Sort specimens under microscope	2	N
	R	Secondary containment bin	Spill containment	1	N
MX104728	R	Specimen cup	Chemical waste containment during rinse	1	N
EB07680000	R	Filter assembly	Rinse samples	1	N
	R	Tupperware container	Organize each bout of Whirl-Pak bags	10	N
MX100308	R	Unitary wash bottle	Rinse samples	1	N

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Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Consumable items					
	S	Adhesive label	Label sample tubes externally	As needed	N
MX100213	R	Ethanol, 190 proof (95%)	Preserve samples		Y
EB07690000	R	Filter cloth	Rinse samples	Variable	N
MX100686	R	Whirl-Pak bags, 13 oz.	Contain samples	Variable	N
MX108277	R	Falcon tubes, 50 mL	Contain samples	Variable	N
	R	Jar, 16 oz. wide mouth jars	Contain samples	50	N
MX103249	R	Label paper, ethanol-safe	Create sampleID labels		N
	S	Nitrile gloves, powderless	Protect hands	Variable	N
	R	Permanent marker	Label sample containers	5	N
MX104422	R	Permanent marker, archival ethanol-safe	Record sampleID number on sampleID labels	5	N
MX103145	R	Transfer pipette	Remove ethanol from petri dish and Whirl-Pak during transfer	1	N

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Item No.	R/S	Description	Purpose	Quantity*	Special Handling
	R	Tube or container	Contain vertebrate bycatch while sorting		N
	R	Tube or container	Contain invertebrate bycatch while sorting		N
	R	Tube or container	Contain Carabids while sorting		N
Resources					
	R	Determination label	Label samples	Variable	N
RD[05]	R	Sorting datasheet	Record data		

R/S=Required/Suggested

Table 6. Equipment list – Laboratory pinning and pointing of Carabids

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items					
MX100270	R	Dual gooseneck light	Illuminate specimens under microscope	1	N
	R	Forceps	Manipulate insects	3	N

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Item No.	R/S	Description	Purpose	Quantity*	Special Handling
MX100720	R	Insect pinning block	Position point and labels on pin	1	N
	R	Microscope	Aid in pinning/pointing specimens	2	N
MX104422	R	Permanent marker, archival ethanol-safe	Record sample information on labels	5	N
MX102811	R	Point punch	Create points	1	N
Consumable items					
MX104417	S	Balsa wood	Hold specimens or pins/points and attach points/labels to pins	4	N
	S	Copy paper, white	Aid in pinning/pointing specimens under microscope	1	N
MX102810	R	Fumigant box	Contain moth crystals within Cornell cabinet	25	N
MX100682	R	Insect pin, No 3	Mount insects	10	N
MX100582	R	Moth crystals	Prevent dermestid damage to specimens	1 lb., ~6.5 g per box	Y
MX100595	R	Multi-purpose glue	Attach specimens to points	2	N

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Item No.	R/S	Description	Purpose	Quantity*	Special Handling
MX104421	R	Schmitt box	Store mounted specimens	Variable	N
MX104505	R	Strathmore paper	Paper for points	1 sheet	N
MX104981		Yellow cardstock	Print individual ID labels		
Resources					
	R	Determination label	Label samples	1 per specimen	N
	R	Locality label	Label samples	1 per specimen	N
RD[05]	R	Pinning datasheet	Record data	1	N

R/S=Required/Suggested

Table 7. Equipment list - Insect labels

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Locality and Determination Labels					

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Item No.	R/S	Description	Purpose	Quantity	Special Handling
MX103249	R	Label paper, ethanol-safe	Printing labels	Variable	N
individualID Labels					
MX104981	R	Yellow cardstock	Printing individual ID labels	Variable	N
All Labels					
MX100503	R	Scissors	Cutting apart labels	1	N
MX104758	R	Cutting mat	Cutting apart labels	1	N
MX104740	R	X-acto knife	Cutting apart labels	2	N
MX103934	R	Ruler, Metal, 18 inch	Cutting apart labels	1	N

Table 8. Equipment list - Shipping to taxonomic facility

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Consumable items					

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Item No.	R/S	Description	Purpose	Quantity	Special Handling
MX104423	R	Cardboard specimen mailers	Organizing specimens for shipment	Variable	N
	R	Cardboard insert with tape tab	Extra stability and protection for pinned specimens during shipment	Variable	N
	S	Packing foam	Fill in extra head space between cardboard insert and specimen mailer lid	Variable	N
MX100682	R	Insect pin, No 3	Brace-pinning specimens for shipping	2	N
	R	Packaging tape	Handle for cardboard insert AND shipping	Variable	N
	R	Address label	Shipping	Variable	N
	R	Cardboard box	Shipping	Variable	N
		Cushioning material (e.g. bubble wrap)	Shipping	Variable	N

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6.2 Training Requirements

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

Training for field techs will include practice analyzing datasheets at various levels of completion or with errors. Training will also include discussions on data interpretation, the importance of uploading data in a timely fashion, and how to recognize the distinction between missing data and true zeroes.

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

6.3 Specialized Skills

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

6.4 Estimated Time

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

Sampling is estimated to require 2 technicians: 12 min per trap x 40 traps = 480 min = 4 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.

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7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

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

1. Identify the locations of sampling plots and access routes
2. Prepare pitfall trap materials, including required labels
3. Cut/drill holes in bottom (exterior) container (16 oz. deli cup)
4. Draw 150 mL and 250 mL fill lines on upper (interior) container
5. Locate other trap materials including pitfall trap spacers to hold up cover and filter squares.
6. Review/prepare checklist of materials needed for pitfall sampling.

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

A.2 Prior to a sampling bout:

1. Prepare bench and fume hood space in the lab for the preparation of field materials (e.g., ethanol, PG). Ensure that space in freezer, refrigerator or flammable materials cabinet is available for samples. Coordinate with potentially conflicting activities (e.g. soil sieving).
2. Assemble field equipment at least one day prior to field sampling.
3. Label each Whirl-Pak bag with the plotID and trapID of each beetle pitfall using an ethanol-safe marker. Label 10 large resealable bags with each plotID and collection date. Place labeled Whirl-Pak bags inside the larger resealable bag according to plotID.
4. Print blank datasheets (see Ground Beetle Field Datasheet in (RD[05])).
5. Prepare locality labels (see APPENDIX A).
6. Charge all electronic equipment (e.g., GPS unit, rangefinder).
7. Prepare one liter bottles of propylene glycol: water solution (500 mL PG:500 mL water, either distilled or deionized). A minimum of six 1 L bottles of this solution is required to fill 40 pitfall traps with 150 mL of preserving fluid. Be sure to prepare more of this solution than you think you will need in the field to ensure that there will still be enough to set or reset

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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.

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

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SOP B Initial Deployment of Pitfall Traps

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

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

1. Locate the first plot where sampling is to occur.
2. Find two corners of the plot using the GPS points collected during plot establishment (e.g., the NE and NW corners). Find the mid-point between those corners (approximately 20 meters from each corner) using a Rangefinder or measuring tape and verify that your position is correct. For the Northern trap, you may move $\pm 2\text{m}$ to the east, west, or south of the mid-point location if there is a natural feature (e.g., a rock or stream) in the target trap location. *You may not place the trap outside of the plot boundaries, due to permitting constraints.*
3. Dig a small hole with a soil knife to accommodate the pitfall trap (**Figure 2**). Start small because it is much easier to enlarge the hole than fill in the edges to maintain a tight fit. Outline the container in the dirt and then dig just inside the line to ensure a snug fit. The exact tools most useful for this step will vary based on soil type, roots, rocks, etc.
4. If the ground is particularly rocky or hard, use a template pitfall trap lid and spikes to pre-bore (with a hammer) the holes for the spikes in the actual trap. Push the bottom container (with holes) into the hole. Slide the top container into bottom container. **Ensure that the lip of the top container is flush with the ground** so no lip is sticking up above the ground surface (this ensures that insects fall into the trap rather than walk around it, and accumulate in the top container only). Also ensure that there is no gap between the containers and the ground. The containers should fit snugly into the hole.
5. Use the pre-drawn line to fill the top container with 150 mL of PG:water solution. See contingency section (**4.4**) above if you have failed to bring an adequate amount of PG to fill pitfalls. If it is not possible to fill all traps to the 150 mL volume (e.g., spilled in field), revisit traps with additional preserving fluid in the following days. If re-visitation is not possible, and the amount of PG was insufficient to preserve specimens captured during the bout, the sample will become degraded and it is unlikely that any of the specimens will be identifiable.



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

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Figure 2. Digging a hole for the pitfall trap.

6. Position cover with four plastic spikes and PVC spacers (**Figure 3-Figure 4**) so that it is visually level and 1.5 cm above the surface of the containers (**Figure 5**).
 - a. Label the top of the cover with the name of the plot and N, S, E or W to indicate the trap location within the plot. This will facilitate matching locality labels with traps during subsequent visits
7. Trap cover deployment will vary based on local topography and vegetation cover. Keep the following purposes of the cover in mind while installing the cover. The cover:
 - a. Prevents vertebrate bycatch while allowing ground beetles to enter
 - b. Shades the trap to lower fluid temperature, thus reducing evaporative loss and decomposition rate
 - c. Prevents precipitation accumulation in the trap, thus reducing dilution of the preserving fluid

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Figure 3. Positioning cover over pitfall trap. Note the pitfall is not sufficiently dug into the ground in this image. The lip should not stick up from the ground.



Figure 4. Installing pitfall lid with spacers. Note that the container is flush with the ground.

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Figure 5. Fastening down pitfall cover. Note the spacer below the cover.

- d. Once one trap in one of the cardinal directions has been set (in this example, the north trap), work along the edge of the 40 m² plot to find the next corner, repeating this procedure until all 4 traps are installed. If possible, reuse the same holes for pitfall traps from year to year (backfilled holes can be excavated anew each year).
- e. For each trap, record the date in the setDate field on the field datasheet.

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SOP C Field Sampling

C.1 Collecting samples from the trap

Upon arrival at the trap:

1. Record data on the same datasheet as used when setting the traps (see Ground Beetle Field Datasheet in (RD[05]), or find the trap activity record on the PDA.
2. Note the relative fluid level remaining in the container (high, low, or ok) in the ‘fluidLevel’ field, and whether the lid (the 20 x 20 cm trap cover) and/or upper container are missing or have been disturbed (in the ‘lidStatus’ and ‘cupStatus’ fields). If the upper container is missing and the bottom container does not obviously contain beetles or fluid, skip to ‘Resetting the Pitfall Trap’. Otherwise, even if the trap appears empty, continue to the next steps to collect a sample.
3. Place three copies of the appropriate locality labels into the container, even if it appears to contain no specimens. The labels can be treated in the same way as the trap contents (e.g., rinsed along with specimens in the trap) in subsequent steps without harm.

*Note: To speed up collections, it is recommended that technicians prepare locality labels preprinted with the collection date and sampleIDs for each trap to save time (see **APPENDIX A** to learn more about how these are created). In case of errors, technicians must bring at least one sheet with preprinted labels that leave the date and sampleID blank as a precaution.*

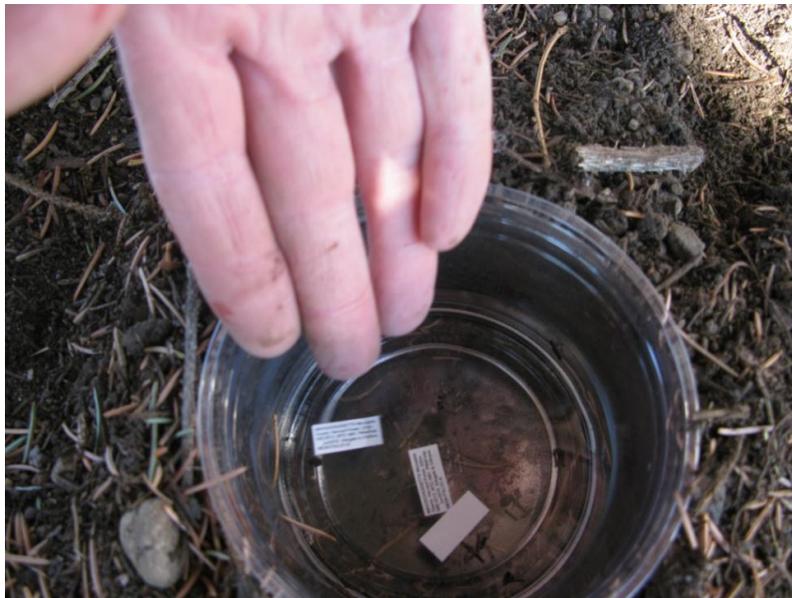


Figure 6. Adding locality labels to trap

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- If there are no fully pre-printed labels available for the previous step, fill in the blanks in the spare locality label sheet (i.e., trap, date and sampleID) for three locality labels and place those into the cup. This involves writing 1) the day (on which the trap collection is occurring) in the space preceding the month and year on the fourth line of the label; 2) the sampleID on the last line of the label, which includes the trapID (see Error! Reference source not found.). Use an archival ethanol-safe pen, and record the date (in the collectDate field, YYYYMMDD format) and sampleID (plotID.trapID.collectDate) on the field datasheet.

USA, GEORGIA Baker County.
Jones Ecological Res Ctr. 47m
N31.2034 W84.4631 Pitfall trap
16 Jul2014. RNelson
NEON.JERC_400. N-20140716

Figure 7. Example label format for a trap where a preprinted label cannot be used.

- [Optional step] Put on disposable gloves to protect your hands from the materials used. Gloves can be reused.
- Pick up the upper container containing the sample, leaving the lower container in the ground.
- 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 removed.
- 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, anesthetize the animal with isoflurane and perform cervical dislocation. All dead vertebrate bycatch should be collected with the rest of the trap contents. Note: Personal protective equipment such as gloves *must* be worn when directly physically handling vertebrate bycatch and performing cervical dislocation. Technicians must be trained in the administration of isoflurane and performance of cervical dislocation prior to implementing this protocol.
- If filtering directly after collecting each pitfall trap, proceed to the section on filtering samples. If collecting all traps and then filtering at once at the end of sampling, place the PG solution from the pitfall trap directly into the Whirl-Pak container and seal tightly. In the latter case, the Whirl-Pak should contain any beetles, dead bycatch, and 3 locality labels (ideally all fully pre-printed) in the PG solution.
- 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).

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C.2 Filtering the sample

After each sample is collected, the PG solution needs to be removed and replaced with ethanol that same day. Technicians can decide if it is easier to filter the samples at each trap between trap collection and trap reset or to place the PG solution from each trap directly into the labelled Whirl-Pak and perform filtering on all 40 traps at the end of collection at the car. If filtering occurs at the end of the collection bout, then place the PG solution with sample from the Whirl-Pak directly into the filter assembly, rather than from the cup as is indicated below.

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

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



Figure 8. 300 μ m mesh filter fitted to the top of a Nalgene bottle

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

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Figure 9. Preparing to filter out specimens from collecting fluid.



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

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

3. After sample collection is finished, dispose of used PG solution back at the lab.
4. Rinse the sample (in the filter cup) using distilled or deionized water (**Figure 12**). Rinse above the waste container so rinse water/PG is efficiently collected instead of spilling onto the ground.



Figure 12. Rinsing sample with water.

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



Figure 13. Rinsing sample with 95% ethanol.

6. Place insects in a Whirl-Pak bag (**Figure 14**), rinse the filter contents into the same Whirl-Pak bag (**Figure 15**), and add additional ethanol until the contents of the bag are covered with approximately 100 mL of 95% ethanol. Finally, remove the filter from the filter cup and place the filter in the Whirl-Pak (**Figure 16**), verifying that the filter cup is free from all sample materials before using it to process another sample. Use forceps to gently push the filter into the Whirl-Pak without tearing the Whirl-Pak.



Figure 14. Placing sample in Whirl-Pak bag.

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



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

- 7. Roll the Whirl-Pak bag shut and tightly close the bag’s twist ties to prevent leaks (Figure 17).

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Figure 17. A packed sample.

8. 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.
9. Store the Whirl-Pak samples in the shade (e.g., in a backpack) until you return to the field vehicle (see **C.5** for additional instructions).

C.3 Resetting the Pitfall

1. Using new solution prepared in the lab, refill the PG mixture in the container to 150 mL line. If the PG solution was under the 150 mL line when the trap was collected (more likely when conditions are hot and dry), add PG up to the 250 mL line to prevent potential trap drying.
2. Position cover with four plastic nails so that it is visually level and 1.5 cm above the surface of the containers.
3. Record that the trap was reset on the field datasheet.
4. Repeat this procedure until the contents of all 40 traps have been collected and each trap have been reset.

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

1. Return all trap components to the lab.
2. Backfill holes with local substrate. These same holes will be excavated in following years for pitfall trapping.
3. Indicate that the final trap processed was not reset, on the field datasheet.

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C.5 Sample preservation

1. Place all 4 Whirl-Pak bags from a single plot into a resealable plastic bag that has been labelled with date and plot ID (includes siteID) before leaving the plot. You may choose to pre-label these bags in the lab.
2. Place bags into Tupperware container, ensuring that the Whirl-Pak bag openings are upward.
3. Store samples in a cooler (with ice packs lining the bottom) in the field vehicle to prevent exposure to direct sunlight or extremely high temperatures during the remainder of the field work. When transporting coolers back to the lab avoid exposure to heat (e.g., direct sun) and wind to the extent possible. In the lab, samples must be stored in the fridge prior to removal of vertebrate bycatch.
4. Change ethanol within 1 day of collection and remove vertebrate bycatch (see **SOP D**).
5. Samples can be stored at room temperature until invertebrate bycatch and carabids are sorted.

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

D.1 Sample processing timing

The ethanol in which each sample is stored must be changed within 1 day of collection (< 24 hours). At this stage, bycatch is sorted from carabids. All vertebrate bycatch must be removed and sorted, with any rare, threatened or endangered (RTE) species reported prior to the following sampling bout. Invertebrate bycatch may also be removed during this rinse (if time allows; see **SOP E** for details). For each trap, record the date of the ethanol change on the sorting datasheet (RD[05]).

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

NOTE: A problem ticket must be created in JIRA when 15 or more individuals *of a given non-RTE vertebrate species* have been collected, cumulatively, within a single plot. See below for example scenarios that do, and do not, require the issuance of a JIRA ticket:

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

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

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

Example 4: Eight leopard frogs and 8 shrews are caught in HARV_001 pitfall traps. No problem ticket is required.

The bycatch of RTE species will involve specialized handling that depends on the permitting at each site. Should any RTE species be caught, consult those permits and notify both NEON Science and NEON Permitting of the RTE species that was captured. Captures of RTE species may involve limited action, such as the temporary suspension of sampling at the plot in which that species was captured, or extensive remediation, such as inactivation of a whole site for the remainder of the field season. Any response will necessarily be site- and species-specific.

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 on an ethanol safe label (and using an archival ethanol-safe pen) that is placed inside the Whirl-Pak bag at the time of the rinse. In addition, all vertebrate bycatch must be removed from the pitfall sample at this time, so that large, soft-bodied animals do not degrade the quality of the etOH in the pitfall sample.

Figure 18 illustrates the steps for etOH rinse and removal of vertebrate bycatch, with detailed instructions in the following text.

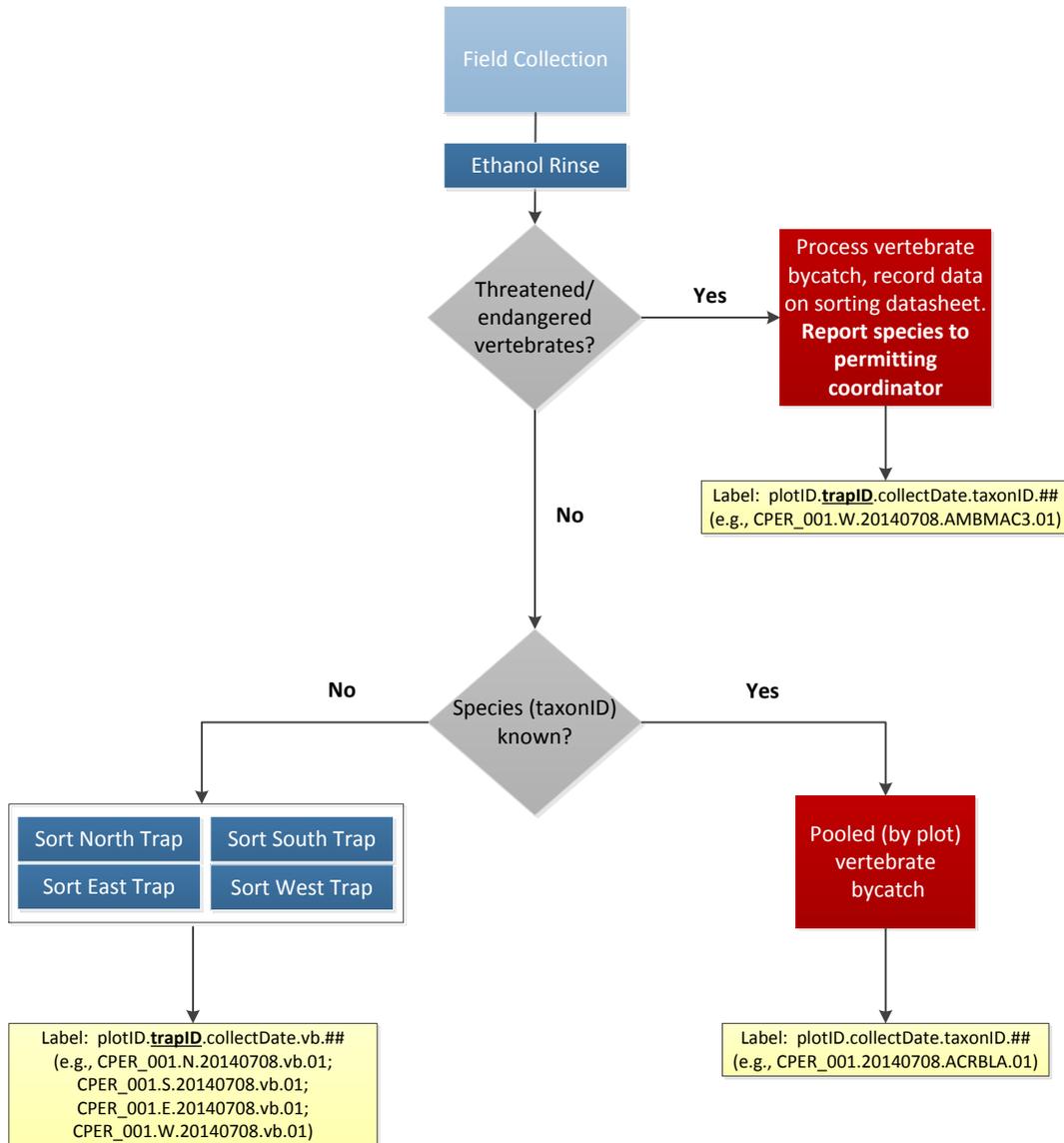


Figure 18. Workflow of the ethanol rinse and vertebrate bycatch removal processing step. Activities are represented by blue boxes, decisions by grey triangles, and labels by yellow boxes. If taxonID is known, all vertebrates of that taxon are kept in a pooled vial (or set of vials). If taxonID is not known, vertebrates OF THE SAME MORPHOSPECIES are maintained in separate vials at the trap level.

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1. [Recommended but optional step] Put on nitrile gloves.
2. For each trap, pour the sample (including the specimens and the initial ethanol added to the Whirl-Pak in the field) through a filter cloth and into an ethanol waste container (**Figure 19**). Remove the used filter cloth from the Whirl-Pak and rinse, with 95% ethanol, over the new filter cloth until there is nothing visible on the filter cloth (**Figure 20**). Place the filter cloth back into the original Whirl-Pak, keeping the original filter with the sample in case small beetles and bycatch are stuck to it.



Figure 19. Filtering diluted ethanol off sample into a specimen cup (a temporary waste container).



Figure 20. Pouring sample through filter directly into larger waste container.

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3. Remove all vertebrate bycatch and record associated data on the sorting datasheet (RD [05]) (**local and/or state permits should be consulted for reporting requirements of vertebrate bycatch**).
4. Record vertebrate bycatch from each plot on the sorting datasheet such that each *taxon* from each trap has its own record in the datasheet which accurately reports 1) the trap it originated from, 2) the final sample ID of the destination tube, and 3) the number of vertebrates of that species that were transferred from that pitfall trap to the destination tube.
 - a. In general, this means that at least **one** record will be needed for each taxon/trap combination. However, *extra* records are required if a species from a single trap is split between two vials. In that case, the ‘individual count’ for each record is the number of vertebrates of a taxon that contributed to the tube labelled with a given ‘final sample ID’.
 - b. Likewise, pooling of known species within a plot is encouraged to save on tubes and space. However, vertebrates are often unevenly distributed across the plot (i.e., East trap has 20 individuals and West trap has 1 individual) or may be present in just one trap. In that case, it is possible that some tubes may contain individuals sourced from just one of the traps even after pooling across plot (e.g., specimens from tube 1 are all from East trap, specimens in tube 2 come from a mix of traps). In that case, record the tubes that have specimens from multiple sources as pooled, but record tubes with specimens from a single trap source as though they are not pooled (because they aren’t). Pooling occurs to save on archival and materials costs. However, if it is possible to keep tubes filled with a single trap’s contents, then the data need to reflect that more specific origin.

Example: The CPER_001 ‘N’ trap has 2 *Myodes californicus* and 1 *Peromyscus californicus*. The CPER_001 ‘E’ trap has 1 *Myodes californicus*. In the datasheet, these would be entered as three rows (one per taxon/trap combination) and the ‘Sample pooled?’ box would be checked for both the *Myodes californicus* rows.

On the datasheet, the sampleIDs for each *Myodes californicus* record would be written CPER_001.20151030.MYCA.01 and *Peromyscus californicus* record will have a sampleID CPER_001.N.20151030.PECA.01.

Note – the end result is that there are three entries and two tubes.

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5. If the species identity is known, then the process of recording and pooling will occur as described below.
 - a. Vertebrates from each trap receive their own entry in the sorting datasheet. For each tube:
 - i. Record the trapID field. (These specimens are pooled and the sampleID will not include 'trapID'. However, the database will save the trapID information in another column.)
 - ii. Record the 'final sample ID (**sampleID**)' (see **APPENDIX D**).
 - iii. The identity of the individuals in the tube, to the finest taxonomic level possible (preferably species), in the 'Sci. Name OR taxonID'. (see **SOP F.2** for information about handling uncertainty in taxonomic identifications)
 - iv. Record the number of individuals in the tube in the 'Individual Count' field that were derived from a given trapID and assigned the listed 'final sampleID'
 - v. If it can be determined whether Carabid beetles are present in the rest of the pitfall sample, record 'Y' or 'N' in the 'Carabids (**targetTaxa**) Present' field; otherwise leave this field blank
 - vi. Record the etOH rinse date in the 'etOH change date' field
 - vii. All other data that can be filled out (e.g., sample type, date of identification, etc.) on the sorting datasheet
 - b. Record the taxonID and number of individuals caught from *each trap* separately into the sorting datasheet, as described above. Be sure to include plotID, collectDate, and taxonID in the 'Final Sample ID' field of the data sheet.

Example: Two tubes are required to hold a vertebrate bycatch species for the plot. SampleIDs for these tubes are plotID.collectDate.taxonID.01 and plotID.collectDate.taxonID.02.

Note – although sampleIDs are automatically generated by the WebUI, the WebUI requires that you specify the destination tube number. In this case, it would need two entries to specify how many animals went into tube 01 and tube 02, respectively.

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- c. Pool all individuals (from the same plot) of the same taxon into as few 50 mL tubes as are required. Label each pooled vial with final sampleID. For each new vial used increase the tubeNumber by 01. The tube number listed in the 'Final Sample ID' should indicate the final destination of the specimen. Note: if pooling results in splitting vertebrates from one trap into multiple tubes, this will require extra records as described above.
- d. 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.
- e. Include a locality and determination label inside each tube. The final sampleID in the format **plotID.collectDate.taxonID.tubeNumber** is also included, where tubeNumber is two digits, starts with 01, and increases for as many tubes as contain the same taxon. The sampleID may be preprinted on archival paper in ethanol-safe ink and insert a label into each tube or the sampleID can be written on a freezer-safe label placed on the outside of the vial in archival ink.

NOTE: If the vertebrate is a known taxon, but all specimens come from only one trap in a particular plot then record plotID.trapID.collectDate.taxonID.tubeNumber on the sampleID label.

- 6. If the species identity is unknown, then specimens will be separated by taxa from each trap and then maintained separately at the *trap level*. The details of this process are outline below.
 - a. Vertebrates from each trap receive their own entry in the sorting datasheet. For each tube, record:
 - i. The sample ID in 'final sample ID' field (see **APPENDIX D**).
 - ii. The morphospecies ID in the 'morphospecies ID' field (see **section 7F.2** for instructions on morphospecies naming conventions).
 - iii. The number of individuals in the tube, in the 'Individual Count' field
 - iv. If it can be determined whether Carabid beetles are present in the rest of the pitfall sample, record 'Y' or 'N' in the 'Carabids (**targetTaxa**) Present' field; otherwise leave this field blank.
 - v. The etOH rinse date in the 'etOH change date' field

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- vi. All other data that can be filled out (e.g., sample type, date of identification, etc.) on the sorting datasheet
- b. Place all individuals of a given taxon, and taken from a single *trap*, into as few 50 mL tubes as are required
- 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.
- d. Include a locality label in each tube, and insert a label into each tube on which the following sample ID is recorded (on an ethanol safe label and using an archival ethanol-safe pen): plotID.trapID.collectDate.vb.tubeNumber (where vb stands for 'vertebrate bycatch' and the tubeNumber is two digits, starts at 01, and increases for as many tubes as contain unknown vertebrate bycatch) in the trap. Because the tube suffix is just 'vb.tubeNumber' (i.e., vb.01, vb.02, etc), every tube created from a single trap MUST have a unique tube number or else sampleIDs will not be unique.

Example: One tube is required to hold a particular species of unknown vertebrate bycatch for trap S, and two tubes are required to hold a different unknown vertebrate bycatch species for trap S. SampleIDs for these tubes are: plotID.S.collectDate.vb.01; plotID.S.collectDate.vb.02; plotID.S.collectDate.vb.03.

Note – sampleIDs are automatically generated by the web user interface (WebUI) when the sample Type is 'vertebrate bycatch' and the Taxon ID field contains *no* value

- 7. Store processed vertebrate samples at room temperature in the flammables cabinets unless there is ample space in refrigerators (4°C) or freezers (-20°C).
- 8. Transfer invertebrates and locality labels back into the same Whirl-Pak bag from the field.
 - a. Optional step. Traps containing a large amount of mud, silt or sand (a condition common when trapping periods overlap with rain events) can become time consuming to sort later. If time allows during the field season, the invertebrates can be separated from the sand, silt, mud, or debris. The invertebrates and their locality labels can be stored in the original Whirl-Pak bag from the field, while their

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extraneous debris is discarded. However, extreme care must be taken that no invertebrates are inadvertently part of the discarded sand/silt/mud/etc.

9. Pour fresh 95% ethanol into each Whirl-Pak bag, ensuring that all of the insects are submerged, and close the bag, leaving as little airspace as possible.
10. Confirm that the appropriate labels (including that with the etOH rinse date) are still in the Whirl-Pak bag.
11. Keep the Whirl-Pak bags from all traps within a plot together in a small bag and put each sampling bout (all the plots from a single date) into a labeled airtight plastic container or re-sealable bag and seal tightly until further processing (sorting, pinning, etc., detailed in the following steps).



Figure 21. Samples from one sampling bout stored in a sealed container. Label containers using lab tape and permanent markers.

12. Store processed invertebrate samples at room temperature in the flammables cabinets unless there is ample space in refrigerators (4°C) or freezers (-20°C).

D.3 Details about species identifications

The NEON master taxon list of vertebrate species codes can be found on the NEON intranet on the FOPs page in the taxonTables folder under the TOS tab. Technicians must use ONLY the NEON master code on all datasheets. The NEON master 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

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taxa is selected, an identification qualifier is not needed, unless the lowest taxonomic rank indicated (e.g., family, genus) is uncertain.

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

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SOP E Laboratory Processing – Sorting Invertebrate Bycatch

E.1 Preparation for Sorting

1. Clear lab bench space for beetle sorting and processing. Coordinate with potentially conflicting activities (e.g. soil sieving). Work beside fume hood/extractors to diminish inhalation of ethanol fumes.
2. Secure access to:
 - a. Propylene glycol and ethanol waste storage.
 - b. Sink for washing materials.
 - c. At least one dissecting microscope
 - d. Storage space for samples (e.g., cold storage for tubes of individuals)
 - e. Storage space for pinned insects in Cornell drawers or Schmitt boxes.

E.2 Sorting Invertebrate Bycatch

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

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

Steps for sorting invertebrate bycatch are detailed in **Figure 22**, with instructions in the following text.

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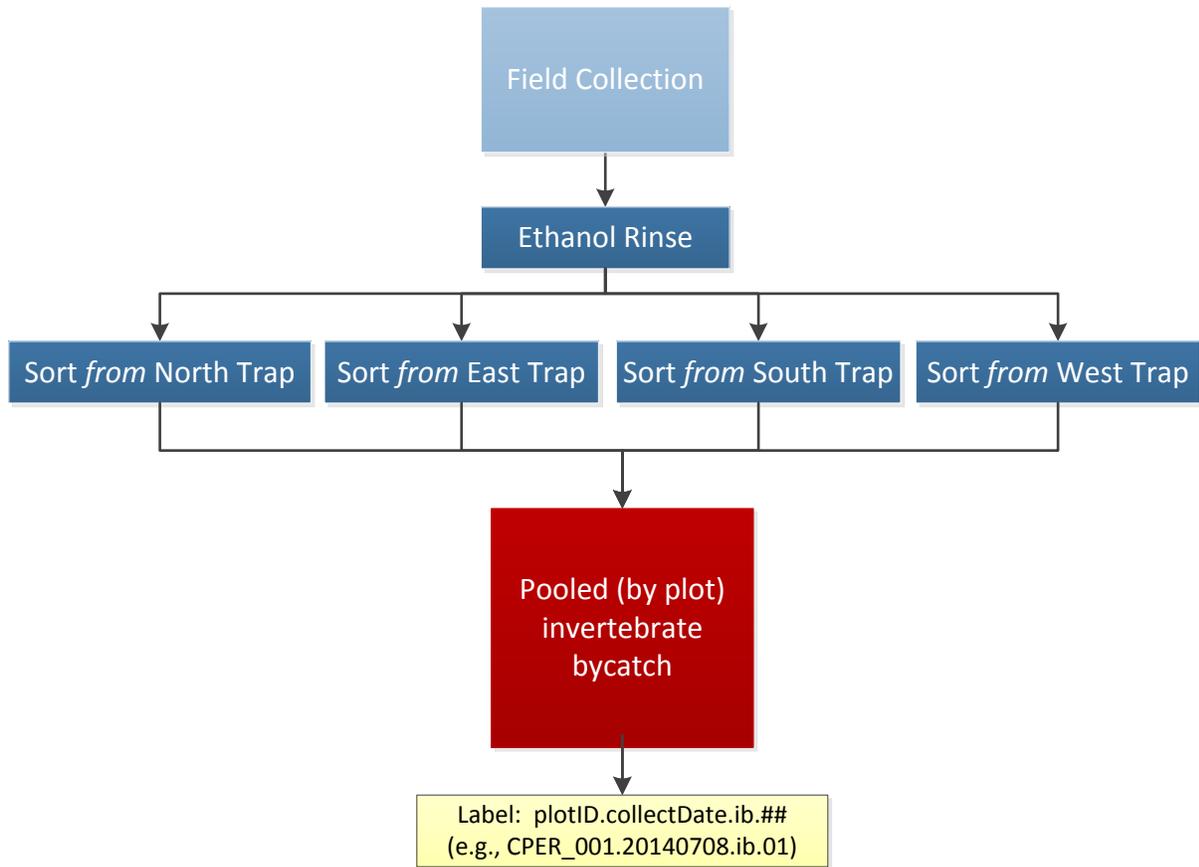


Figure 22. Workflow for processing invertebrate bycatch. Activities are represented by blue boxes, intermediate and final products by red boxes, decisions by grey triangles, and labels by yellow boxes.

1. Sort each sample individually, from one trap at a time.
2. Suspend specimens in ethanol in the Whirl-Pak bag so that they are uniformly floating throughout. Jostle the bag and use wash bottle to add 95% ethanol if necessary.
3. Pour specimens into a large Petri dish marked with a grid (hereafter referred to as the sorting dish). Remove or add 95% ethanol as needed to keep specimens submerged in the sorting dish, using a transfer pipette and a secondary container for used ethanol (removal) or wash bottle of ethanol (addition).
4. Remove the filter cloth from the Whirl-Pak and inspect it for any small invertebrates that may be attached. Rinse invertebrates into the sorting dish, with 95% ethanol, and then discard the filter.
5. Remove all non-beetle invertebrates, larval beetles, and beetle body parts that cannot be confidently identified as belonging to an individual Carabid, and place these into a

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temporary container marked 'invert bycatch' and the trapID. 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.

6. Using a dissecting microscope, sort all remaining beetle specimens into either the temporary 'invert bycatch' container OR a second temporary container that is marked 'Carabids' and into which a single locality label, from those in the initial pitfall trap, has been placed (see **APPENDIX B** and **APPENDIX C** for guides for delineating which beetles are Carabids). If a specimen cannot be confidently identified to the family Carabidae, but is a member of the suborder Adephaga, treat as if it is a Carabid.
7. If, at the end of sorting, no Carabids have been found in the sample, mark 'N' on the sorting datasheet for 'Carabids (**targetTaxa**) Present'.
8. Record the presence of invertebrate bycatch at the trap-level (circle 'invert bycatch' in the sampleType field), and fill in the 'processingDate', 'recordedBy', and 'etOH Change Date' fields. *Neither counts nor taxonomic identifications are expected for invertebrate bycatch specimens.*
9. When all samples from a plot have been fully sorted, pool invertebrate bycatch by the plotID and collectDate.
 - a. Transfer all invertebrate bycatch specimens from the four traps within a plot into as few 50 mL tube as are required, leaving ½ inch empty at top of tube.
 - i. Be sure to move the locality labels into the new storage tubes (e.g., if the invertebrate bycatch from traps N and S fit into one 50 mL storage tube, place the locality labels for both traps into the new storage tube).
 - ii. If multiple tubes are required from the same trap, generate additional locality labels and place a locality label in each tube.
 - b. Insert a new sampleID into each tube: plotID.collectDate.ib.tubeNumber (where tubeNumber is two digits and starts at 01).
 - c. Pooling of invertebrate bycatch within a plot is intended to save on tubes and space. However, invertebrates are often unevenly distributed across the plot (i.e., East trap has 200 carrion beetles and West trap has 1 grasshopper) or may be present in just one trap. In that case, it is possible that some tubes may contain invertebrates sourced from just one of the traps even after pooling across plot (e.g., specimens from tube 1 are all from East trap, specimens in tube 2 come from a mix of traps). In that case, record the tubes that have specimens from multiple sources as pooled,

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but record tubes with specimens from a single trap source as though they are not pooled (because they aren't). Pooling occurs to save on archival and materials costs. However, if it is possible to keep tubes filled with a single trap's contents, then the data need to reflect that more specific origin. Dealing with this is examined more in step 10.

Example: Three tubes are required to hold invertebrate bycatch for a plot, one with specimens from traps N and S, another with specimens from traps E and W, and a final tube with samples that came from trap S only. SampleIDs for these tubes are plotID.collectDate.ib.01, plotID.collectDate.ib.02, and plotID.S.collectDate.ib.01.

Note – sampleIDs are automatically generated by the WebUI when the Sample Type is 'invertebrate bycatch'.

- d. Fill each tube with 95% ethanol, with at least ½ inch of ethanol above the fully submerged specimen(s). Split the specimens into multiple tubes if necessary to ensure that there is ½ inch or more of ethanol above the specimens in each tube (**Figure 23**).
- e. Some samples may contain debris that will be stored with the bycatch (large items, e.g., leaves or twigs, may be removed and discarded, but be careful that small invertebrates are not attached to them).

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Figure 23. Bycatch samples stored in a 50 mL centrifuge tubes, the amount of ethanol in a bycatch sample will vary by sample size.

10. If invertebrate bycatch has *not* been pooled by plotID and collectDate:

- a. Fill out all trap-level metadata (e.g., processingDate, etOH Change Date, etc.) as above.
- b. Insert a new sampleID label into each tube:
plotID.trapID.collectDate.ib.tubeNumber (where tubeNumber is two digits and starts with 01, then increases for as many tubes as contain the invertebrate bycatch for the trap).

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

Note – sampleIDs are automatically generated by the WebUI when the Sample Type is ‘invertebrate bycatch’ and the box for ‘Not a pooled sample’ is checked.

- c. Fill each tube with 95% ethanol, with at least ½ inch of ethanol above the fully submerged specimen(s). Split the specimens into multiple tubes if necessary to ensure that there is ½ inch or more of ethanol above the specimens in each tube

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- d. Some samples may contain debris that will be stored with the bycatch (large items, e.g., leaves or twigs, may be removed and discarded, but be careful that small invertebrates are not attached to them).
- e. Record the ‘final sample ID (**sampleID**)’ field of each record and circle “Y” or “N” in the targetTaxaPresent field (see **APPENDIX D**).

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

12. Transfer Carabids (if any) from the temporary container marked ‘Carabids’ and one locality label back into the original Whirl-Pak bag from the field.

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

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SOP F Laboratory Processing - Sorting Ground beetles

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

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

Carabid beetles should be initially sorted from invertebrate bycatch, as described in **SOP E**. After non-Carabidae invertebrates are removed from Whirl-Pak, the carabids (at this point still not identified to species) are returned to their original, trap-level Whirl-Pak (see 12 from above). During the season, only the initial sorting phase discussed in **SOP E** will be conducted. Data concerning invertebrate bycatch generated from separating bycatch from Carabids may be digitally transcribed during the field season, but information about carabids themselves (e.g., individual counts, etc) should not be input into the sorting datasheet until the end of the season when either the species or morphospecies is known. Species-level sorting should occur on a trap-by-trap basis so that specimens from individual traps are kept separate through the entire sorting process.

During the sorting process specimens will be identified to species and either 1) be individually pinned or pointed, or 2) grouped by species in vials of ethanol at the *trap-level*. For domain support facilities with multiple sites, identification of beetles should happen for one site at a time, completing the identification of all beetles at one site before moving on to another site. This cuts down on the number of temporary vials that are in storage at any one time. Only after an entire site has been processed can groups of the same species be pooled by plot and collection date. The general process of sorting Carabid beetles is illustrated in

Figure 24, with detailed instructions in the following text. Do *not* fill out any carabid sort information into the WebUI until *after all* pinning, pooling, and QAQC procedures have been completed for that site.

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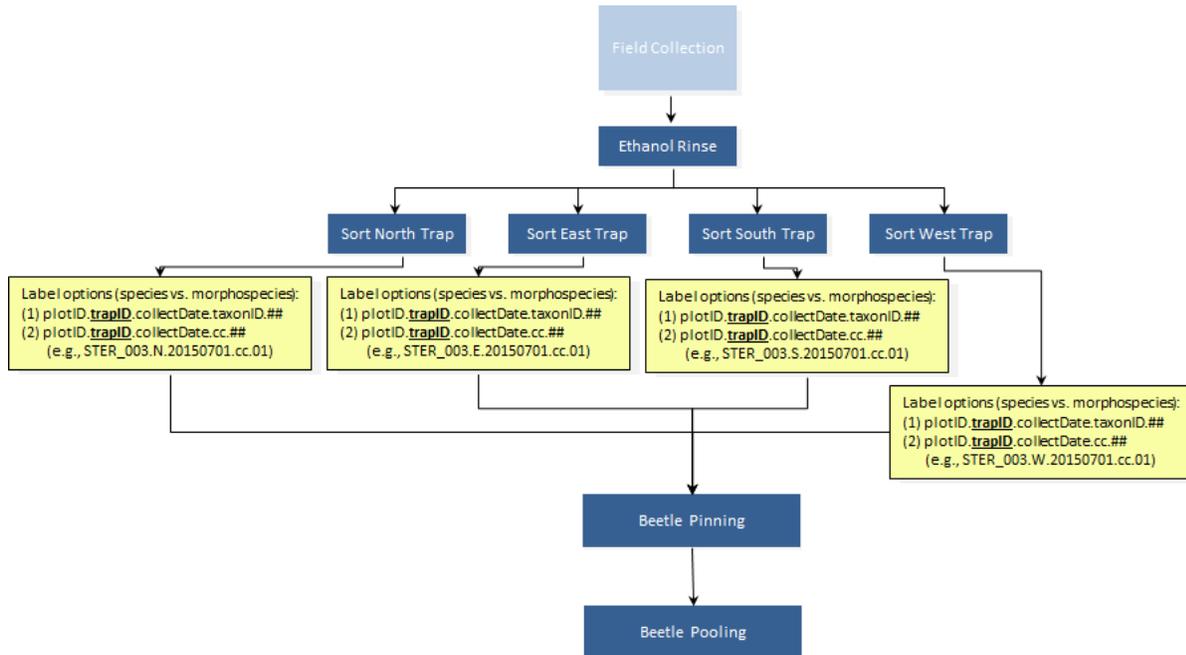


Figure 24. Workflow for processing Carabid beetles. Carabids are sorted by species within each trap, pinned as necessary. At the end of the season, more pinning may be required to get a representative mixture of variation within the pool of beetles. After all identifications and pinning are complete, then Carabids are pooled as necessary. Activities are represented by blue boxes and labels by yellow boxes.

1. 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.
 - a. Place all carabids from the same trap of the same species or morphospecies, into as few 50 mL tubes as required. If there are only a few small individuals, you may use smaller (10 mL) tubes, pending availability.
 - b. Fill each tube with enough 95% ethanol that there is at least 1/2 inch of ethanol above the fully submerged specimen(s). If there are too many specimens to allow for 1/2 inch of ethanol above the level of the specimens, split the specimens into multiple tubes to ensure that there is 1/2 inch or more of ethanol above the specimens in each tube.
 - c. Include a locality and determination label in each tube.
2. For specimens that can be assigned a species determination, whether with some uncertainty (using an identification qualifier) or poorly preserved specimens (will only receive a coarse level of identification, proceed as follows:
 - a. Insert a label into a new tube on which the following sample ID is recorded (recorded on ethanol safe paper and using an archival ethanol-safe pen):

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plotID.trapID.collectDate.taxonID.tubeNumber (where tubeNumber is two digits, starts with 01, and increases for as many tubes as contain the same taxon).

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

Example: Two tubes are required to hold a particular carabid species for trap W. SampleIDs for these tubes are plotID.W.collectDate.taxonID.01 and plotID.W.collectDate.taxonID.02.

Note – sampleIDs are automatically generated by the WebUI when the Sample Type is ‘Common Carabid’, there is an entry in the ‘Taxon ID’ field.

3. For species in good condition whose identities are completely unknown (morphospecies):
 - a. Insert a label into a new tube on which the following sample ID is recorded (recorded on ethanol safe paper and using an archival ethanol-safe pen):
plotID.trapID.collectDate.cc.tubeNumber (where ‘cc’ signifies ‘Carabid’ and the tube number is two digits, starts at 01, and increases for as many tubes as contain morphospecies in the trap).

Example: Trap E yields two species of Carabids (Morph A and Morph B) and each species is too numerous to fit in one tube. SampleIDs for these tubes are plotID.E.collectDate.cc.01, plotID.E.collectDate.cc.02, plotID.E.collectDate.cc.03, and plotID.E.collectDate.cc.04.

Note – This sampleID is automatically generated by the WebUI when the Sample Type is ‘Common Carabid’ and the ‘switch to morphospecies’ button has been pressed.

4. For each tube, record on the paper sorting datasheet:
 - a. The sample ID generated in step F.1. **Error! Reference source not found.** or F.1.e.e.a above in the ‘final Sample ID’ field (see **APPENDIX D**).
 - b. The number of individuals in the tube, in the ‘Individual Count’ field
 - c. ‘Y’ in the ‘Carabids (**targetTaxa**) Present’ field

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- d. 'Common Carabid' in the sampleType field
 - e. The etOH rinse date in the 'etOH change date' field
 - f. The identity of the individuals in the tube, to the finest taxonomic level possible (preferably species), in the 'Sci. Name OR taxonID' or 'morphospecies ID' field (see section D.8 for instructions on morphospecies naming conventions).
 - g. All other data that can be filled out (e.g., date of identification, etc.)
5. Close the lid on the tube(s) tightly so no ethanol leaks out (can result in smeared labels).
 - a. While samples are being sorted to determine their IDs (either to species or morphospecies), a subset of Carabids may be selected for pinning according to the guidelines in **SOP G**. Once all Carabids that are being pinned have been removed from a vial, *verify that the 'individualCount' number 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 datasheet indicates that 2 *Pasimachus sublaevis* were pinned from CPER_001.W.20160401.PASSUB1.01, then that will be interpreted as 10 beetles being present in the West trap of plot 001 at CPER on the 1st of April 2016.

6. After an entire site's samples have been processed from a field season and no more individuals are going to be pinned, then individuals of the same species can be pooled by the plotID and collectDate:
 - a. Transfer all individuals of the same species (from all four traps in the plot) into as few 50 mL tubes as are required. Be sure to move the locality labels into the new storage tubes

Example: If the individuals from traps N and S fit into one 50mL storage tube, place the locality labels for traps N and S into the new storage tube.
 - b. Insert a new sampleID label (recorded on ethanol safe paper and using an archival ethanol-safe pen) into the tube with: plotID.collectDate.taxonID.tubeNumber (where tubeNumber is two digits and starts with 01, then increases for as many tubes as contain the individuals of that taxon for the plot).

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- c. For each record, write the sample ID from step (b above) in ‘final sample ID’ field of the sorting datasheet (see **APPENDIX D**). At this point, the pinning and sorting data can be transferred from the paper datasheets to the WebUI.
 - d. Fill each tube with enough 95% ethanol that there is at least 1/2 inch of ethanol above the fully submerged specimen(s). If there are too many specimens to allow for 1/2 inch of ethanol above the level of the specimens, split the specimens into multiple tubes to ensure that there is 1/2 inch or more of ethanol above the specimens in each tube.
7. Some samples may contain debris that will be stored with the bycatch (large items, e.g., leaves or twigs, may be removed and discarded, but be careful that small invertebrates are not attached to them).
 8. Store processed samples at room temperature in the flammables cabinets unless there is ample space in refrigerators (4°C) or freezers (-20°C)

F.1 Equipment maintenance, cleaning, and storage

1. After all samples have been sorted:
 - a. Empty, wash, and dry all waste receptacles (e.g. ethanol and PG containers).
 - b. Return all flammables to the appropriate cabinets.
 - c. Pack equipment and consumables for subsequent field work and store neatly.

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

F.2 Handling uncertainty in species identifications

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

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can indicate the finest known level of taxonomic information in one of two ways: 1) recording an identification qualifier or 2) assigning a morphospecies.

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

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

Table 9. Codes for identification qualifier entries

idQ Code	Identification Qualifier Description*
CS	cf. species
AS	aff. species
CG	cf. genus
AG	aff. genus
CF	cf. family
AF	aff. family

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

Morphospecies designations must be used when the specimen is in good condition, but technicians cannot narrow the field of possible identifications to just two or three choices. Keep notes in the morphospecies tracking sheet that include descriptive information about the specimen plus any known

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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).

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 FOPs page in the morphospeciesTracking folder under the TOS tab.

Cryptic species issues arise when two species that are morphologically indistinguishable in the field co-occur (or might co-occur) at a site. NEON intends to add these species pairs 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 entered in the crypticSpeciesGroups spreadsheet on the NEON intranet on the FOPs page in the taxonTables folder under the TOS tab.

Finally, the WebUI currently limits technicians such that only species thought to be present in a domain are available for selection within the WebUI. 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 WebUI 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. The taxon table will be updated for the subsequent year to make that species name available to technicians within that domain.

F.3 About morphospecies designations

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

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

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

Note: Because domain 13 is split across two support facilities, the MorphA assigned by the Boulder office will not be the same as the MorphA assigned by the support facility in Utah. To avoid confusion, the Utah domain support facility will put an extra letter (“Z”) between “Morph” and their unique letter combinations. Unique letters will be used as described above. The first morphs would be called MorphZA, MorphZB, MorphZC, etc. The 27th morphospecies at the Utah facility will be MorphZAA.

Record and describe each morphospecies on the morphospecies datasheet (found on NEON Intranet page), along with the year and name of the person who designated it on the NEON intranet on the FOPs page in the morphospeciesTracking folder under the TOS tab. If a taxonomist is able to identify some individuals of a morphospecies to species, the identity of all the other individuals of that morphospecies should be updated on both the sorting and ID and pinning datasheets (or in the respective web user interface (WebUI)).

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

G.1 Pinning and pointing large and small ground beetles

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

The number of individuals pinned from a species will depend on its abundance and the level of uncertainty in the identification. Species are considered abundant if more than 100 are found per site in a season, moderately common if 20 to 100 individuals occur per site in a season, and uncommon if less than 20 occur per site in a season. Species-level identifications that contain more uncertainty will require more individuals of that species group to be pinned. Decision trees in Figure 25 and Figure 26 describe the minimum number of specimens that must be pinned in different situations.

However, in general, pinning or pointing is required for:

1. Up to 20 individuals per species per site per year of uncommon species (see Figure 25: directions for pinning moderately common to uncommon species).
2. At least 20 individuals, if available, for each easily identified species (**APPENDIX A**).
3. At least 100 individuals, if available, for each species or genus on the list of difficult taxa (**APPENDIX B**).
4. If species is moderately common to abundant, does not occur on any list of species (**APPENDIX A, APPENDIX B**), and the technician is confident in their identification, then pin at least 20 but no more than 100 (see Figure 25 and Figure 26 for more detailed instructions).

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

Data for each pinned or pointed specimen should be recorded on the ID and Pinning datasheet (RD[05]) and later transcribed as a Pin Activity of the web user interface (WebUI). Most of the data for the datasheet can be found on labels in the tubes from which the specimens are obtained, or in the record in the sorting datasheet that corresponds to this tube.

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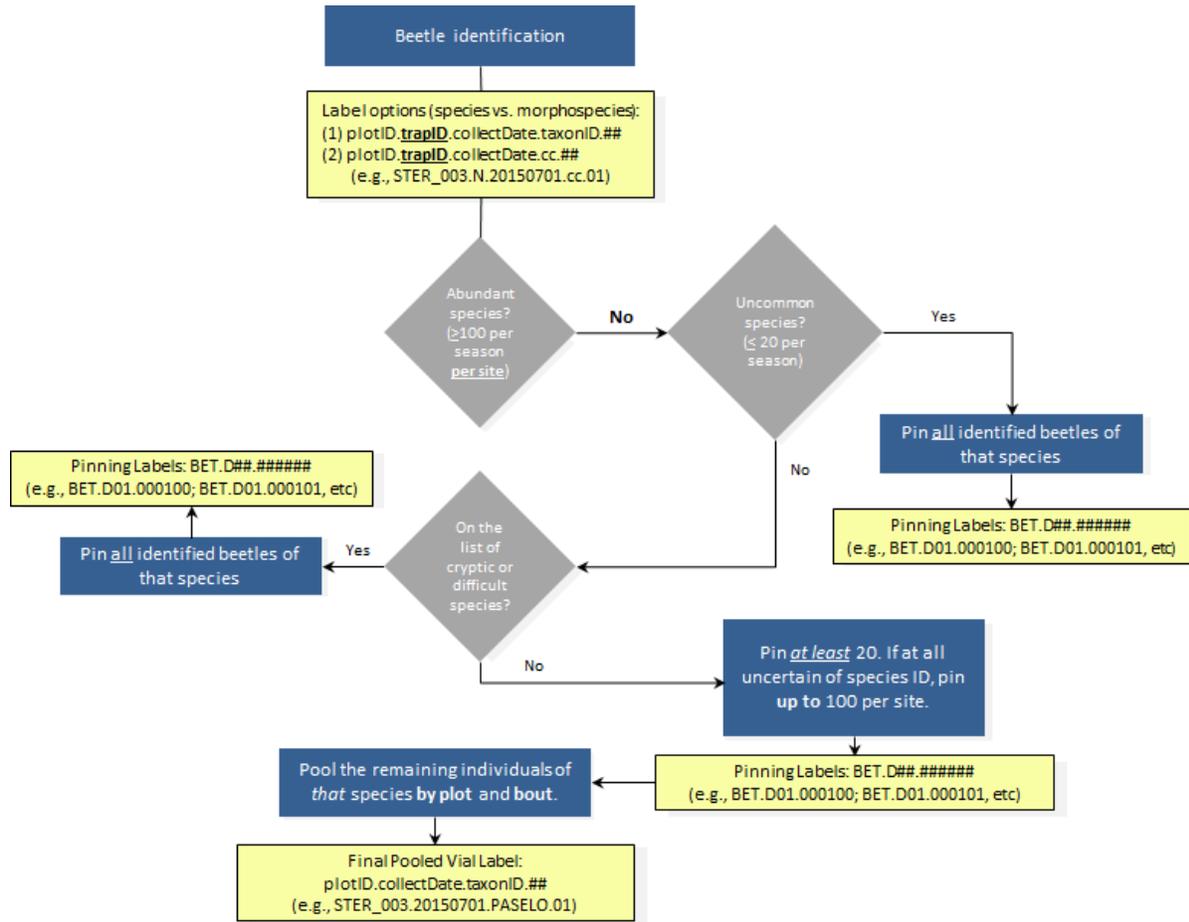


Figure 25. Pinning instructions for moderately common and uncommon species (less than 100 over the season)

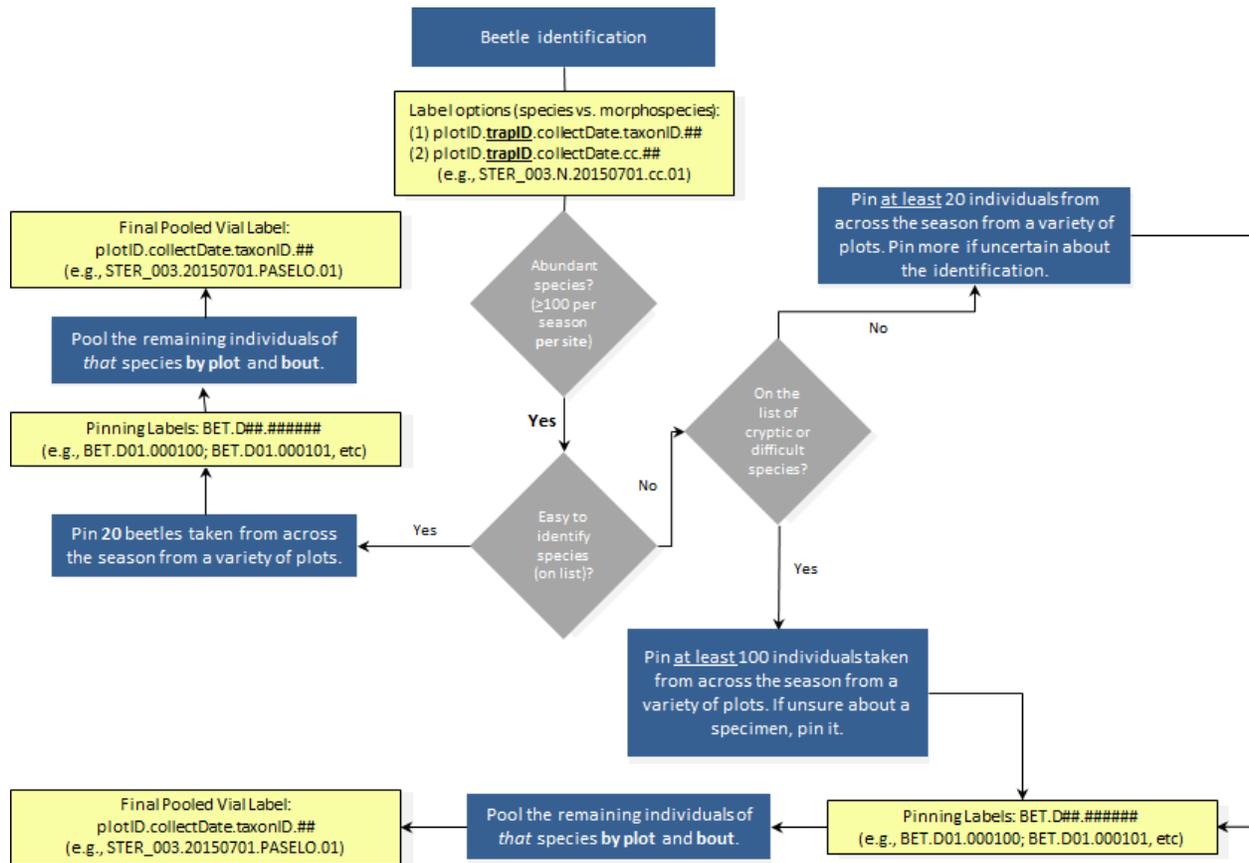


Figure 26. Instructions for pinning abundant species (more than 100 present at a site over the entire season).

G.2 Determine whether to pin or point the specimen

1. Generally specimens 5 mm and greater are pinned, and specimens smaller than 5 mm are pointed (Table 10; Figure 27).
2. Pointing is a good default option for any specimen you are concerned will be destroyed by pinning (Table 10; Figure 27).

Table 10. 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

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Figure 27. Pinned (left) and pointed (right) beetle specimens

G.3 Prepare points (if pointing)

1. Prepare a batch of points prior to beginning pointing specimens.
2. Using 100% rag cotton paper (Bristol or equivalent), prepare ~40 triangles using the point punch.
3. Over a balsa wood surface, for each point, press a No. 3 archival quality pin into the point at the center of the point’s base (see the “X” in **Figure 28**). Ensure that the point is at a 90 degree angle from the pin and neither angled up nor down.



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

4. Use a pinning block (**Figure 29**) to push the point up the pin until it is 15 mm from the top (non-pointed end) of the pin. This leaves enough space above the insect for the pin to be

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

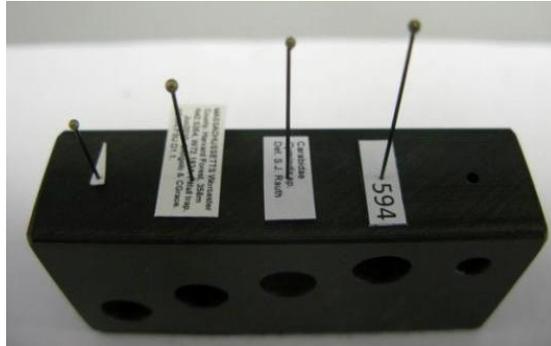


Figure 29. A pinning block with a point and the various labels that will be affixed to a pinned or pointed specimen. This shows the heights at which points and various labels should be placed on a pin.

G.4 Prepare labels

1. See **APPENDIX A** for instructions on creating locality labels. These are created for all mounted beetle specimens during field preparation and will be placed on the pin below each specimen.
2. See **APPENDIX B** for instructions on creating determination labels. These are created for all mounted beetle specimens during field preparation and will be placed on the pin below the locality label located below each specimen. Ground beetles that have not yet been identified, however, may be mounted on pins with locality labels but no determination labels.
3. See **APPENDIX C** for instructions on creating insect individual ID labels. These will be created for all mounted beetle and specimens at the time of preparation and will be placed on the pin below the determination label (which is below the locality label) located below each specimen.



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

G.5 Mount Specimens

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

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- b. After removing a specimen from the tube, allow it to dry for a few minutes. It is easier to work with dried specimens when pointing.
- c. Use forceps to put the beetle specimen on its left side on a slip of plain white paper, so that its head is facing the right and its legs are toward the edge of the bench (**Figure 30**)



Figure 30. A ground beetle on its side in preparation for pointing

- d. Slide the slip of paper with the beetle to the center of the microscope stage.
- e. Examine the slope of the ground beetle's thorax to determine if the tip of the point needs to be bent to ensure that the specimen will lie flat on the point. Use forceps to bend the point if needed (**Figure 31**).
 - i. **NOTE:** The sides of a beetle's thorax can slope inward to differing degrees, therefore, the tip of the point can be bent down with forceps to accommodate the size and shape of each specimen. For example, a specimen that has a flat-bottomed thorax can be mounted on a flat (unbent) point. A specimen on which the sides of the thorax are vertical must have the tip of the point bent downward at a right angle. See **Figure 31** for thorax slope scenarios and **Figure 32 - Figure 33** for examples of well-pointed and poorly-pointed specimens, respectively.

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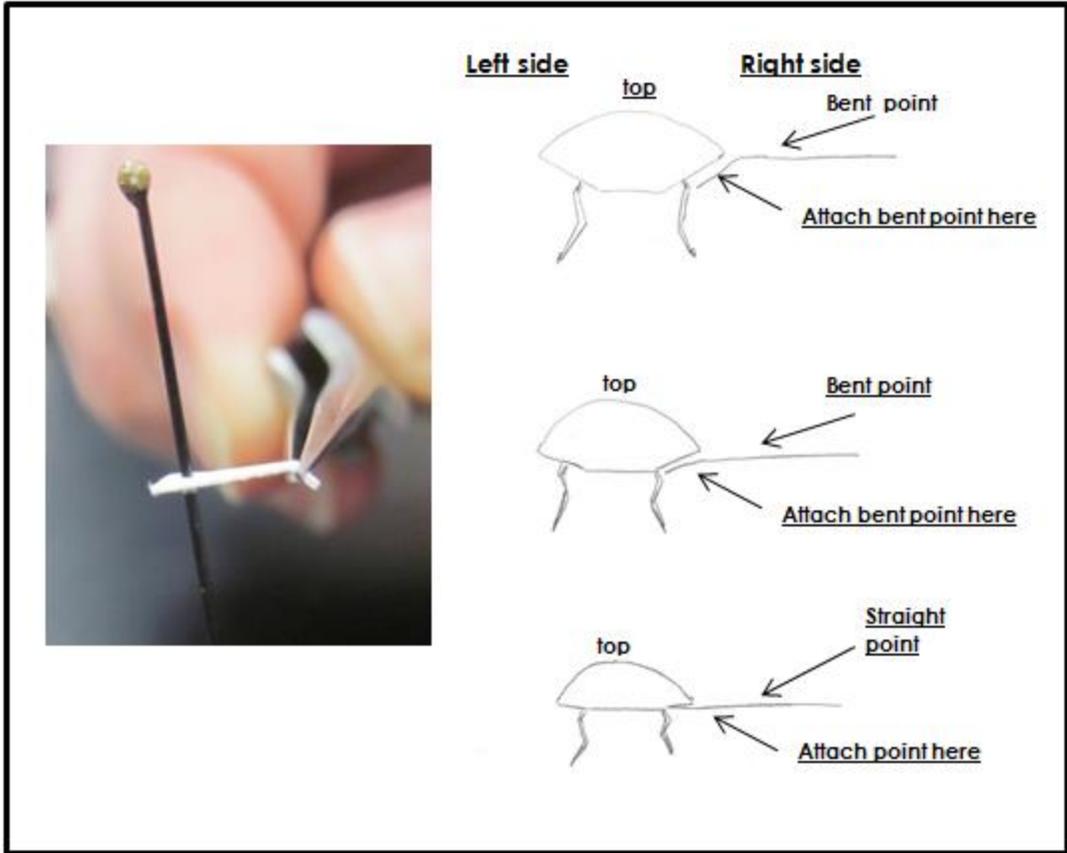


Figure 31. Examples of bending a point tip to maintain a level body position of the specimen

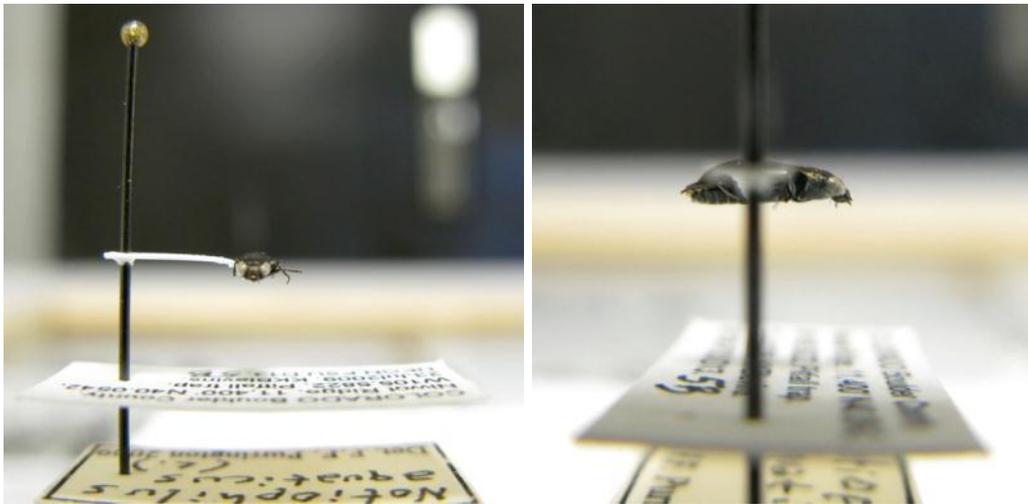


Figure 32. A well-pointed beetle specimen, with associated labels

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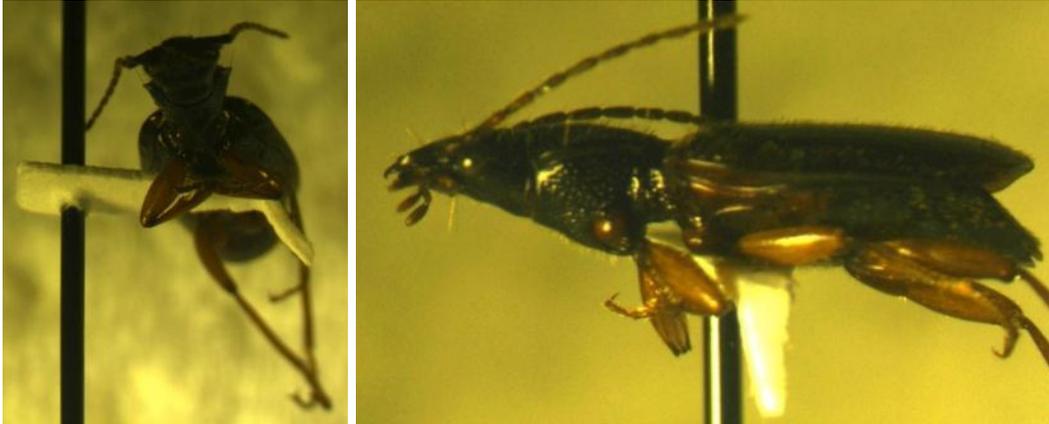


Figure 33. A poorly pointed beetle specimen

- f. Take the prepared point and touch the tip of the point in a bit of glue that has been squeezed out onto a paper towel or piece of paper (**Figure 34**). Use the minimum amount of glue needed in order to attach the specimen firmly to the point.



Figure 34. Preparing to glue a specimen to a point by dipping the tip of the point in glue.

- g. Under a microscope, zoom in on the target location for the tip of the point, this being on the **RIGHT** side (from a dorsal perspective) of the specimen's thorax and between the bases of the 2nd and 3rd pairs of legs (see "X" on **Figure 35**). Gently press the glue-covered tip of the point to the beetle's body (**Figure 36**), and wipe off excess glue in order to avoid obscuring any part of the beetle with large amounts of glue.
 - i. **Note:** Although the point and glue will obscure one side of the thorax, the other side needs to remain unobscured for examination. Neither the point nor the glue should extend onto the top or the bottom of the beetle, nor touch the head, abdomen, or wings.
 - ii. **Note:** Be sure to place the point on the *right* side of the specimen

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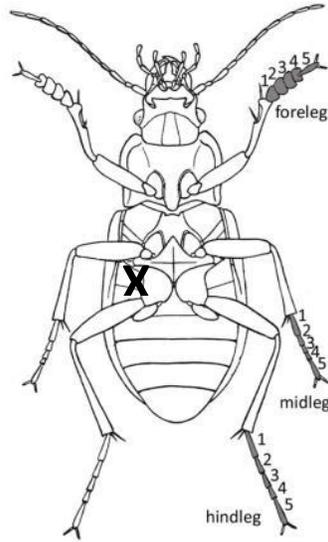


Figure 35. Diagram of ventral side of a beetle, with and X indicating where to place point



Figure 36. Attaching a specimen to a point, and pointed specimens left to set.

- h. Wait for the glue to set slightly, so that the beetle stays relatively still when the pin is moved. Then lift and angle the pin so that the weight of the specimen is leaning directly into the point and the specimen is properly aligned on the point, as in **Figure 31 - Figure 32**.
- i. Place the pin in a block of Styrofoam such that gravity is holding the specimen down on the point at the correct angle.
- j. Adjust the specimen as needed with forceps or a pin to ensure the specimen binds firmly to the point. A heavy specimen that rotates on the point should be straightened as the glue thickens but before it completely hardens.
- k. Slide the locality label (instructions in **APPENDIX A**) onto the pin below the specimen, and use the second-deepest hole in the pinning block to position the

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label at the correct height (**Figure 37**). Position the specimen perpendicular to the text on the label, with the back edge of the point slightly inset from the short edge of the label and the specimen centered between the two longer edges of the label (**Figure 37**).

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



Figure 37. Attaching a locality label below a pointed beetle.

- l. Attach the determination label (instructions in **APPENDIX B**) below the locality label. Use the third-deepest hole in the pinning block to position the label at the correct height. Orient the label in the same manner as the locality label.
 - m. Attach the insect individual ID label (instructions in **APPENDIX C**) below the determination label using the fourth-deepest (shallowest) hole. Orient the label in the same manner as the locality label.
 - n. When all of the specimens are mounted and labeled, examine them under the microscope to confirm that they are all attached securely to the points. To test this, place the specimens in a Schmitt box or unit tray and tap the box or tray. The specimens should remain affixed. Re-attach any specimen that do not stay affixed to the points, as long as it is clear which locality label belongs with the specimen.
2. For pinned beetle specimens:
- a. **Note:** if you are new to pinning, start with larger specimens as they are easier. Keep specimens in ethanol until ready to pin.
 - b. Use only No. 3 archival quality pins. Finer pins are too flexible and vibrate in the specimen, destroying them from within. Thicker pins damage specimens as they are inserted.
 - c. Press your thumb and index finger together in order to form a “v”, which you will use to support the specimen as you insert the pin.

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- d. Place the specimen into the “v” and position it so that it is straight in your fingers and the legs are facing downward.
- e. Insert the pin vertically through the top layer of the specimen’s body (**Figure 38**).
 - i. **Note: It is important to insert the pin in the correct location in the beetle’s body.** A properly placed pin is positioned on the **RIGHT** side of the beetle (when viewed dorsally), halfway between the midline of the beetle’s body and the edge where the elytra (wing shells) begin to round behind the thorax (see the “X” in **Figure 38**).
 - ii. **Note:** Be sure to place the pin through the *right* side of the specimen

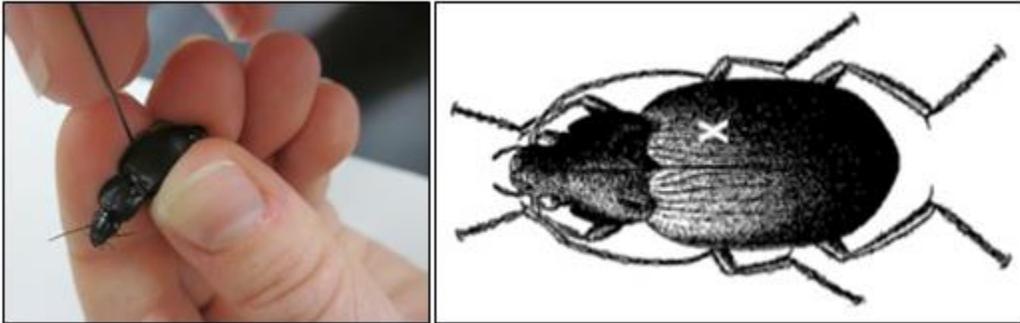


Figure 38. Ground beetle pinning and dorsal side schematic showing where to place the pin (marked by an “X”)

- f. Place the specimen on top of the pinning block and gently slide the pin through the body of the specimen. Push the pin slowly and with constant pressure.
- g. Ensure that the pin is at a right angle to the beetle’s body and push the specimen up the pin until its top surface is 15 mm from the top of the pin. This leaves enough space above the specimen for the pin to be handled and below for labels. Additionally, specimens at a uniform height are easier to examine and compare with one another. See **Figure 39** for examples of well-pinned and poorly-pinned specimens.

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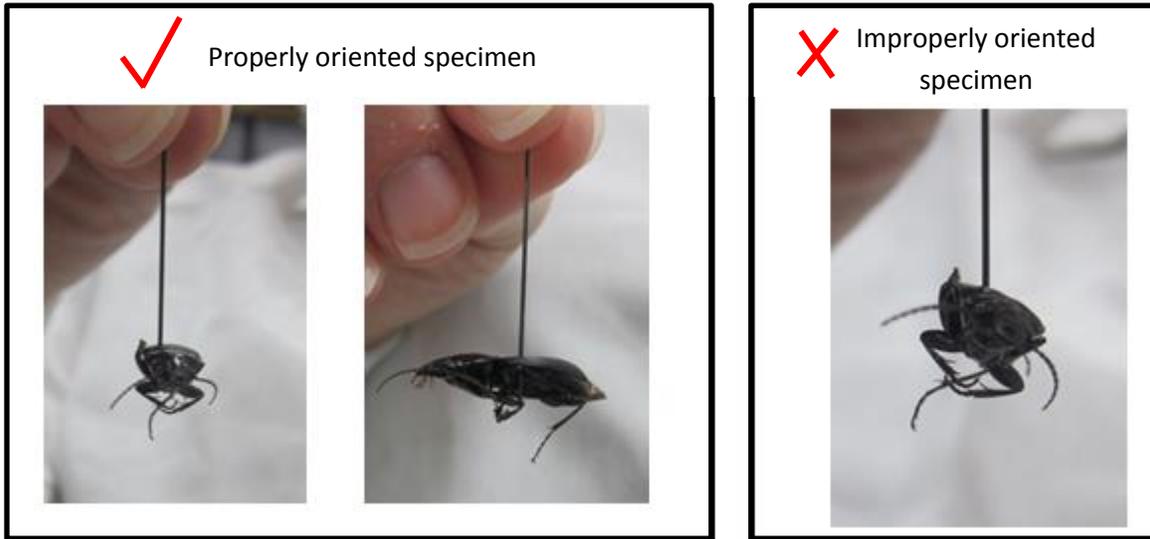


Figure 39. Correctly and incorrectly pinned beetle specimens

- h. Slide the locality label (instructions in **APPENDIX A**) onto the pin below the specimen, and use the second-deepest hole in the pinning block to position the label at the correct height (**Figure 40**). Position the specimen in the middle of the locality label and with the head pointing to the left side. The specimen should be lined up with the long axis of the body parallel to the print on the label (**Figure 40**).

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



Figure 40. Attaching a locality label below a pinned ground beetle

 - i. Attach the determination label (instructions in **APPENDIX B**) 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.

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

G.6 Loss of Body Parts

1. If a part of a specimen, such as a leg, falls off during pinning, save it and re-attach it to the beetle (provided you are certain which specimen the body part belongs to; **Figure 41**). To do this:
 - a. Hold the part being reattached with forceps.
 - b. Touch the top of the body part to a bit of glue.
 - c. Place the body part back into correct location and orientation and hold until the glue has set.



Figure 41. A leg being inserted back into its socket, using forceps and a dab of glue

G.7 Sample Preservation

1. Pinned or pointed ground beetle specimens are to be stored in airtight Schmitt boxes or in unit trays in Cornell cabinet drawers, with a small amount of moth crystals (≈ 6.5 g) in a cardboard fumigant box.
2. After a specimen has been pinned or pointed, and any glue has dried, it should be placed in a box or tray so that locality labels are oriented in the same direction as those of the other specimens in the box or tray (
3. Figure 42).

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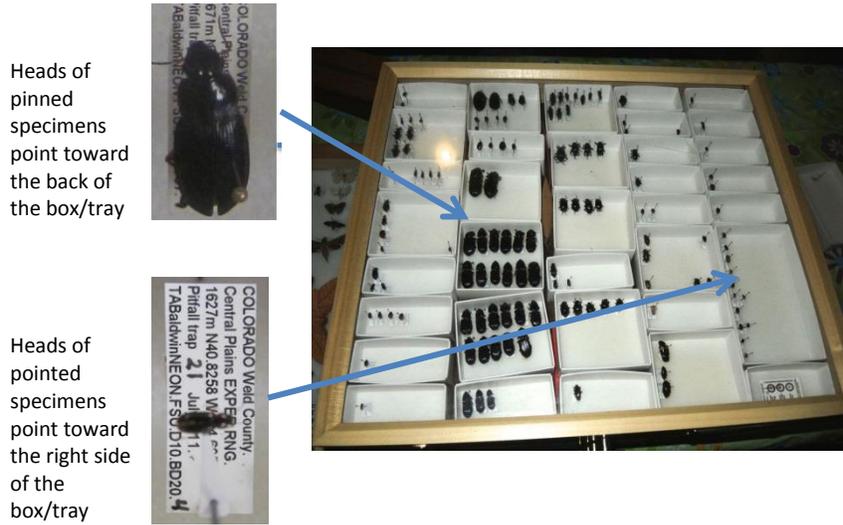


Figure 42. Proper orientation of mounted specimens in unit trays within a Cornell drawer.

4. Specimens should be grouped into unit trays within each Cornell drawer or Schmitt box by species or morphospecies, leaving space to place specimens mounted at a later date/from different sampling bouts. If there are empty areas in the Cornell drawer, fill them with empty unit trays to keep those holding specimens from sliding around.
5. If there are not enough unit trays, containing specimens, to fill a Schmitt box or Cornell drawer, add empty unit trays so that filled trays are unable to slide around.
6. A fumigant box should be secured in the corner of one of the unit trays with pins, so that it does not slide around and damage specimens (**Figure 43**).

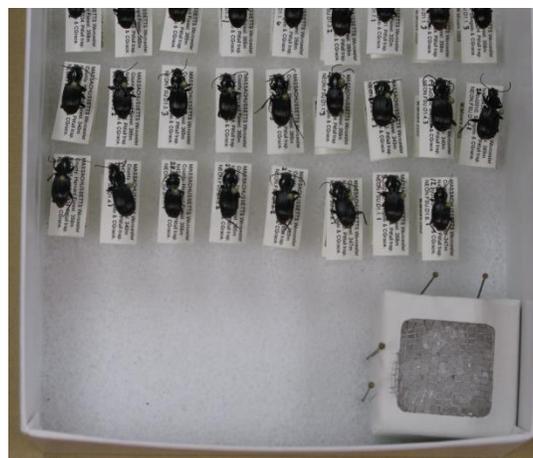


Figure 43. Fumigant box filled with moth crystals and secured in the corner of a unit tray with pins.

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7. Specimen storage containers and workspaces should be kept clean to prevent pests. Dermestid beetles are common pests in insect collections. If you notice dermestid beetle activity, such as debris under a specimen or larval skins of dermestid beetles, freeze the drawer/box for two weeks and clean up the damage.
8. Check/replace moth crystals monthly.
9. Samples can be stored in this manner indefinitely.
10. Maintain fumigant in every drawer, including those without specimens. The fumigant acts as a repellent, but will not kill pests if they get into the collection.
11. Never leave boxes or drawers sitting out, put them away when you are not working on them
12. Keep counters clean, always wipe down with ethanol before putting a box or drawer on a countertop.
13. Minimize amount of time lids are off drawers; never put drawers or lids on the floors
14. Keep all dead insects away from light, e.g. lights and windowsills. If sticky traps are used to capture pests in/around the boxes or drawers, replace these traps frequently.

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SOP H Data Entry and Verification

Field data (i.e., the ‘trapping activity’ on the WebUI) and **bycatch** sorting data (i.e., the ‘sorting activity’ on the WebUI) collected on paper datasheets shall be digitally transcribed within 14 days of collection (where applicable). Vertebrate sorting takes place within 24 hours of trap collection, thus digital transcription shall happen within 14 days of collection. Invertebrate bycatch may not be processed until the end of the field season; digital data transcription of *invertebrate* bycatch is therefore not required until 14 days after sorting of invertebrates occurs. Do not digitally transcribe any carabid sorting and pinning data for a site until sorting and pinning of all carabids at that site is completed. See RD[04] for complete instructions regarding manual data transcription.

Before entering data, all personnel must read RD[04] for complete instructions regarding manual data transcription. Prior to entering data via a web user interface (WebUI), each technician shall enter a plot (or subplot) of data from one bout into the protocol-specific WebUI housed on the Training portal, as described in RD[04]. Protocol-specific instructions and the associated data ingest workbook for entering beetle data can be found on the NEON intranet in the FOPs folder under the TOS tab. Be sure to enter data for all plots within a bout even if traps were not set as scheduled, due to unforeseen circumstances. However, if an entire bout is missed then no data need to be entered.

Ensure that all metadata from the paper datasheets are entered into the PDA (for field – collected data only) or web user interface (WebUI). Metadata include field or lab notes that detail any deviations from the protocol. Explain what was done and why, so that any questions/problems/inconsistencies can be understood. The sooner these data are entered, the more detail from the lab/field will be remembered and recorded. This improves data quality.

H.1 Field datasheets

1. At the end of each field day or as soon as possible after data collection (14 day limit), according to instructions in the NEON Protocol: manual data transcription (AD[04]), field data should be promptly and carefully transcribed into the WebUI (if PDAs are not available). Remarks that apply to an entire plot should be recorded for each trap separately (e.g., ‘mowing disturbed all traps at the plot). NOTE: any field data must receive QAQC checks *prior* to submission via the WebUI.
2. Scan datasheets and save in PDF file format.
3. Save paper copy of datasheets.

H.2 Sorting and ID & Pinning datasheets

1. Data from **bycatch** sorting datasheets (mammal and herptile bycatch; invertebrate bycatch) can be entered into the WebUI through the season prior to entry of any carabid sorting or

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pinning data, as time and staff resources allow. NOTE: bycatch sorting data must receive QAQC checks *prior* to submission via the WebUI.

2. Beetle sorting and pinning data from a site may not be entered until sorting, pinning and pooling of all beetles from that site is complete. Data from sorting datasheets should be carefully transcribed into the WebUI *after* all beetle processing (including identifying and pinning) is complete and all data are recorded and/or updated (e.g., records have a corrected final sampleID if specimens were pooled, counts updated and/or new records generated following any taxonID revisions due to initial misidentification) on the datasheet.
3. Following data sheet finalization, data from ID and Pinning datasheets should then be promptly and carefully transcribed into the WebUI.
4. Scan datasheets and save in PDF file format.
5. Save paper copy of datasheets.

H.3 Morphospecies datasheet

1. Promptly and carefully transcribe data into the [beetleMorphospeciesTracking datasheet](#), in NEON Intranet (FOPS/TOS/morphospeciesTracking/beetleMorphospeciesTracking).
2. Scan datasheets and save in PDF file format.
3. Save paper copy of datasheets.

Refer to NEON Protocol and Procedure: Manual Data Entry (RD[04]) for instructions regarding manual transcription and data entry.

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SOP I Sample Shipment

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

Information included in this SOP conveys science-based packaging, shipping, and handling requirements, not lab-specific or logistical demands. For that information, reference the [CLA's NEON intranet site \(https://neoninc.sharepoint.com/sites/cla/SitePages/Home.aspx\)](https://neoninc.sharepoint.com/sites/cla/SitePages/Home.aspx) and the Domain Chemical Hygiene Plan and Biosafety Manual (AD[03]).

I.1 Choosing specimens for shipment

Each year, up to 467 ground beetle specimens *per site* may be sent to a taxonomist for identification. Specimens must be intact, as a badly damaged specimen (e.g., one with a missing head) is not identifiable, even by experts. Specimens should be preferentially selected for shipment to a taxonomist based on the following criteria, and in the following order:

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

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

Use the Beetle ID and Pinning spreadsheet (downloaded from the saved copy of the CSVs created by the WebUI) to create a “pick list” of specimens to be shipped to taxonomists. This can be done by sorting the sheet by scientificName (or taxonID) or morphospeciesID, and sampleID, and choosing a variety of specimens that represent samples from all plots and times of collection.

If all specimens meeting the criteria above have been set to a taxonomist and the limit of 467 total individuals per site has not been met, a JIRA ticket should be issued to the Insect Ecologist so that budgets can be properly allocated between domains with low versus high capture rates.

I.2 Preparing for shipment

1. No hazardous materials or chemical substances will be included in this shipment. The fumigant box containing moth crystals (paradichlorobenzene) shall be **REMOVED** prior to shipment.
2. Supplies/Containers: See **Table 8** for a complete list of shipping materials.
3. Timelines: Beetles must be sent no later than the 1st of February in the year following collection, although more expedited shipment is recommended as other steps in the ground beetle processing workflow are contingent upon the samples being returned to the domain facility. Prior to shipment, dried, pinned/pointed specimens may be stored in an air tight container with paradichlorobenzene (to be removed prior to shipping) without detriment to the integrity of the specimens.

I.3 Packaging specimens for shipment

1. Ship prepared dry (pinned/pointed) beetle specimens to the taxonomic ID facility with a hard copy of the Taxonomy Shipping Manifest (taxonomy in datasheet from RD[05]). Email a digital copy of Taxonomy Shipping Manifest to the taxonomy lab and NEON’s CLA contact.
2. Place specimens in a mailer box so that locality labels are oriented in the same direction and the specimens are neatly aligned (**Figure 44**)

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Figure 44. Specimens prepared for shipping

3. Large beetles (typically 1.5 cm or larger, but this step may also be required for smaller specimens with heavy bodies) must be brace-pinned on either side of the label to prevent them from spinning on their pin during transit (Figure 45).



Figure 45. Large beetle that is brace-pinned for shipment

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4. When the mailers are ready for shipping, take a photo of each box (with specimens visible) for tracking purposes (**Figure 44**). Save these photos to the domain's local drives for future reference.



5. **Do NOT include moth crystals in shipments.**
6. Examine specimens before sealing the box. Make sure all specimens are appropriately labeled. Ensure that all of the specimens are firmly glued to their points by gently tapping the box to test. A specimen dissociated from its locality label is worthless. Finally, make sure that all pins with specimens are as vertical as possible.
7. Place four pins (#3 size) in the four corners of the specimen mailer, and four more at the midpoint of each of the four sides. These pins will support the cover board (cardboard insert).
8. Cut a cardboard insert to size to place on top of specimens (resting on pins). This cover board should fit **PRECISELY** inside the box with a snug fit. The cover board will contact **ALL** pins in the box. Make a packing tape tab to easily pull off the cardboard insert (otherwise it can become wedged in the box and difficult to remove; **Figure 46**). The cardboard insert must fit exactly to the dimensions of the box because a slightly smaller insert will chatter over the pin heads and ultimately become a source of specimen breakage during shipment.
9. Fill in any additional head space between the cover board and the lid of the specimen mailer with packing or bubble-foam so tightly that it slightly bulges the top of the box when filled.
10. Secure the lid of the box with a rubber band, tape, or other method that will firmly push the lid downward onto the cover board. Gently break off the metal tabs that come as part of the box. The secondary securement backs up the metal tabs which are prone to break off after several uses. If they break during shipment, they are prone to rattling around among the specimens.

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Figure 46. Cardboard insert that serves as extra protection for pinned/pointed specimens, and helps fills dead space between specimens and mailer lid.

11. Ship prepared dry material in cardboard mailers inside of larger cardboard boxes with ample packing material to ensure that the specimens are not jostled (**Figure 47 -Figure 48**).



Figure 47. A mailer placed inside a larger box for shipping

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Figure 48. Mailer within a shipping box, with adequate padding to keep mailer from moving in transit

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

I.4 Creating shipping inventory

Whenever samples are shipped, they must be accompanied by a hard-copy Taxonomy Shipping Manifest enclosed within the shipping container. In addition, a corresponding electronic version of the Taxonomy Shipping Manifest (Excel file) must be emailed to the taxonomic ID facility and NEON’s CLA contact. The Taxonomy Shipping Manifest is the Beetle_shipping_datsheet tab of RD[05]. For locations to which to ship beetle specimens, and CLA contract information, please reference [CLA’s NEON intranet site](#).

The Taxonomy Shipping Manifest (**Figure 49**) lists every specimen in the sample shipment, in the identifier field (for which the entry will be individualID in the case of pinned beetles). Remarks may also be entered for every specimen; for example specimens that the domain facility would like returned in order to add them to the teaching collection may have “Return to NEON facility” in the remarks field. The other required information on the Taxonomy Shipping Manifest, sentTo (name of the facility to which the specimens are being sent), sentDate, and recordedBy, must only be entered once per Taxonomy Shipping Manifest. An electronic version of the Taxonomy Shipping Manifest is an Excel file that should be emailed to the taxonomic ID facility and the CLA contact as soon as possible after the samples have been shipped.

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NEON Ground Beetle Pitfall Sampling: Shipping

sentTo: <u>Insect ID facility of western America</u>		sentDate: <u>12/12/2014</u>	recordedBy: <u>nrobinson@neoninc.org</u>
identifier <i>(e.g., sampleID, individualID, plateID)</i>	remarks <i>(e.g., "Return to NEON facility")</i>		
NEON.BET.D10.001116			
NEON.BET.D10.001117	Return to NEON facility		
NEON.BET.D10.001110			
NEON.BET.D10.001111			
NEON.BET.D10.001112			
NEON.BET.D10.001118	Return to NEON facility		

Figure 49. Example shipping manifest for the taxonomic ID facility

I.5 Laboratory contact information and shipping/receipt days

See [CLA's NEON intranet site](#).

I.6 Return of shipping materials

Taxonomists will return ONLY specimens that are to be added to the domain lab teaching collection or for which a request was made in the remarks field of the Taxonomy Shipping Manifest. As such, include a return shipping label in the original shipment. The format for this is found on [CLA's NEON intranet site](#)

8 REFERENCES

N/A

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APPENDIX A Datasheets

The following datasheets are associated with this protocol:

Table 11. Datasheets associated with this protocol.

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

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

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APPENDIX B Quick References

Part 1: Beetle Workflow

Quick Reference: Getting Ready for Field Sampling

- STEP 1** – Charge all electronic devices (e.g., GPS units)
- 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

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Quick Reference: Collecting Insects from Trap

STEP 1 – Record date and trapID on three locality labels

STEP 2 – Place three locality labels in top container

STEP 3 – Remove top container

STEP 4 – Filter contents over waste container

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

STEP 6 – Rinse sample through filter with 95% ethanol

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

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

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

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

[**ALTERNATIVELY**, complete steps 1-3 and 10. Place contents of trap into Whirl-Pak. Reset trap, complete steps 4-9 at the car on all 40 traps in series]



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

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Quick Reference: Initial Processing and Bycatch Removal

Ethanol Rinse (within 24 hours of sample collection)

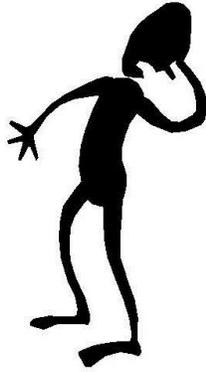
- STEP 1** – Filter contents of Whirl-Pak bag (specimens, labels, and mesh filter), discard ethanol waste, and record date of ethanol rinse on sorting datasheet
- STEP 2** – Remove vertebrate bycatch, record relevant information on sorting datasheet, and store vertebrate bycatch in 50 mL tubes with locality and determination labels. Fill tubes with enough 95% ethanol to submerge their contents.
- STEP 3** – Place vertebrate bycatch in labeled 50 mL tubes, with locality and determination labels. Fill tubes with enough 95% ethanol to submerge their contents.
- STEP 4** – Transfer trap contents (specimens, labels, mesh filter) back into original Whirl-Pak bag
- STEP 5** – Fill Whirl-Pak bag with enough 95% ethanol to completely cover its contents, and seal the bag (leaving as little air space as possible)
- STEP 6** – Store: 1) 50 mL tubes and 2) Whirl-Pak bags from the same sampling bout (in an airtight container)

Sorting Ground Beetles from Invertebrate Bycatch

- STEP 1** – Transfer contents of Whirl-Pak bag into ‘sorting’ dish
- STEP 2** – Rinse mesh filter with 95% ethanol, over sorting dish; discard mesh filter when clean
- STEP 3** – Sort specimens into two temporary containers, one marked ‘invertebrate bycatch’ and the other marked ‘carabids’. Make sure each container contains locality labels and enough 95% ethanol to keep its contents submerged
- STEP 4** – Place invertebrate bycatch in labeled 50 mL tubes, with locality labels
- STEP 5** – Return carabid beetles to the original Whirl-Pak bag until ready for sorting, pinning and identification.

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APPENDIX C Reminders and Checklists



Avoid looking like this guy

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

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

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 95% ethanol)
- Distilled or deionized water
- Map and coordinates uploaded onto GPS
- Ground beetle data sheets

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Checklist: Collecting Quality Samples

Sample collection: Be sure to...

- Use waste containers to catch PG solution
- Double check that your actual location matches the one on the locality label
 - plotID & trapID are written on the trap cover
- Write collection date and trapID (N, S, E, W) on locality label (if not pre-printed)
- Remove twigs, leaves and debris from trap
- Rinse the sample – first with distilled or deionized water, then with 95% ethanol
- Rinse the filter apparatus with 95% ethanol to ensure that all beetles and bycatch are transferred to the Whirl-Pak bag
- Check Whirl-Pak bag for locality labels and mesh filter
- Record all metadata (plotID, date, field staff, etc.) on the datasheet
- 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
- 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 sorting datasheet or WebUI
- Locality labels are with specimens (in Whirl-Pak bag)
- Specimens are stored in airtight containers so the ethanol does not leak out

Sorting beetles and bycatch: Be sure to...

- Only work with one Whirl-Pak bag at a time
- Examine Whirl-Pak bags, mesh filters, and sorting dishes under a microscope, to check for tiny organisms
- Ask for a second opinion, use reference collections, and refer to species/photo lists if unsure whether an insect is a ground beetle. Still unsure? Call it a ground beetle for now
- Keep specimens and associated locality labels together at all times and create new locality labels (with date and trapID) as needed
- Place locality labels (with collection date and trapID) in each 50 mL tube and/or jar, and any temporary container used while working
- Completely cover all organisms with 95% ethanol at all times, and use multiple storage tubes as needed
- Record sorter's name and the date of the sorting event (processingDate) on the sorting datasheet

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APPENDIX D Estimated Dates for Onset and Cessation of Sampling

The dates in **Table 12** are based on MODIS (Moderate Resolution Imaging Spectroradiometer) data from NASA records from the most recently available decade. 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. Estimates for the start and stop dates of sampling are provided for each site. Except for sites with warm temperatures year-round, the start and end of beetle sampling should coincide with the plant phenology protocol.

These dates are estimates and local conditions may vary. If the listed start date passes and no observable green-up has occurred, then the start of the sampling season should be delayed until green-up is observed. Likewise, if green-up occurs earlier than indicated in this chart then sampling will need to occur earlier than the estimated date. Completion of the sampling season should also coincide with the observation of senescence in the field.

Table 12. Estimated seasonal start and end dates based on patterns of historical ‘green-up’

Domain	Site	Start	End	Bouts Expected
1	8.1.2 HARV	Apr-14	Sep-14	8.1.3 10
1	8.1.4 BART	Apr-14	Sep-14	11
2	8.1.5 BLAN	Mar-14	Oct-14	14
2	8.1.6 SCBI	Mar-14	Oct-14	8.1.7 14
2	8.1.8 SERC	Mar-14	Oct-14	14
3	8.1.9 D	Mar-14	Sep-14	15

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		S N Y			
3	8.1.10	J E R C	Mar-14	Sep-14	8.1.11 13
3	8.1.12	O S B S	Mar-14	Sep-14	14
4	8.1.13	G U A N	Apr-14	Oct-14	8.1.14 14
5	8.1.15	S T E I	Apr-14	Sep-14	8.1.16 10
5	8.1.17	T R E E	Apr-14	Sep-14	10
5	8.1.18	U N D E	May-14	Sep-14	9
6	8.1.19	K O N A	Mar-14	Sep-14	13
6	8.1.20	K O N Z	Mar-14	Sep-14	13
6	8.1.21	K	Mar-14	Oct-14	15

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		U F S			
7	8.1.22	G R S M	Mar-14	Sep-14	13
7	8.1.23	M L B S	Apr-14	Sep-14	11
7	8.1.24	O R N L	Mar-14	Oct-14	13
8	8.1.25	C H O C	Mar-14	Oct-14	15
8	8.1.26	D E L A	Mar-14	Sep-14	15
8	8.1.27	T A L L	Mar-14	Sep-14	14
9	8.1.28	D C F S	Apr-14	Sep-14	10
9	8.1.29	N O G P	Apr-14	Sep-14	10
9	8.1.30	W	Apr-14	Sep-14	10

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		O O D			
10	8.1.31	C P E R	Mar-14	Sep-14	13
10	8.1.32	R M N P	Apr-14	Sep-14	10
10	8.1.33	S T E R	Mar-14	Sep-14	12
11	8.1.34	C L B J	Mar-14	Sep-14	14
11	8.1.35	K L E M	Mar-14	Sep-14	13
12	8.1.36	Y E L L	Apr-14	Sep-14	10
13	8.1.37	M O A B	Mar-14	Oct-14	14
13	8.1.38	N I W O	May-14	Sep-14	8
14	8.1.39	J	Mar-14	Oct-14	15

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		O R N			
14	8.1.40	S R E R	May-14	Oct-14	10
15	8.1.41	O N A Q	Mar-14	Sep-14	13
16	8.1.42	A B B Y	Apr-14	Sep-14	11
16	8.1.43	W R E F	Apr-14	Sep-14	11
17	8.1.44	S J E R	Sep-14	May-14	9
17	8.1.45	S O A P	Mar-14	Sep-14	12
17	8.1.46	T E A K	Apr-14	Sep-14	11
18	BARO		Jun-14	Aug-14	3
18	8.1.47	T O O L	Jun-14	Aug-14	5

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19	8.1.48	B O N A	May-14	Aug-14	7
19	8.1.49	D E J U	May-14	Aug-14	8
19	8.1.50	H E A L	May-14	Aug-14	7

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APPENDIX A Site Specific List of Easy to Identify Ground Beetle Species

Lists of easily identified ground beetle species will be available after the first year of sampling within each domain. In order for new species to be added to these lists, F OPS must initiate JIRA tickets with recommended additions. Science will then review the recommendations and update the lists accordingly.

A.1 DOMAIN 1

Table 13. D01 easily identified ground beetle species.

Site	Species
HARV	<i>Synuchus impunctatus</i>

A.2 DOMAIN 2

Table 14. D02 easily identified ground beetle species.

Site	Species
	<i>Carabus goryi</i>
	<i>Chlaenius aestivus</i>
	<i>Chlaenius emarginalis</i>
	<i>Cyclotrachelus sigillatus</i>
	<i>Pterostichus coracinus</i>
	<i>Pterostichus stygicus</i>

A.3 DOMAIN 3

Table 15. D03 easily identified ground beetle species.

Site	Species
OSBS	<i>Cyclotrachelus faber</i>
	<i>Pasimachus strenuus</i>
JERC	<i>Pasimachus strenuus</i>
	<i>Tetragonoderus intersectus</i>
DSNY	<i>Cyclotrachelus faber</i>
	<i>Pasimachus strenuus</i>
	<i>Selenophorus fossulatus</i>
	<i>Tetragonoderus laevigatus</i>

A.4 DOMAIN 6

Table 16. D06 easily identified ground beetle species.

Site	Species
	<i>Cratacanthus dubius</i>

A.5 DOMAIN 8

Table 17. D08 easily identified ground beetle species.

Site	Species
	<i>Cratacanthus dubius</i>

A.6 DOMAIN 10

Table 18. D10 easily identified ground beetle species.

Site	Species
CPER	<i>Amara carinata</i> <i>Anisodactylus rusticus</i> <i>Cicindela punctulata punctulata</i> <i>Cratacanthus dubius</i> <i>Euryderus grossus</i> <i>Pasimachus elongatus</i>
STER	<i>Amara carinata</i> <i>Cicindela punctulata punctulata</i> <i>Cratacanthus dubius</i> <i>Pasimachus elongatus</i> <i>Poecilus scitulus</i>
RMNP	<i>Carabus taedatus agassii</i>

A.7 DOMAIN 11

Table 19. D11 easily identified ground beetle species.

Site	Species
	<i>Cratacanthus dubius</i>

A.8 DOMAIN 13

Table 20. D13 easily identified ground beetle species.

Site	Species
	<i>Cratacanthus dubius</i>

A.9 DOMAIN 14

Table 21. D14 easily identified ground beetle species.

Site	Species
	<i>Cratacanthus dubius</i>

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APPENDIX B Domain Specific List of Species that are Difficult to Identify

Some species of ground beetles are inherently difficult to parse because the morphological characters that are useful for telling these species apart vary across that beetle’s range. There are also cryptic species or small species that are fundamentally difficult to differentiate. Although these lists contain many of the more difficult ground beetle taxa, they are by no means exhaustive. Each list will continue to grow in one of two ways after the first year of sampling within each domain. Field operations can initiate JIRA tickets with recommended additions of species combinations they find tough. Science will then review the recommendations and update the lists accordingly. If there are species where the taxonomist identifications and field technician identifications never match, then those species will also be added to the list on an annual basis. As teaching collections grow, there may be cause to remove a species from this list or to modify the list for certain domains. In that case, Field operations can initiate JIRA tickets with recommended modifications to the species on this list.

Table 22. Difficult taxa

Domains	Taxa
1	8.1.51 <i>Harpalus texanus</i> , <i>Harpalus pennsylvanicus</i>
8,10,14,15,16,17	8.1.52 <i>Microlestes</i> (all species)
5,8,9	8.1.53 <i>Pterostrichus</i> (all species)
2,3,8,11	8.1.54 <i>Loxandrus</i> (all species)

Cryptic species issues arise when two species that are morphologically indistinguishable in the field co-occur (or might co-occur) at a site. NEON intends to add these species pairs 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 entered in the crypticSpeciesGroups spreadsheet on the NEON intranet on the FOPs page in the taxonTables folder under the TOS tab.

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APPENDIX A Site-Specific Protocol Modifications

DOMAIN 04

In Guanica forest (core site), a few modifications will be implemented in the first year of sampling as a safeguard against the capture of the endangered Puerto Rican crested toad. However, unless detailed in this paragraph, sampling will occur at Guanica in a way that is identical to sampling conducted at any other site. First, a deeper trap (32 oz. Deli cup) of the same lip circumference will be used in lieu of the shallower cups described above, but the deeper cup traps will be created exactly the same way as the shallower traps. A larger amount of PG and water preserving fluid (500 mL; same concentration) and a dowel rod (1 inch diameter and 4.5 inches length) will be placed into each cup. The dowel rod will allow Puerto Rican crested toads to survive a fall into the cup. Traps will be collected once every 14 days, but checked for the presence of Puerto Rican crested toads on a weekly basis. Through close coordination with the Puerto Rican Department of Natural and Environmental Resources, all or some traps may be temporarily inactivated during Puerto Rican crested toad breeding events or toadlet migration.

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.

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APPENDIX B Insect Taxonomic Identification Aids

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

B.1 GENERAL KEYS

- [Catalogue of Geadephaga \(Coleoptera, Adephaga\) of America, north of Mexico](#)
Bousquet Y. 2012. ZooKeys 245: 1–1722.
- [The ground-beetles \(Carabidae, excl. Cicindelinae\) of Canada and Alaska, parts 1–6](#)
C.H. Lindroth. 1961. Opuscula Entomologica Supplementa XX, XXIV, XXIX, XXXIII, XXXIV, XXXV.
- Pearson, D.L., Knisley, C.B., and Kazilek, C.J. 2006. A Field Guide to the Tiger Beetles of the United States and Canada: Identification, Natural History, and Distribution of the Cicindelidae. Oxford University Press, New York, NY. Available from: <http://www.amazon.com/Field-Beetles-United-States-Canada/dp/0195181565>
- Tiger Beetles of the United States (website: <http://www.npwrc.usgs.gov/resource/distr/insects/tigb/index.htm>). Coordinated by W. Wyatt Hoback and John J. Riggins

B.2 REGIONAL KEYS

- [Illustrated Identification Guide to Adults and Larvae of Northeastern North American Ground Beetles \(Coleoptera: Carabidae\)](#) Yves Bousquet. 2010. Pensoft Publishers.
- [An annotated checklist of Wisconsin ground beetles \(Coleoptera: Carabidae\)](#)
Peter W. Messer. 2009. The Great Lakes Entomologist 42(1-2): 30-61.
- [Key to Florida Ground Beetles](#)
- [Ground Beetles and Wrinkled Bark Beetles of South Carolina](#)
Janet Ciegler. 2000. Clemson University.
- [University of New Hampshire Insect and Arachnid Collections](#)
- [Checklist of the Coleoptera of Oklahoma](#)
- [California Beetle Project](#)
- [The Beetles of the Pacific Northwest](#) Hatch, M. 1953. University of Washington publications in biology, Volume 16. University of Washington Press, Seattle, Washington.
- Choate, P.M. 2001. Manual for the Identification of the Ground Beetles (Coleoptera: Carabidae) (including tiger beetles) of Florida http://www.entnemdept.ufl.edu/choate/florida_Carabidae_new.pdf
- Insects of Hawaii, Volume 16: *Coleoptera: Carabidae*: Part 1: Introduction and Tribe Platynini
- Liebherr, J.K., and Zimmerman, E.C. 2000. University of Hawai'i Press, Honolulu, HI. Available from: <http://www.uhpress.hawaii.edu/p-2012-9780824823566.aspx>

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APPENDIX C Guide to Distinguishing the Family Carabidae

The suborder Adephaga includes ground beetles and some aquatic groups. Adephagan beetles are easily identified by the manner in which the last pair of legs articulates with the beetle’s underside (**Figure 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.

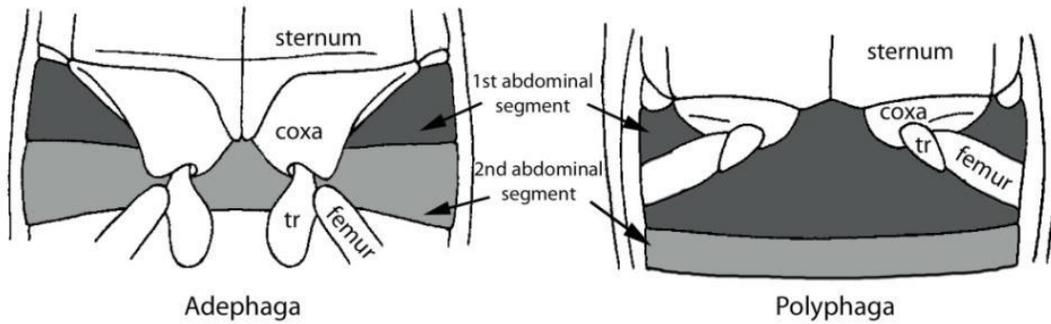


Figure 50. Hind leg articulation in Adephaga and Polyphaga.

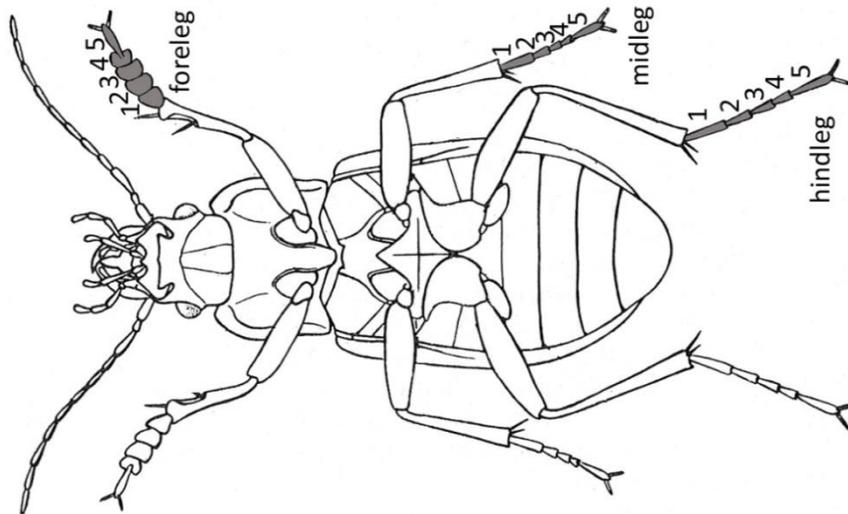


Figure 51. Tarsal formula of adephagan beetles.

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

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Figure 52). Otherwise these beetles lack easily recognizable and distinct morphological features. However, several other families of adephagan beetles do have distinct morphological features, by which they can easily be identified as *not* Carabids. Below are some descriptions and illustrations of these identifying features (**Figure 53 - Figure 58**), which will allow for the identification of non-carabid adephagans, (a.k.a. invertebrate bycatch).

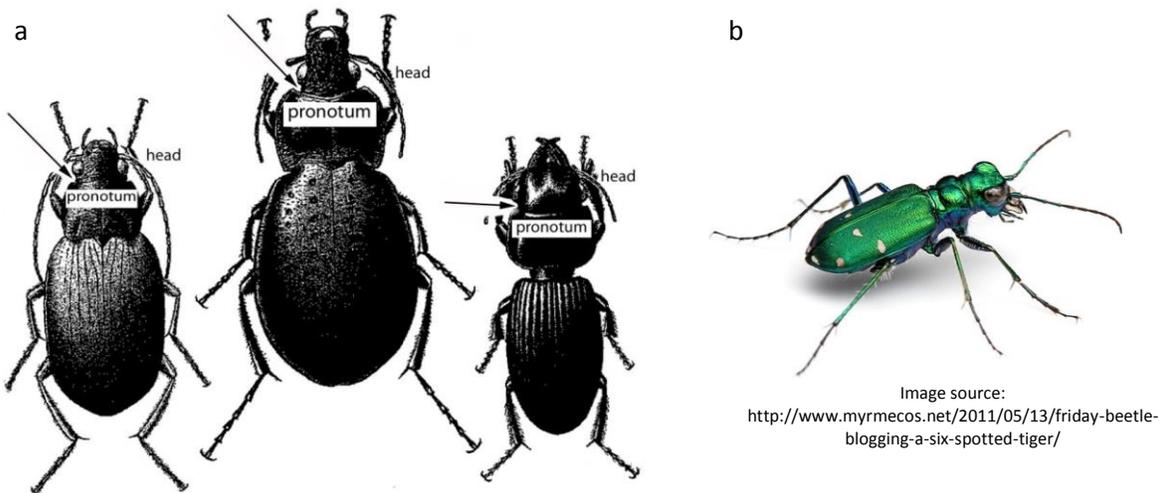


Figure 52. a) Ground beetle specimens with head and pronotum labeled; arrow shows division between head and pronotum. b) Tiger beetles (a group within the Carabidae family) are frequently metallic in coloration.

- 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**).

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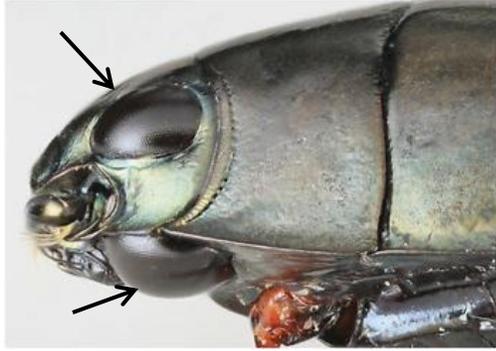


Figure 53. Lateral view of a beetle in the family Gyrinidae, showing dorsal and ventral portions of the compound eye.

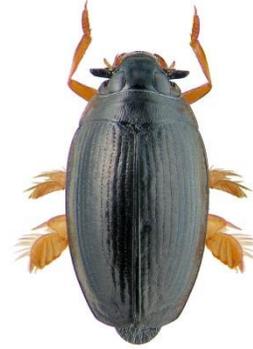


Image source:
<http://www.zin.ru/animalia/coleoptera/rus/g>

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 aedeagus-specific hind leg articulation described above).

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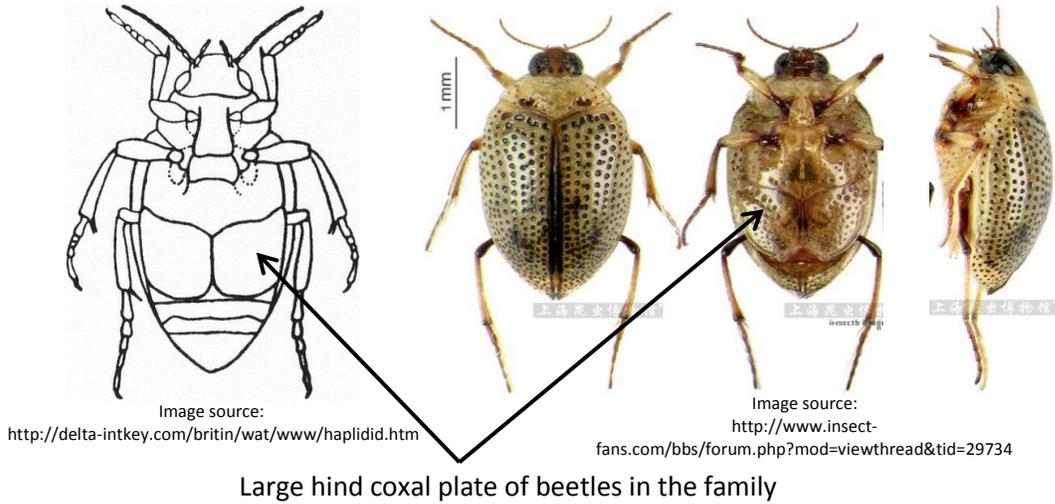


Figure 56. Diagram and photo of a Halplidid beetle, showing the large coxal plates that are characteristic for this family.

- 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**).

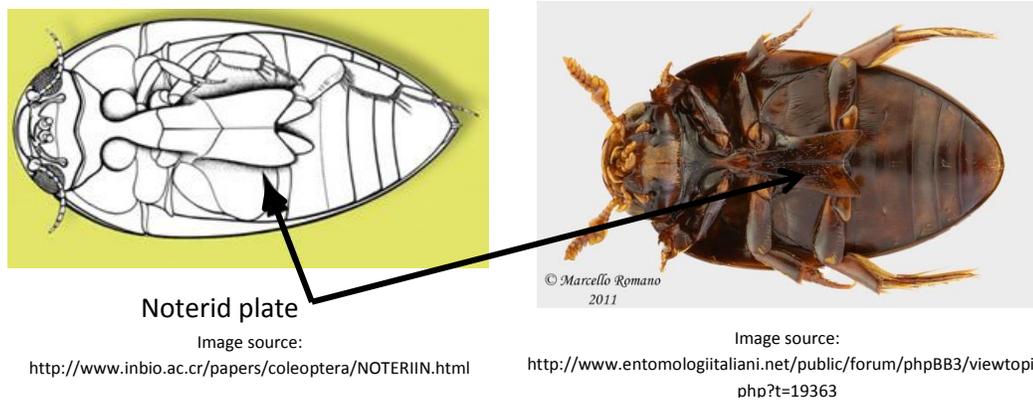


Figure 57. Diagram and photo of a noterid beetle, showing the noterid plate between the second and third pairs of legs, on the ventral side.

- 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**).

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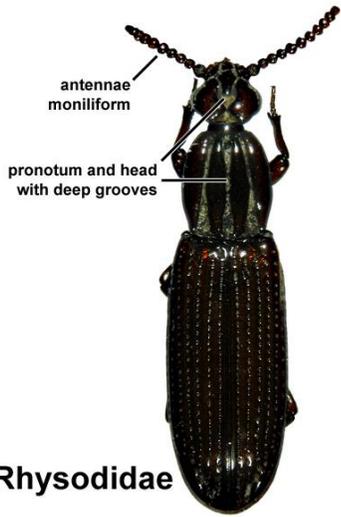


Image source:
<http://www.entomology.umn.edu/museum/links/coursefiles/Coleop%20characters.html>



Image source:
http://www.biodiversity.ubc.ca/entomology_pictures/Coleo

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.

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APPENDIX D Intermediate and Final Sample IDs per sample type

Table 23 contains information on generating sampleIDs (intermediate and final) for different types of samples generated during beetle sorting. How sampleIDs are generated depends on the type of sample and its fate (e.g., how it will be stored in the long term).

Table 23. Sample IDs for various types of samples generated during beetle sorting

Sample Type ¹	Fate of Sample	Intermediate (associated) sampleID	Final sampleID (sampleID)
Known vert. bycatch ²	Pooled (by plot) for storage	plotID.trapID.collectDate.taxonID.tubeNumber	plotID.collectDate.taxonID.tubeNumber ^{3,5}
Unknown vert. bycatch	Stored (by trap) until positively identified	plotID.trapID.collectDate.vb.tubeNumber	plotID.trapID.collectDate.vb.tubeNumber ⁴
Invert bycatch	Pooled (by plot) for storage	plotID.collectDate.ib.tubeNumber	plotID.collectDate.ib.tubeNumber ⁵
Any Carabid	Pinned	plotID.trapID.collectDate.taxonID.tubeNumber	8.1.55 NA ⁶
Known Carabid	Pooled (by plot) for storage	plotID.trapID.collectDate.taxonID.tubeNumber	plotID.collectDate.taxonID.tubeNumber ^{4,5}
Morphospecies	Pooled (by plot) for storage	plotID.trapID.collectDate.cc.tubeNumber	8.1.56 plotID.collectDate.cc.tubeNumber ^{4,5}

¹ The WebUI has a designation of 'other carabid'. However, this is an obsolete sample type and we are not using it.

² Assuming that the vertebrate in question is not endangered, threatened, or otherwise protected

³ The tube number in the final sampleID may be different from that in the intermediate sampleID. If vert bycatch is not pooled for some reason, the trapID will still be in the final sampleID.

⁴ After unknown specimens have been identified they can be pooled for long term storage, and given a pooled sampleID that follows the format of the final sampleID in row 1 (including a taxonID and without a trapID).

⁵ If the specimens are *not* pooled by plot, a trapID should be inserted between the plotID and collectDate in this final sampleID

⁶ Individuals that are pinned will have an individualID, so no final sampleID is required

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APPENDIX A Locality Labels

Locality labels are used to identify when and from where a sample was collected. They are used in the field to label traps, and in the laboratory to label whole samples and individual 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, mosquitoes and ticks.

The locality label-sheet has 8 columns, each with 27 labels. Each column of labels is identical and contains the information for one plot and one month. If more than 27 labels per plot are needed, print additional copies of this document.

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

Figure 59. Example label for pitfall trap sample. Size shown is larger than size of actual label.

A.1 COMPILE LABEL DATA

1. Compile the label data for all relevant plots in a Microsoft Excel spreadsheet.

a. Enter label data into a Microsoft Excel spreadsheet (

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	Country	comma	State	County	period	Site	period	Plot	period	Elevation	Lat	Long	Trap type	Month	Year	period	Collector	NEON	Plot code	period	
2	USA	,	COLORADO	Weld County	.	Central Plains Expm Rng	.	CPER_001	.	1639m	N40.8509	W104.6995	Pitfall trap	Jul	2015	.	TBaldwin	NEON	CPER_001	.	
3	USA	,	COLORADO	Weld County	.	Central Plains Expm Rng	.	CPER_002	.	1625m	N40.8109	W104.7292	Pitfall trap	Jul	2015	.	TBaldwin	NEON	CPER_002	.	
4	USA	,	COLORADO	Weld County	.	Central Plains Expm Rng	.	CPER_003	.	1625m	N40.8184	W104.7072	Pitfall trap	Jul	2015	.	TBaldwin	NEON	CPER_003	.	
5	USA	,	COLORADO	Weld County	.	Central Plains Expm Rng	.	CPER_004	.	1621m	N40.8127	W104.6969	Pitfall trap	Jul	2015	.	TBaldwin	NEON	CPER_004	.	
6	USA	,	COLORADO	Weld County	.	Central Plains Expm Rng	.	CPER_005	.	1665m	N40.8531	W104.7280	Pitfall trap	Jul	2015	.	TBaldwin	NEON	CPER_005	.	
7	USA	,	COLORADO	Weld County	.	Central Plains Expm Rng	.	CPER_006	.	1651m	N40.8148	W104.7481	Pitfall trap	Jul	2015	.	TBaldwin	NEON	CPER_006	.	
8	USA	,	COLORADO	Weld County	.	Central Plains Expm Rng	.	CPER_007	.	1647m	N40.8619	W104.6838	Pitfall trap	Jul	2015	.	TBaldwin	NEON	CPER_007	.	
9	USA	,	COLORADO	Weld County	.	Central Plains Expm Rng	.	CPER_008	.	1647m	N40.7988	W104.7492	Pitfall trap	Jul	2015	.	TBaldwin	NEON	CPER_008	.	
10	USA	,	COLORADO	Weld County	.	Central Plains Expm Rng	.	CPER_009	.	1657m	N40.8261	W104.7459	Pitfall trap	Jul	2015	.	TBaldwin	NEON	CPER_009	.	
11	USA	,	COLORADO	Weld County	.	Central Plains Expm Rng	.	CPER_010	.	1644m	N40.8500	W104.6814	Pitfall trap	Jul	2015	.	TBaldwin	NEON	CPER_010	.	

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1	Country	comma	State	County	period	Site	period	Plot	period	Elevation	Lat	Long	Trap type	Day	Month	Year	period	Collector	NEON	Trap Code
2	USA	,	COLORADO	Weld County	.	Central Plains Expm Rng	.	CPER_001	.	1639m	N40.8509	W104.6995	Pitfall trap	4	Jul	2015	.	TBaldwin	NEON	CPER_001.N.20150704
3	USA	,	COLORADO	Weld County	.	Central Plains Expm Rng	.	CPER_001	.	1639m	N40.8509	W104.6995	Pitfall trap	4	Jul	2015	.	TBaldwin	NEON	CPER_001.E.20150704
4	USA	,	COLORADO	Weld County	.	Central Plains Expm Rng	.	CPER_001	.	1639m	N40.8509	W104.6995	Pitfall trap	4	Jul	2015	.	TBaldwin	NEON	CPER_001.W.20150704
5	USA	,	COLORADO	Weld County	.	Central Plains Expm Rng	.	CPER_002	.	1625m	N40.8109	W104.7292	Pitfall trap	4	Jul	2015	.	TBaldwin	NEON	CPER_002.E.20150704
6	USA	,	COLORADO	Weld County	.	Central Plains Expm Rng	.	CPER_002	.	1625m	N40.8109	W104.7292	Pitfall trap	4	Jul	2015	.	TBaldwin	NEON	CPER_002.W.20150704
7	USA	,	COLORADO	Weld County	.	Central Plains Expm Rng	.	CPER_002	.	1625m	N40.8109	W104.7292	Pitfall trap	4	Jul	2015	.	TBaldwin	NEON	CPER_002.S.20150704
8	USA	,	COLORADO	Weld County	.	Central Plains Expm Rng	.	CPER_002	.	1625m	N40.8109	W104.7292	Pitfall trap	4	Jul	2015	.	TBaldwin	NEON	CPER_002.N.20150704
9	USA	,	COLORADO	Weld County	.	Central Plains Expm Rng	.	CPER_003	.	1625m	N40.8184	W104.7072	Pitfall trap	4	Jul	2015	.	TBaldwin	NEON	CPER_003.E.20150704
10	USA	,	COLORADO	Weld County	.	Central Plains Expm Rng	.	CPER_003	.	1625m	N40.8184	W104.7072	Pitfall trap	4	Jul	2015	.	TBaldwin	NEON	CPER_003.W.20150704
11	USA	,	COLORADO	Weld County	.	Central Plains Expm Rng	.	CPER_003	.	1625m	N40.8184	W104.7072	Pitfall trap	4	Jul	2015	.	TBaldwin	NEON	CPER_003.S.20150704
12	USA	,	COLORADO	Weld County	.	Central Plains Expm Rng	.	CPER_003	.	1625m	N40.8184	W104.7072	Pitfall trap	4	Jul	2015	.	TBaldwin	NEON	CPER_003.N.20150704
13	USA	,	COLORADO	Weld County	.	Central Plains Expm Rng	.	CPER_003	.	1625m	N40.8184	W104.7072	Pitfall trap	4	Jul	2015	.	TBaldwin	NEON	CPER_003.W.20150704

Figure 60) with one column for each piece of information required for the locality label, as follows:

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- i. Column Header = “Country”, content = The country in which the sample was collected, **in all capital letters** (i.e., USA)
- ii. Column Header = “comma”, content = “,” (without quotes)
- iii. Column Header = “State”, content = The state in which the sample was collected, **in all capital letters**
- iv. Column Header = “County”, content = The county in which the sample was collected, with the county name capitalized, followed by “County” (without quotes)
- v. Column Header = “Period”, content = “.” (without quotes)
- vi. Column Header = “Site”, content = The abbreviated site name. Instructions for generating this abbreviated site name are as follows (with examples in **Table 24**):

- A. Use the following standardized abbreviations for words in the list below:

Environmental – Env
 National – Natl
 Laboratory – Lab
 Mountain – Mtn
 Biological – Biol
 Station – Stn
 University – Univ
 Ecological - Ecol
 Research - Res
 Center – Ctr
 Experimental – Expm
 Conservation - Cons
 Biology - Bio
 Institute - Inst
 Range – Rng
 Long Term Ecological Research – LTER

- B. Eliminate specific locational information that will be expressed elsewhere on the label (e.g., ‘North’ and ‘,CO’ are not needed for North Sterling, CO)

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- C. Generate the abbreviated site name with a period at the end (e.g., 'Jones Ecol Res Ctr. '); examples in **Table 24**)
- D. If you are unsure of the abbreviated name, contact NEON Science for verification

Table 24. Examples of site name abbreviations for locality labels

Domain	Site Code	Site Name	Site Name Abbreviation
1	HARV	Harvard Forest	Harvard Forest
10	RMNP	Rocky Mountain National Park	Rocky Mtn Natl Park
10	STER	North Sterling, CO	Sterling
10	CPER	Central Plains Experimental Range	Central Plains Expm Rng
3	OSBS	Ordway-Swisher Biological Station	Ordway-Swisher Biol Stn
3	DSNY	Disney Wilderness Preserve	Disney Wilderness Preserve

- vii. Column Header = "Period", content = "." (without quotes)
- viii. Column Header = "Elevation", content = The elevation (in meters and to the nearest whole number) of the location at which the sample was collected.
This is provided by NEON Headquarters
- ix. Column Header = "Latitude", content = The latitude (in decimal degrees and with four digits after the decimal) of the location at which the sample was collected. *This is provided by NEON Headquarters*
- x. Column Header = "Longitude", content = The longitude (in decimal degrees and with four digits after the decimal) of the location at which the sample was collected. *This is provided by NEON Headquarters*
- xi. Column Header = "Trap Type", content = The type of trap used to collect the sample (Pitfall trap)
- xii. Column Header = "Day", content = The day on which the specimen was collected
- xiii. Column Header = "Month", content = The month, abbreviated to three letters (e.g., Jun OR Sep), in which the sample/specimen:
 - A. Will be collected, in the case of labels for field use
 - B. Was collected, in the case of labels for pinning/pointing/storing insects

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- xiv. Column Header = “Year”, content = The year in which the sample was collected, in numeric format
- xv. Column Header = “Period”, content = “.” (without quotes)
- xvi. Column Header = “Collector”, content = The first initial and last name of the field operations manager, with no spaces and the initial and last name capitalized (e.g., TBaldwin)
- xvii. Column Header = “NEON”, content = The name of the organization (NEON)
- xviii. Column Header:
 - A. “plotID”, for labels for field use, content = the plotID from which the sample will be collected, with a period at the end
 - B. “Trap Code”, for labels for pinning/pointing/storing insects, content = plotID.trapID.collectDate, where collectDate is in the format YYYYMMDD.

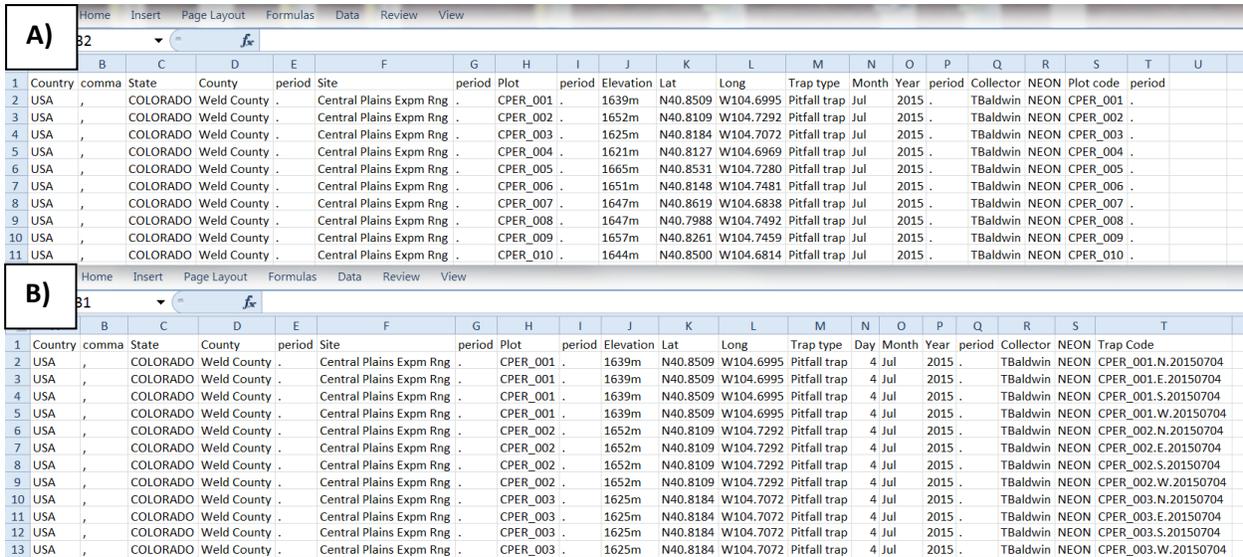


Figure 60. Example of spreadsheets containing information for locality labels. (A) Spare labels including the “plotID” field. (B) Fully pre-prepared labels include the “Day” and “Trap Code” fields. Pinned and pointed locality labels follow the latter spreadsheet.

- b. The spreadsheet should contain four rows for each plotID within the domain, with identical information and corresponding to the four pitfall traps deployed at each plot

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2. Name the sheet using a meaningful name such as 'BetLoc2015'. Save and close the file – if you do not close the file, the Mail Merge may not work correctly.
 - a. *Note – it may be helpful to create one locality label document for labels to be used in the field, and another for labels to be used for pinning/pointing/storing insects. In such cases, name the files appropriately (e.g., 'BetFieldLoc2015' and 'BetPinLoc2015').*

A.2 EXPORT THE DATA INTO MICROSOFT WORD USING MAIL MERGE



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

1. Open a Microsoft Word document and under **Mailings**, select **Start Mail Merge** and then select **Labels**.
2. In the **Labels** menu select **New Label** and enter information for the dimensions of the label in the "Label Details" box, exactly as in **Figure 61**. Give the template a name that makes it easy to locate when creating a new sheet of labels (e.g. 'insect determination labels').

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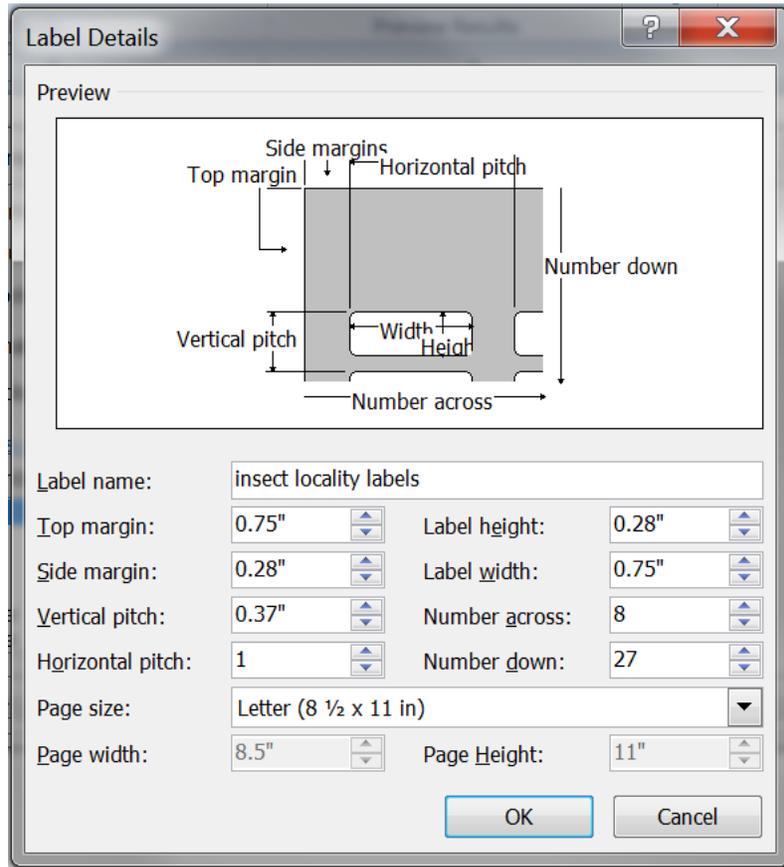


Figure 61. Dimensions for creating a locality label template in Microsoft Word

- When label dimensions have been specified, a blank template document will appear. In the **Mailings** tool bar, click on **Select recipients** and select **Use existing list**. A file navigation window will appear. Navigate to and select the Microsoft Excel spreadsheet created in step **A.1**.
- A “Select Table” pop-up window (**Figure 62**) will appear, showing a list of the sheets within the Excel workbook created in step **(8.1.56A.1)**. Select the worksheet containing the determination label information. Make sure to check the box that says ‘First row of data contains column headers’.

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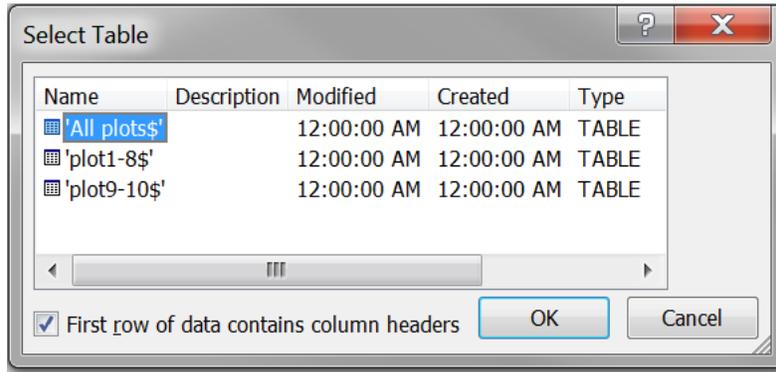


Figure 62. Selecting recipients from a Microsoft Excel file during Mail Merge

A.3 PERFORM FINAL FORMATTING IN MICROSOFT WORD.

1. Place the cursor in the top left cell of the label sheet.
2. Populate the labels by clicking on **Insert Merge Field** in the **Mailings** menu. An “Insert Merge Field” drop-down menu will appear, containing the names of the column headings from the Microsoft Excel spreadsheet. Add the headers to the cell as follows:
 - a. Highlight “Country” in the dropdown, then click “Insert”
 - b. Highlight “comma” in the dropdown, then click “Insert”
 - c. Highlight “State” in the dropdown, then click “Insert”
 - d. Highlight “County” In the dropdown, then click “Insert”
 - e. Highlight “Period” in the dropdown, then click “Insert”
 - f. Highlight “Site” in the dropdown, then click “Insert”
 - g. Highlight “Period” in the dropdown, then click “Insert”
 - h. Highlight “Elevation” in the dropdown, then click “Insert”
 - i. Highlight “Latitude” in the dropdown, then click “Insert”
 - j. Highlight “Longitude” in the dropdown, then click “Insert”
 - k. Highlight “Trap Type” in the dropdown, then click “Insert”
 - l. If labels are going to be completely pre-printed (required for pinned specimens):
 - A. Highlight “Day” in the dropdown, then click “Insert”

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- m. Highlight “Month” in the dropdown, then click “Insert”
- n. Highlight “Year” in the dropdown, then click “Insert”
- o. Highlight “Period” in the dropdown, then click “Insert”
- p. Highlight “Collector” in the dropdown, then click “Insert”
- q. Highlight “plotID” or “Trap Code” in the dropdown, then click “Insert”
- r. Click “Close” and you will see that the top left cell contains:

(Fully pre-printed labels)

«Country»«Comma»«State»«County»«Period»«Site»«Period1»«Elevation»«Latitude»«Longitude»«Trap_Type»«Day»«Month»«Year»«Period2»«Collector»«Trap_Code»

OR

(Back up labels)

«Country»«Comma»«State»«County»«Period»«Site»«Period1»«Elevation»«Latitude»«Longitude»«Trap_Type»«Month»«Year»«Period2»«Collector»«Plot_Code»«Period3»

Note – The numbers appearing after “Period” are Excel’s way of dealing with multiple columns that have the same name, do not worry about this.

3. Insert line breaks and spaces as follows:
 - a. Place the cursor at the end of “«Comma»” and hit the spacebar
 - b. Place the cursor at the end of “«State»” and hit the spacebar
 - c. Place the cursor at the end of “«Period»” and hit the spacebar
 - d. Place the cursor at the end of “«Elevation»” and hit the spacebar
 - e. Place the cursor at the end of “«Latitude»” and hit the spacebar
 - f. Place the cursor at the end of “«Longitude»” and hit the spacebar
 - g. Place the cursor in front of “«Day»”
 - h. Place the cursor in front of “«Month»”

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- i. Place the cursor at the end of “«Period2»” and hit the spacebar
- j. Place the cursor at the end of “«Collector»” and hit the spacebar
- k. The top left label will now contain (notices the spaces, highlighted grey below):

(Fully pre-printed labels)

«Country»«Comma»|«State»|«County»«Period»|«Site»«Period1»|«Elevation»|«Latitude»|«Longitude»
«Trap_Type»|«Day»«Month»«Year»«Period2»|«Collector»|«Trap_Code»

OR

(Back up labels)

«Country»«Comma»|«State»|«County»«Period»|«Site»«Period1»|«Elevation»|█ «Trap_Type»
█ «Month»«Year»«Period2»|«Collector»

4. Highlight the contents of the top left cell and set the font to Arial, font size 3.5
5. After finishing the first cell, click **Update Labels** – this will copy formatting into remaining labels.
6. Preview the labels by clicking on **Preview Results**.
7. Confirm that the first row in each label starts with the country, the date information starts on a new line, and the plotID OR Trap Code starts on a new line.
8. In the **Mail Merge** menu, select **Finish & Merge**, then **Edit Individual Documents**. A pop-up menu will appear. Choose the ‘All’ option. This will create a Microsoft Word document with the labels.
9. Save the document. Use a meaningful name such as ‘BetLoc2015’.
 - a. *Note – it may be helpful to create one locality label document for labels to be used in the field, and another for labels to be used for pinning/pointing/storing insects. In such cases, name the files appropriately (e.g., ‘BetFieldLoc2015’ and ‘BetPinLoc2015’).*
10. Copy and paste each label as needed, in order to generate additional locality labels for samples and/or specimens and/or storage containers. Group all labels for the same plotID and/or Trap Code within the same column.

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- a. *Note – it may be helpful to paste the same label all of the way down an entire column, as several identical locality labels are required for containers during trapping and sorting, and for pinned/pointed specimens that were captured from the same traps at the same times.*

11. Re-save the document after editing

A.4 PRINT LABELS

1. Print the labels on ethanol-safe archival paper (e.g., Bioquip 1223RA).
2. Determine how many labels are needed for each plot for the selected activity (see **Table 25**). If more than one page of labels per plot are required, print the document multiple times until enough labels are printed.
3. **NOTE:** The calculations and quality checks listed below for field work are based on current plans. If the schedule or frequency of trapping changes, the numbers will have to be recalculated using the following formula:

$$A * B * C = D; D/27 = E$$

where

- A** = # of labels/trap
- B** = # of traps/plot
- C** = # of trap collections/plot during a month
- D** = # of labels needed/plot during a month
- E** = # of strips of labels needed
- (for fractions, round up to the nearest whole number)

Table 25. Number of labels needed for the difference activities requiring insect locality labels.

Activity	Variables	Label Calculation	Label Sheet Calculation
Beetle field work	A = 3 labels/trap B = 4 traps/plot C = 2 trap collections per month** 10 plots/site	D = 3 labels per trap x 4 traps/plot x 2 collection events = 24 labels per plot E = 24/27 = .88 print 1 strip of labels per plot	Each label sheet contains 8 strips (columns). Using 1 strip per plot, for 10 plots you would need 2 sheets of labels (10 strips / 8 strips per sheet = 1.25 sheets). There are enough labels in each strip to complete 2 bouts (at the core site).

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		Print 1 set of labels (10 plots = 120 labels per bout)
Beetle pinning	1 label/specimen or pooled sample	Print labels as needed. Refer to field datasheet.

A.5 CUTTING LABELS

Cut labels into long columns 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.

1. Cut labels with as little white space on all sides of the text as possible (**Figure 63**).

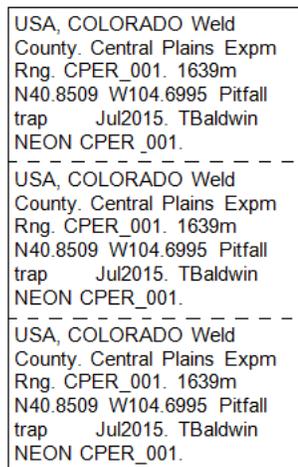


Figure 63. Example of a strip of labels, dashed lines indicate where the labels should be cut (when/if they are cut into individual labels). In this example, these are back-up labels that don't have date or trapID pre-printed

2. These cuts result in many labels in a vertical strip.
3. Store labels in strips. When needed, cut individual labels across the row with scissors, leaving as little white space around the text as possible.
4. Use ethanol-safe pens whenever adding information to labels to ensure longevity of markings.

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APPENDIX B Determination Labels

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

<i>Cratacanthus dubius</i>	D10.2015.MorphA
det. J. S. Smith	det. J. S. Smith
2015	2015

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

B.1 COMPILE DETERMINATION LABEL DATA

1. Compile the label data in a Microsoft Excel spreadsheet.
 - a. Enter label data into a Microsoft Excel spreadsheet (**Figure 65**) with one column for each piece of information required for the determination label, as follows:
 - i. Column Header = “Species”, content = scientific name or morphospecies ID for the taxon.
 - A. For scientific names: text needs to be italicized and with the first letter of the genus capitalized
 - B. For morphospeciesIDs: text should not be italicized
 - ii. Column Header = “det.”, content =the characters “det.” (with the period at the end and without quotes)
 - iii. Column Header = “Identifier”, content =First and middle initials and last name of the person who identified the individual or group of individuals
 - A. Initials should be capitalized and followed by a period
 - B. Last names should be capitalized
 - C. All initials should be separated by a space, and there should be a space between the middle initial and the last name

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

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

Figure 65. Spreadsheet containing information for determination labels

- b. If labels are printed prior to insect sorting, leave the identifier blank so that it can be filled in as sorting occurs
- Name the sheet using a meaningful name such as ‘BetDet2015’. Save and close the file – if you do not close the file, the Mail Merge may not work correctly.

B.2 EXPORT THE DATA INTO MICROSOFT WORD USING MAIL MERGE



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

- Open a Microsoft Word document and under **Mailings**, select **Start Mail Merge** and then **Labels**.
- Dimensions for these labels are the same as for locality labels, so the correct label may already exist in the list. If not, in the **Labels** menu select **New Label** and enter information for the dimensions of the label in the “Label Details” box, exactly as in **Figure 66**. Give the template a name that makes it easy to locate when creating a new sheet of labels (e.g. ‘insect determination labels’).

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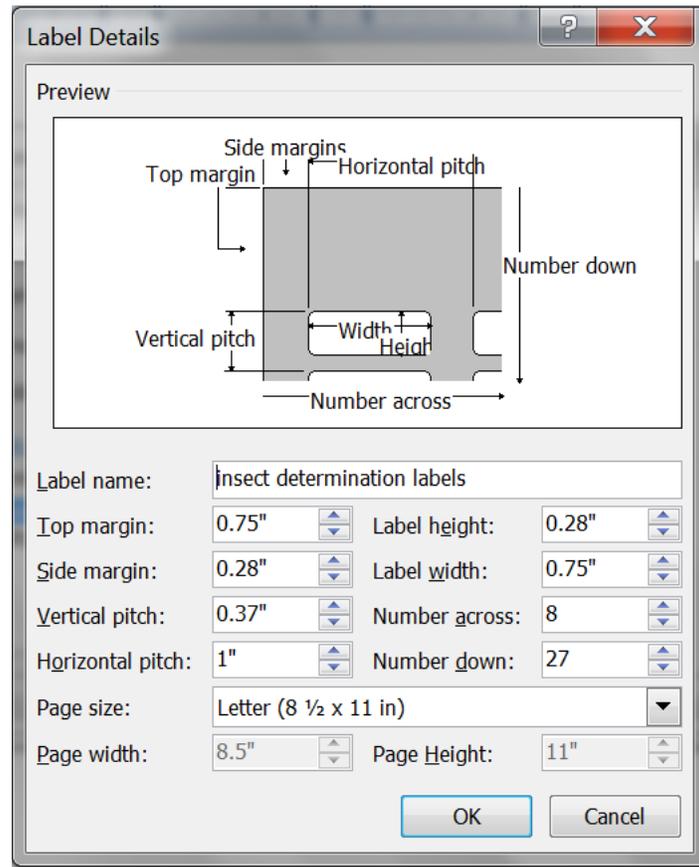


Figure 66. Dimensions for creating a determination label template in Microsoft Word

- When label dimensions have been specified, a blank template document will appear. In the **Mailings** tool bar, click on **Select recipients** and select **Use existing list**. A file navigation window will appear. Navigate to and select the Microsoft Excel spreadsheet created in step (B.1).
- A “Select Table” pop-up window (**Figure 67**) will appear, showing a list of the sheets within the Excel workbook created in step (B.1). Select the worksheet containing the determination label information. Make sure to check the box that says ‘First row of data contains column headers’.

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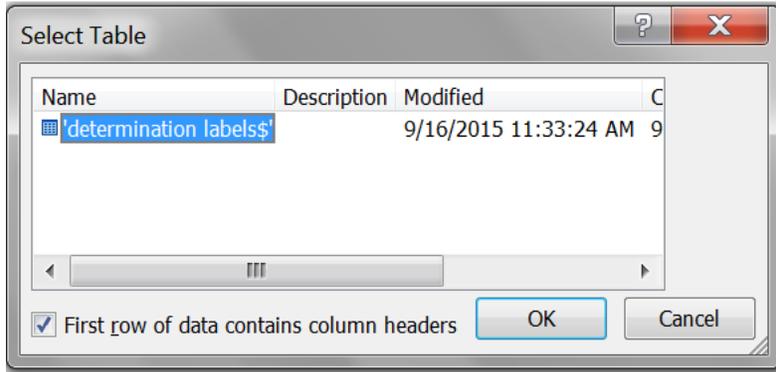


Figure 67. Selecting recipients from a Microsoft Excel file during a Mail Merge

B.3 PERFORM FINAL FORMATTING IN MICROSOFT WORD

1. Place the cursor in the top left cell of the label sheet.
2. Populate the labels by clicking on **Insert Merge Field** in the **Mailings** menu. An “Insert Merge Field” drop-down menu will appear, containing the names of the column headings from the Microsoft Excel spreadsheet. Add the headers to the cell as follows:
 - a. Highlight “Species” in the dropdown, then click “Insert”
 - b. Highlight “det.” In the dropdown, then click “Insert”
 - c. Highlight “Identifier” in the dropdown, then click “Insert”
 - d. Highlight “Year Identified” in the dropdown, then click “Insert”
 - e. Click “Close” and you will see that the top left cell contains:

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

3. Insert line breaks and spaces as follows:
 - a. Place the cursor at the end of “«Species»” and hit enter
 - b. Place the cursor at the end of «det» and hit the spacebar
 - c. Place the cursor at the end of «Identifier» and hit enter
 - d. Place the cursor at the end of «Year_Identified» and hit enter

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e. The top left label will now contain:

«Species»
«det» «Identifier»
«Year_ Identified»

4. Highlight the contents of the top left cell and set the font to Arial, font size 3.5.
5. *Note: Formatting such as italics does not get transferred from Microsoft Excel to Microsoft Word during a Mail Merge; italics will have to be applied to scientific names after the labels have been created*
6. After finishing the first cell, click **Update Labels** – this will copy formatting into remaining labels.
7. Preview the labels by clicking on **Preview Results**.
8. Confirm that the first row in each label has either a scientific name OR morphospeciesID, the second line has ‘det.’ and an identifier’s name, and the third row has the identification year.
9. In the **Mail Merge** menu, select **Finish & Merge**, then **Edit Individual Documents**. A pop-up menu will appear. Choose the ‘All’ option. This will create a Microsoft Word document with the labels.
10. Save the document. Use a meaningful name such as ‘BetDet2015’.
11. In the Microsoft Word document, italicize scientific names (but not morphospeciesIDs).
12. Copy and paste each label as needed, in order to generate the required number of labels for specimens of each taxon. Group all labels for the same taxon within the same column.
13. Re-save the document after editing

B.4 PRINT LABELS

1. Print the labels on ethanol-safe archival paper (e.g., Bioquip 1223RA).
2. Cut the labels as detailed in **APPENDIX A**

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APPENDIX C IndividualID Labels

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

BET.D10.000139

Figure 68. IndividualID label for a beetle specimen

C.1 INDIVIDUALID SPECIMEN LABELS

Note that the individualID label omits the “NEON” prefix that is part of an individualID. This is to save space on the label.

1. Use the Microsoft Excel file titled “Insect Individual ID Labels”
 - a. “Domains” (first) tab of the file: This tab contains the correctly formatted domainIDs, which are the first component of the individualID labels. Select the domain ID that corresponds to the location in which labels are being printed.
 - b. “BET_IndividualID” (second) tab: Each page in this tab contains a total of 329 individualID labels. The column width is 0.91”, row height is 0.21”, and font is Calibri size 7. All the borders are turned ON around each cell – borders will help keep straight lines when cutting out the labels.
 - i. The first 6 pages (through label 001974) have already been created, although the domainID may need to be altered to reflect the correct location. To do this:
 - A. Select all labels on all pages in the “BET_IndividualID” tab
 - B. In the “Find and Replace” menu, select the “Replace” tool
 - C. In the “Find what” box, type “D15” (without quotes)
 - D. In the “Replace with” box, type “DXX” (without quotes and where XX is the correct domain number)
 - E. Click the “Replace All” button

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

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

- iii. Keep careful track of the page of labels, and pay close attention to the last number used during the previous bout of printing individualID labels, even if that bout was in a previous sampling year.
- iv. More label pages will be added to the “Insect Individual ID Labels” spreadsheet in future years, as requested.

2. Insert a sheet of yellow cardstock into the manual feed tray of the printer. Select all labels on the page of individualID labels to be printed. Under the print menu, select “Print Selection”. Under Printer Properties, set the Paper source to Manual Feed.
3. Cut the labels as detailed in **APPENDIX A**