FIELD AND LAB PROTOCOL FOR GROUND BEETLE ABUNDANCE AND DIVERSITY

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REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
-	03/30/2011	ECO-00160	Initial Draft Release
A_DRAFT	09/23/2011	ECO-00280	Update to new document numbers/template throughout
B_DRAFT	06/25/2012	ECO-00443	Draft release for 2012 updates
C_DRAFT	01/10/2014	ECO-01138	Updates from 2013. Will be finalized in next rev.

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1 DESCRIPTION

1.1 Purpose

The primary purpose of this document is to provide a change-controlled version of NEON protocols and procedures. This document provides the content for training and field-based materials for NEON staff and contractors. 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.

This document is a detailed description of the field data collection, relevant pre- and post-field tasks, and safety issues as they relate to this procedure and protocol.

These protocols are to be followed precisely. If a deviation is considered, it should be brought to the attention of the relevant staff scientist. Any deviation from written protocols should immediately be brought to the attention of the staff scientist.

1.2 Scope

This document relates the tasks for a specific field sampling or laboratory processing activity and directly associated activities and safety practices. This document does not describe:

- general safety practices (e.g. how to drive a boat)
- site-specific safety practices (e.g. how to safely walk in a stream)
- general maintenance (e.g. refuel the field vehicle)

It does identify procedure-specific safety requirements such as safe handling of small mammals or safe use of required chemicals and reagents.

1.3 Acknowledgements

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

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain information that shall be applied in the current document. Examples are higher level requirements documents, standards, rules and regulations.

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.001155	NEON Training Plan
AD [05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD [06]	NEON.DOC.014002	TOS Science Requirements

2.2 Reference Documents

Reference documents contain information complementing, explaining, detailing, or otherwise supporting the information included in the current document.

RD [01]	NEON.DOC.000008	NEON Acronym List
RD [02]	NEON.DOC.000243	NEON Glossary of Terms
RD [03]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD [04]	NEON.DOC.014051	Field Audit Plan
RD [05]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan
RD [06]	NEON.DOC.001100	Common Insect Lab Protocols
RD [07]	NEON.DOC.005005	NEON Level 0 Data Products Catalog
RD [08]	NEON.DOC.000909	TOS Science Design for Ground Beetle Abundance and Diversity
RD [09]	NEON.DOC.000724	Chemical Hygiene Plan and Biosafety Manual
RD [10]	NEON.DOC.001025	NEON Field Protocol and Procedure: Plot Establishment

2.3 Acronyms

TOS	Terrestrial Observation System
BOLD	Barcode of Life Database
SDS	Safety Data Sheets
FOPS	Field Operations
PG	Propylene Glycol
GPS	Global Positioning System
PDA	Personal Digital Assistant

2.4 Definitions

A **protocol** is a formal summary description of a procedure and its related rationale, and includes information on knowledge and resources needed to implement the procedure. A procedure is a set of prescribed actions that must take place to achieve a certain result, and can also be called a method. It differs from a science design in that science designs provide a more complete description of the

rationale for selecting specific protocols. It differs from a training manual in that training manuals provide materials in support of skills acquisition in the topic areas including information on how to best train staff rather than detailing only the steps of the procedure.

3 BACKGROUND AND OBJECTIVES

3.1 Background to Ground Beetle Sampling

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. They are easy to sample and well known taxonomically. They are relatively common in many habitats and form well-defined gradients in species richness across North America. They have been used as indicators of environmental change as they are sensitive to habitat disturbance. They are generally predacious and can influence trophic structure. They also represent other feeding types (scavengers, frugivores, etc.), and are consumed by other predators, therefore, changes in the proportion of each guild can indicate significant changes in the local ecological 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" RD [08].

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 them. The animals that fall into the trap become preserved by a liquid mixture of DNA-safe preservative in the bottom of the trap. Animals collected in these traps other than ground beetles are termed "bycatch." In addition, this protocol describes laboratory processing of the collected animals, appropriate storage, and shipment of samples to contracted experts for morphological identifications or Genetic Analytical Laboratories for DNA sequencing.

3.2 NEON Science Requirements

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.

3.3 NEON Data Products

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

4 PROTOCOL

Ground beetle sampling involves the collection of ground beetles using pitfall traps, processing samples in the lab to identify and record individuals by species, and sending a subset to external facilities for DNA barcode analysis.

The pitfall trap design currently consists of two wide, shallow plastic bowls (7 cm deep with an 11 cm diameter, 540 mL total volume) nestled within one another. One bowl ensures that the hole stays in the ground and enables efficient collection and resetting of the trap. This lower bowl has holes drilled into it so that any excess moisture can drain rather than building up and causing the top bowl to float. The second bowl has lines at the 150 and 250 mL marks and contains the preserving fluid. This top bowl is picked up and changed during sampling. The bowls are protected from weather (e.g., dilution from rain, drying from sun) by a cover.

The preserving fluid used in the pitfall traps is a 1:1 mixture of distilled water and propylene glycol (abbreviated PG). Propylene glycol is a non-toxic antifreeze (SDS: mild irritant, non-toxic).

Pitfall traps will be placed adjacent to Distributed Plots (outside of the 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 labeled “B” for beetles. Plots for pitfall sampling will be identified prior to the field season.

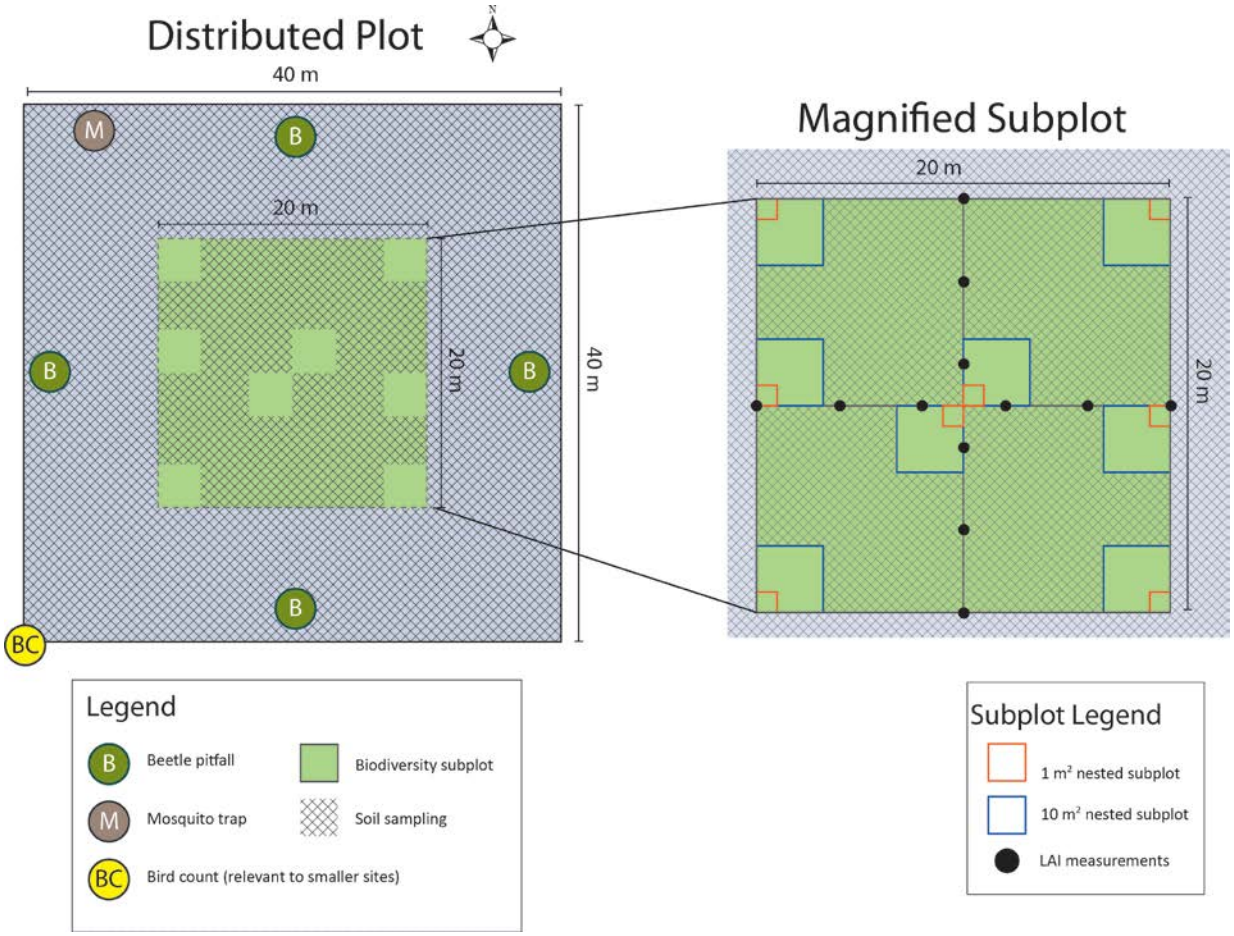

Figure 1. Standard plot layouts

5 QUALITY ASSURANCE AND CONTROL

The procedures associated with this protocol will be audited according to the Field Audit Plan (RD[04]). Additional quality assurance will be performed on data collected via these procedures according to the NEON Data and Data Product Quality Assurance and Control Plan (RD[05]).

Because of the wide range and variance of ground beetle abundance, algorithms that check data for irregularities may catch some errors but will not be a dependable way to fully quality control ground beetle data from the field. In order to ensure data quality, "hot checks" conducted by someone with extensive field experience who knows the protocols well and observes data collection will be conducted and reported on a regular basis per the Field Audit Plan (RD [04]). For work done by external laboratories, QA/QC plans will be developed based on pre-existing laboratory protocols modified as needed to meet NEON requirements.

5.1 Contingency Decisions

The following table describes how to respond to delays in the sampling schedule 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.

Table 1. Contingent decisions for field sampling

Delay	Action	Adverse Outcome?	Outcome for Data Products
hours	Collect traps asap; note duration & cause	None	None
day	Collect traps asap; note duration & cause; resume standardized sampling (2 weeks)	YES. Data not comparable to standard collection events	Cannot calculate diversity indices if traps collections are not comparable. If ALL traps move by a day this is less of an issue.
2-13 days	Collect traps asap; note duration & cause; resume standardized sampling (2 weeks)	YES. Data not comparable to standard collection events; integrity of specimen DNA becoming compromised	May not be readily able to obtain DNA barcodes; affects Abundance & Diversity measurements
2 or more	Collect traps asap; note duration & cause; resume	YES. Data not comparable to standard	May not be readily able to obtain DNA barcodes; affects

Delay	Action	Adverse Outcome?	Outcome for Data Products
weeks	standardized sampling (2 weeks)	collection events; integrity of specimen DNA suspect	Abundance & Diversity measurements

1.1.1 Pitfall Fluid Shortage

If it is not possible to fill all traps to the 150 mL volume, revisit traps with additional preserving fluid in the following days. If this is not possible you may reuse recently collected PG to reset traps (only in case of emergency, note on datasheet if this measure is taken). If the trap dries out and specimens are not preserved, the entire sample is lost. Comparability between samples relies on sufficient preserving solution in each pitfall cup. Bring more preserving fluid out to the field than you anticipate using.

6 SAFETY

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, and paradichlorobenzene (moth crystals). Whenever chemicals are used, follow requirements of the site-specific Chemical Hygiene and Biosafety Plan (RD[09]).

7 PERSONNEL REQUIREMENTS

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

8 TRAINING REQUIREMENTS

All technicians must complete required safety training as defined in the NEON Training Plan (RD[03]). Additionally technicians complete protocol specific training for safety and implementation of protocol as required in Field Operations Job Instruction Training Plan (RD[04]).

Training for field techs will include attempted analysis of datasheets of various levels of completion or with errors. Training will include discussion of how to interpret these things and the importance of uploading data in a timely fashion and will include the distinction between missing data and true zeros.

9 FIELD STANDARD OPERATING PROCEDURE

9.1 Sampling Frequency and Timing

Pitfall traps will be checked, emptied, and reset every 14 days. See section 5.1 regarding contingencies for delays in the sampling schedule.

Table 2. Approximate sample dates for beetle sampling at all NEON domains during Operations

Domains	Start Week	Stop Week (inclusive)	Total No. Weeks	Approx. Calendar Start Date	Approx. Calendar Stop Date
1	17	38	22	23-Apr	17-Sep
2	15	38	24	9-Apr	17-Sep
3,4,7,9,11,14,17,20	14	38	25	2-Apr	17-Sep
5,10	20	38	19	14-May	17-Sep
6	18	38	20	30-Apr	17-Sep
8,15	21	38	18	21-May	17-Sep
12	25	38	14	18-Jun	17-Sep
13,16	19	38	20	7-May	17-Sep
18,19	21	35	15	21-May	27-Aug

9.2 Criteria for Determining Sampling Dates

Ground beetles should be sampled during the growing season, when biological activity is highest. The start and end of sampling each season will be triggered by biologically relevant thresholds. Sampling should begin within 2 weeks of the 10-day running average night temperature being > 4 °C, but no earlier than April 15. Sampling should end when the 10-day running average night temperature is < 4 °C. If this season-ending threshold has not been reached by 17 September, sampling will automatically stop for the season. The approximate time we expect to sample in each domain is estimated in Table 2.

9.3 Sampling Frequency

Beetle sampling is to occur every 14 days within 6 hours of a standard time of day. 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., they are collecting beetles for the same amount of time). The selected day of the week is discretionary; however, the sampling should occur on the same weekday every 2 weeks for the entire field season. Sampling is estimated to require 2 technicians 12 min per trap x 40 traps = 480 min = 4 hours per person each day of sampling, plus hiking time and travel to and from site.

9.4 Equipment and Materials

Materials and supplies required for the Beetle Sampling Procedure, at one site, for one sampling day are listed in Table 3.

Table 3. Beetle Sampling Procedure materials and supplies

Maximo Item No.	Item Description	Quantity per sampling event	Hazardous Chemical
	Pitfall trap locality labels	3 per trap; 3*40=120	N
	Ice packs	2	N
	Chests coolers	1	N
	Trowel or soil knife	2	N
	Permanent marker	4	N
	1.5 cm PVC pipe (for pitfall cover spacers)	200	N
	Pitfall trap bowls	80 (in ground)	N
	Pitfall covers	40 (in ground)	N
	Plastic nails for pitfall covers	200	N
	Propylene glycol	75 mL per trap	N
	Distilled water	75 mL per trap	N
	Ethanol	2L	Y
	Archival, ethanol-safe pens (Pigma brand, size 01)	4	N
	Scissors	2	N
	Forceps, fine point	2	N
	Whirl-Pak bags, 13 oz	40	N
	300 µm filter mesh organdy pre-cut (9 cm square)	40	N
	Hard-bottomed container of appx. 10 cm width (e.g., Tupperware)	10	N
	Modified wide mouth Nalgene bottles (bottom portion of bottle cut off)	3	N
	Modified wide mouth Nalgene lids as filter tops (top cut out of lid, filter screws onto bottle	3	N
	Ethanol squeeze bottle	2	N
	Water squeeze bottle	2	N
	Carry-all craft tote bag or other field pack	2	N
	Specimen cups for temporary pitfall waste storage	3	N
	1-gallon ziploc bags	12	N
	Ethanol waste wide mouth bottle (1000 mL Nalgene)	2	N
	PG waste wide mouth bottle (4000 mL jug)	2	N
	Surgical gloves	1 pair	N
	Handwipes	optional	N
	1000 mL wide mouth Nalgene bottles	8	N
	Duct tape	1 roll	N
	Flagging	1 roll	N

Maximo Item No.	Item Description	Quantity per sampling event	Hazardous Chemical
	Digital Camera, 10 megapixels	1	N
	GPS/Trimble	1	N

* Quantities are the minimum required to implement protocols and additional items should be on hand, in case of equipment failure.

9.5 Sample Labels

Seven different types of labels are required and described briefly in turn below.

1. LOCALITY LABELS: Locality labels for samples should be created before the field campaign begins in order to expedite processing and reduce opportunities for error. Locality labels are inserted into pitfall samples in the field and remain with samples throughout the lab processing steps and storage (mounted on pins or put into alcohol vials).

   Storage containers for long-term sample archive in voucher collections (e.g., 50 mL centrifuge tubes) will require locality labels inserted into them in triplicate (three individually cut labels). Technicians are responsible for generating field/lab locality sample labels on archival, ethanol-safe paper. Directions and a template file for the preparation of labels are provided in the Common Insect Lab Protocols RD [07]. New labels must be made whenever plot information changes (e.g., plots are moved or different plots are selected).

2. DETERMINATION LABELS: These labels are mounted on a pin below a pinned/pointed specimen and communicate the taxonomic designation of the specimen, as well as who identified it and when. Details about how to prepare determination labels are described in the Common Insect Lab Protocols RD [07]. Vials that contain multiple members of a single species also require a determination label.
3. DNA BARCODE UNIQUE NUMBER LABELS: A file will be provided from NEON headquarters to each domain prior to each field season with the reserved unique numbers for specimens that are to be DNA barcoded. These numbers are to be applied to pins below locality labels on each specimen to be DNA barcoded. Thus, each pinned specimen that will be / has been barcoded will have an extra label on its pin with a DNA barcode number on it.
4. UNIQUE NUMBER LABELS: For sample tracking purposes every identified individual or group of individuals requires a unique number label. These are labels that go on pinned/pointed specimens or in alcohol vials of specimens of the same species that are used to label and track specimens from a given sample plot from a given sampling period. Instructions for generating unique number labels are provided in the Common Insect Lab Protocols RD [07].
5. TEMPORARY LABELS: Consumables that only require temporary labels (e.g., waste ethanol cups, cardboard mailers) will be marked with laboratory tape and permanent makers.
6. MICROPLATE LABELS: Ninety-six well microplates will have designated numbers on labels when they arrive from BOLD.

7. SHIPPING LABELS: Shipping labels with the relevant details for addressor (Domain contact) and addressee (the external facility) may be pre-printed to expedite shipping throughout the field season**.**

9.6 Preparation

2.1.1 Deploying Pitfall Traps

1) Identify the locations of sampling plots and access routes.
2) Prepare pitfall trap materials
   a) Cut/drill holes in bottom cup
   b) Draw the 150 mL and 250 mL fill lines on top cup
   c) Cut 1.5 cm PVC sections to hold up cover
   d) Cut filter squares (10 cm) of mesh organdy (as needed, may do all at the beginning of the season)

1) Select the first trap location by moving 20 m north of the center plot marker. Orient yourself between the corner markers, in the middle of the northern edge of the plot (see RD[10]).
2) Dig a small hole with a soil knife to accommodate the pitfall bowl (Figure 2). Start small because it is much easier to enlarge the hole than fill in the edges and maintain a tight fit. You may outline the bowl 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.

Figure 2. Digging a hole for the pitfall bowl.

3) 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.
4) Push the bottom bowl (with holes) into the hole. Slide the top bowl into bottom bowl. **Ensure that the lip of the top bowl 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 trap). Also ensure that there is no gap between the bowls and the ground. The cups should fit snugly into the hole. The top bowl must be flush with ground level so that specimens accumulate in the top bowl only. The top bowl is the only part of the trap where specimens should be collected for the sample.
5) Use the pre-drawn line to fill top bowl with 150 mL of PG:water solution. Initially fill all traps to equal volume.
6) Position cover with four plastic nails and plastic spacers (see Figure 3 and Figure 4) so that it is visually level and 1.5 cm above the surface of the bowls (Figure 5). This serves several purposes including: 1) preventing vertebrate bycatch, 2) reducing evaporative loss and decomposition rate by shading and thus lowering fluid temperature, and 3) preventing precipitation which dilutes preserving fluid.
7) Cover deployment will vary based on local topography and vegetation cover. Keep the purposes of the cover in mind while installing the cover.

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 at all.

Figure 4: Installing pitfall lid with spacers. Note lower position of cup.

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

8) Install remaining traps in other cardinal directions (east, south and west) 20 m of the center plot marker. If possible, reuse the same holes for pitfall traps from year to year (backfilled holes can be excavated anew each year).

3.1.1 Prior to a Sampling Bout

1) Assemble field equipment at least one day prior to field sampling.
2) Transfer files/Load PDA or gather plot location information.
3) Print datasheets if PDA is not available (see Ground Beetle Field Datasheet, Appendix B).
4) Prepare locality labels:
   a) Prepare template to print 0.28 in x 0.75 in label.
   b) Prepare data for label.

Table 4 Required Information for Locality Label

Label Field	Format	Example
State	All capital letters	GEORGIA
County	See example	Baker County
Site Code	Standard abbreviation	Jones Ecological Res Ctr
Elevation	In meters, to the nearest meter	38m
Latitude	In decimal degrees, to 4 decimal places	N31.1874
Longitude	In decimal degrees, to 4 decimal places	W84.4707
Trap Type	See example	CO2 light trap OR Pitfall trap
Month	Abbreviated to 3 letters	Jun
Year	4 digit year	2013
Collector	Field Operations Manager first initial last name	RNelson
Plot code	NEON.Site Code.Plot Number.	NEON.JERC.000562.

   c) Print labels.(see RD [06] for additional detail on locality labels).

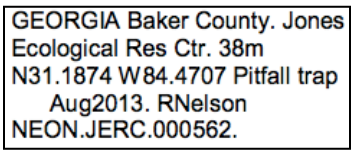

Figure 6 Example of locality label

5) Fully charge all electronic equipment (e.g., PDA, GPS unit).
6) Prepare bench and fume hood space in the lab for the preparation of field materials (e.g., ethanol, PG). Ensure that space in freezer, fridge or flammable materials cabinet is available for samples. Coordinate with potentially conflicting activities (e.g. soil sieving or other dust).
7) Prepare 3 one liter bottles of propylene glycol:water solution (500 mL PG : 500 mL distilled water).
8) Prepare 3 one liter bottles of distilled water.
9) Prepare 2 one liter bottles of ethanol in fume hood.

9.7 Sample Collection in the Field

4.1.1 Collecting Insects from Trap

Upon arrival at the trap

1) Place three copies of locality labels (Figure 6) into the bowl (Figure 7). The labels can be attached or cut into separate labels and can be treated in the same way as the trap catch in the subsequent steps without harm.

Figure 7. Adding locality labels to trap.

2) Pick up the top bowl containing the sample. Disposable gloves may be worn to protect your hands from the materials used. Gloves can be reused.

Figure 8. A pitfall trap containing insect specimens.

3) Remove any large debris (e.g., sticks, leaves) from the trap prior to filtering. Take care no beetles (especially tiny ones) or bycatch are removed.
4) Filter the trap contents using a 300 µm mesh filter and a modified Nalgene bottle with a screw cap lid (Figure 8-Figure 11). Holding the filter above an empty temporary waste container, pour the contents of the bowl through/onto the filter. The PG solution will flow through into a specimen cup while the sample is collected on the filter.

Figure 9. A filter installed on a trap bowl.

Figure 10. Upside down Nalgene filter ready to use.

Figure 11. Preparing to filter out specimens from collecting fluid.

Figure 12. Filtering specimens from trap. The temporary waste container is below the filter (not visible).

Figure 13. Beetle specimens after collecting fluid has been poured off.

5) Pour used PG solution into a 1 L storage bottle for disposal in the lab.
6) Rinse the sample (in the filter cup) using distilled water (Figure 13). Rinse above the (temporary) waste container so rinse water/PG is collected.

Figure 14. Rinsing sample with water.

7) Rinse sample in filter cup with 95% ethanol over an ethanol waste container or directly over the Whirl-Pak bag (Figure 14).

Figure 15. Rinsing sample with 95% ethanol.

8) Place insects and filter in a Whirl-Pak bag **(**Figure 15**)** and cover with approximately 100 mL of 95% ethanol (Figure 16). After removing the mesh filter and placing it in the Whirl-Pak, verify that all sample materials are off the filter cup before using it to process another sample.

Figure 16. Placing sample in Whirl-Pak bag.

Figure 17. Rinsing the sample into the Whirl-Pak and covering sample with 95% ethanol. The mesh is also placed in the bag.

Figure 18. Checking filter for remaining insects. Place filter in bag with sample.

9) Roll the Whirl-Pak bag shut and tightly close the bag's twist ties so that there is minimal opportunity for the ethanol to leak (Figure 19).

Figure 19. Whirl-Pak bag containing sample, mesh and locality labels.

Figure 20. A packed sample.

10) Verify that the appropriate locality labels are in the Whirl-Pak bag.

11) Record metadata (e.g., time of collection, any issues with the trap or sample) in the PDA or paper datasheet (Ground Beetle Field Datasheet; see Appendix B). Write down any irregularities that may be relevant to the ground beetle data in the notes section (e.g. trap was damaged by bears, wind blew cover off, trap flooded with rainwater).
12) Reset pitfall trap.
    a) Using new solution prepared in the lab, refill the PG mixture in the bowl 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 250mL line to prevent potential trap drying.
    b) Position cover with four plastic nails so that it is visually level and 1.5 cm above the surface of the bowls.
13) Place all 4 Whirl-Pak bags from a single plot into a ziploc bag and label the outside of the bag with date and plot # before leaving the plot. You may choose to pre-label these bags in the lab.
14) Place bags into hard-bottom carrying device (e.g., an airtight plastic Tupperware container) ensuring that the Whirl-Pak bag openings are upward.

5.1.1 Collecting the final sample of the field season:

1) After final sample collection, remove traps from holes
2) Backfill holes with local/approved substrate. These same holes will be excavated in following years for pitfall trapping.
3) Return all trap components to the lab.

9.8 Sample Preservation

1) Store samples in a chest 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.
2) Change ethanol within 24 hours of collection (see laboratory procedure).

9.9 Data Handling

If PDAs are not available, data from paper datasheets should be promptly and carefully transcribed into the NEON database as soon as possible after data collection. This should be done by the same people who recorded the data in the field. Note all metadata (deviations from the protocol or any other notes that may be relevant to ground beetle data) in the database. Scan paper datasheets and save originals.

10 LABORATORY STANDARD OPERATING PROCEDURE

10.1 Sample Processing Timing

The ethanol that each sample is stored in must be changed within 24 h after collection. Ideally, bycatch will be removed during this rinse (details in lab section below). Following the rinse, pitfall samples can be stored in ethanol (ideally at -15 to -30 °C) for several months.

Final processing, including identification, pinning, submission of samples to taxonomists, and submission of DNA barcode datasheets must occur within four months of the end of the field season (see RD [06]). The specimen identifications must be verified and domain voucher collection updated before the beginning of the next field season. See the Common Insect Lab Protocols RD [06] for instructions on taxonomic identification, DNA barcode submission, the identification verification process and voucher collection maintenance.

10.2 Equipment and Materials

Table 5. Beetle Lab Procedure materials and supplies

Maximo Item No.	Item Description	Quantity per sampling event	Hazardous Chemical
	Ethanol		Y
	Plastic petri dishes for sorting specimens under microscope	2	N
	Permanent markers	5	N
	Archival, ethanol-safe pens (Pigma brand, size 01)	5	N
	50 mL non-sterile centrifuge tubes	Variable	N
	Specimen cups - bottoms	5	N
	8 oz. plastic jars	50	N
	Specimen cups - lids	5	N
	Jewelers forceps	3	N
	Microscope	2	N
	Fumigant (paradichlorobenzene - moth crystals)	Variable, ~6.5 g per box	Y
	Shipping supplies (boxes, padded envelopes)	Variable	N
	Cardboard specimen mailers	Variable	N
	Scissors	2	N
	Exacto knife	2	N
	Metal ruler	1	N
	Secondary containment bins for pouring liquids	1	N

	Surgical gloves	Variable	N
	Ethanol waste drum	1	N
	PG waste drum	1	N
	Tupperware for temporary storage of each bout of Whirl-Paks	10	N
	Tupperware for photo box of large beetles	1	N
	Styrofoam for photo box	1	N
	Tissue paper for photo box background	1	N
	LED lights (microscope)	3	N

*Quantities are the minimum required to implement protocols and that it is recommended that additional items be on hand, in case of equipment failure.

10.3 Preparation

1) Clear lab bench space for beetle sorting and processing. Coordinate with potentially conflicting activities (e.g. soil sieving or other dust). 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) Storage space for samples.
   d) Storage space for pinned insects in Cornell cabinets or Schmitt boxes.

10.4 Sample Processing in the Lab

6.1.1 Ethanol Rinsing/Processing

The first change of ethanol must occur within 24 h of sample collection from the field. Water leaks out of arthropods into the ethanol, effectively diluting it. Changing the ethanol is important for sample preservation. Remove bycatch during the rinse so there are fewer arthropods diluting the ethanol. Prioritize the removal of vertebrate bycatch.

Figure 21. Preparing for the first ethanol change. Filter cup (sieve) and temporary waste storage container used for sieving sample are shown above the Whirl-Pak.

1) Disposable gloves may be worn to protect your hands from the materials used.
2) Pour the sample (including the specimens and the initial ethanol added to the Whirl-Pak in the field) through a sieve into a temporary waste container. Rinse and inspect the filter cup to ensure none of the sample is left on the mesh. Filter cups in the lab can be reused. Do this for each trap, one at a time. This ensures that specimens from different traps are not mixed.

Figure 22. Filtering diluted ethanol off sample into a specimen cup.

3) Transfer waste ethanol into ethanol waste containers.

Figure 23. Pouring sample through filter directly into larger waste container.

4) Place insects and the original filter along with locality labels back into the same Whirl-Pak bag from the field. Keep the original filter with the sample in case small beetles and bycatch are stuck to it.
5) Pour fresh 95% ethanol into each Whirl-Pak bag, ensuring that all of the insects are submersed, and close bag, leaving as little airspace as possible.
6) Confirm that the appropriate labels are still in the Whirl-Pak bag.
7) Put each sampling bout (all the plots from a single date) into a labeled airtight plastic container or resealable bag and seal tightly until processing.

Figure 24. Samples from one sampling bout stored in a labeled container.

8) Store samples in the freezer (-30 °C to -15 optimal) or refrigerator (2-6 °C) if freezer space is limited.

7.1.1 Sorting Ground Beetles from Bycatch

Sorting requires more time than only changing the ethanol. Do not begin sorting unless certain that the change of ethanol can be completed for all samples. 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.

1) Sort each sample one at a time for ground beetle specimens.
2) Suspend specimens in ethanol in the Whirl-Pak bag so that they are uniformly floating throughout.
3) Pour them into a large Petri dish marked with a grid.

Figure 25. Preparing to sort a sample.

4) Sort beetle samples to select all adephagan beetles.
5) The suborder Adephaga includes ground beetles and some aquatic groups (which are unlikely to fall into pitfall traps) – all adephagan beetles are of interest to NEON. This group is identified by the manner in which the last pair of legs articulates with the beetle's underside, as well as the tarsal formula (5-5-5). 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.

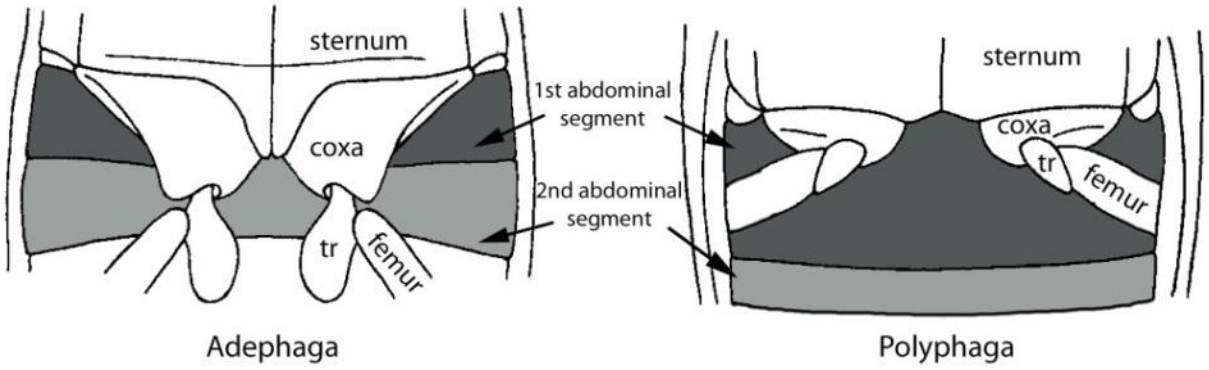

Figure 26. Hind leg articulation in Adephaga and Polyphaga.

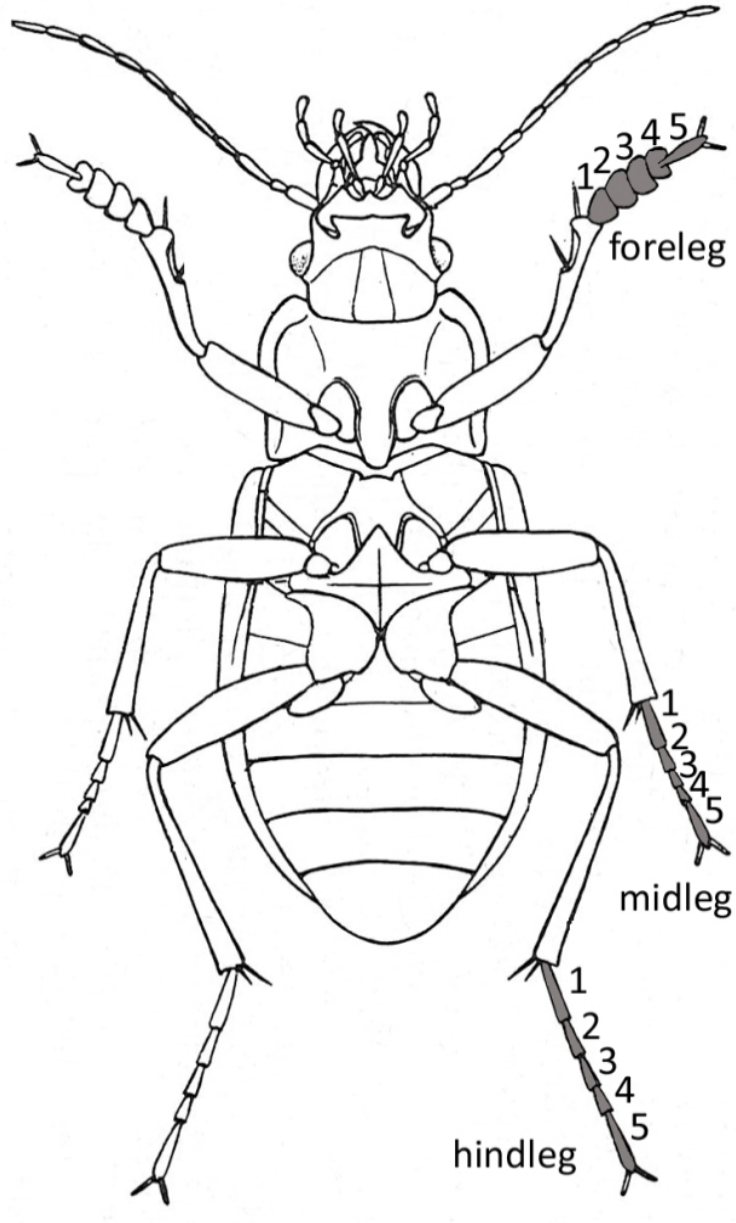

Figure 27. Tarsal formula of adephagan beetles.

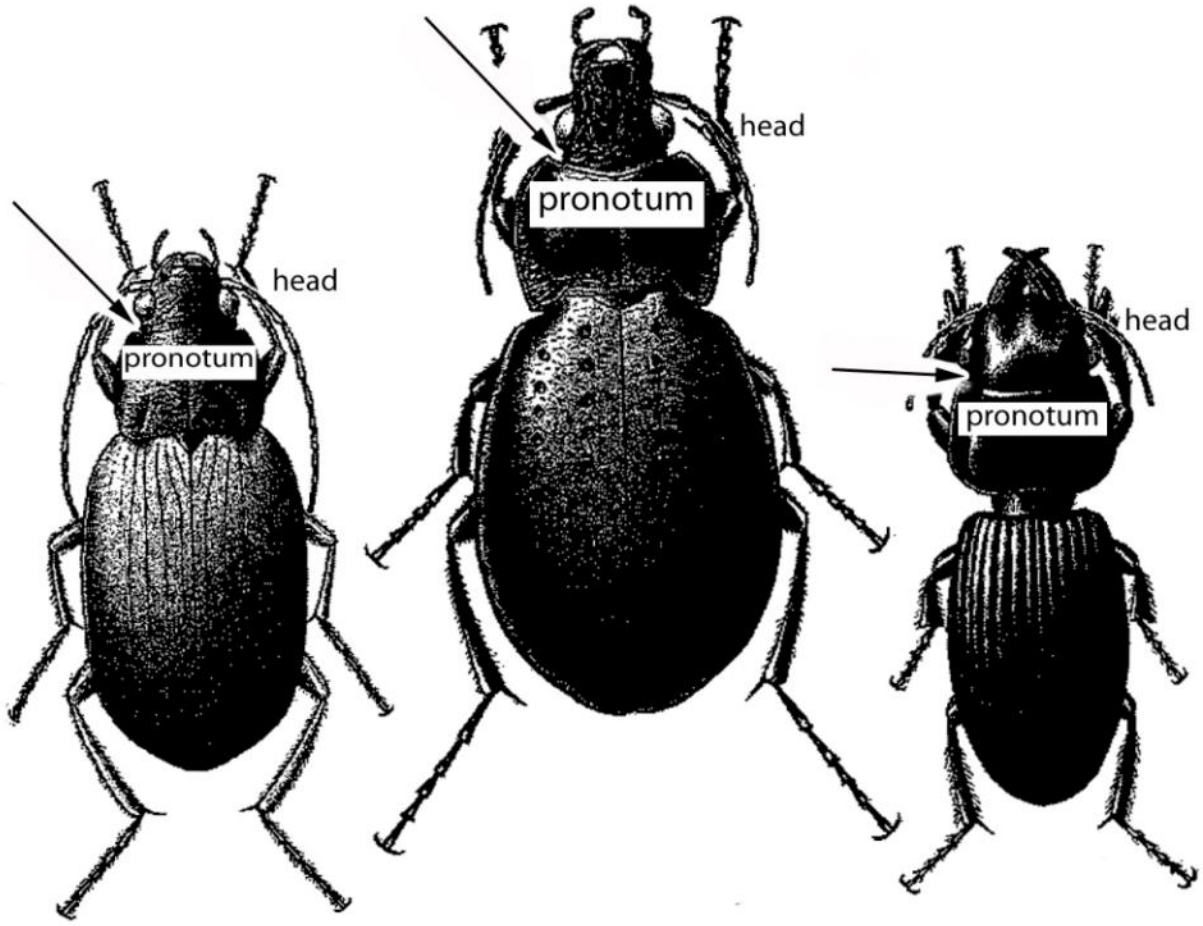

Figure 28. Drawing of three ground beetle specimens with head and pronotum labeled.

6) Sort ground beetles into 50 mL vials or temporary holding cups with a single locality label from the three initially put in the pitfall bowl.
7) The other 2 locality labels remain in the sorting dish with the rest of the specimens at all times.
8) Additional locality labels may be required for large samples with many specimens that do not all fit into a single 50 mL centrifuge tube. If additional 50 mL vials are required to hold the sample, add 3 locality labels to each tube.

8.1.1 Select Specimens for Pinning/Pointing and Taxonomic ID

Many beetles will be pinned/pointed, however in the interest of time and storage efficiency, not all specimens will be pinned. The identity of abundant and easily distinguishable beetles will vary among sites and sites-specific lists are provided for each site. For these species, a minimum of 20 individuals per species will be (pinned/pointed) from each site per year. This select group of common and easily identified ground beetles is listed for each site in the Common Insect Lab Protocol (see RD [06]).

For example, for CPER in D10, pin only the first 20 *Pasimachus elongatus* collected from each site. Some attempt should be made to represent *Pasimachus elongatus* from different plots and sampling bouts

among the 20 pinned specimens. This beetle is extremely abundant at some sites in D10 and is easy to distinguish from other ground beetles. Other ground beetle types (those not on the site-specific list) are more difficult to distinguish and must be pinned/pointed for identification. However, by removing the most abundant easily identified species, the number of beetles to be pinned can be greatly reduced.

1) Select 20 individuals of the pre-defined common species for the site. Attempt to represent species with individuals from different plots and sampling bouts among the 20 pinned specimens.
2) After selecting 20 individuals for pinning, pool additional specimens of these common species by species at the plot level (4 traps).
3) Count and record these individuals and place them in 95% ethanol in a 50 mL plastic centrifuge tube along with a locality label (date filled in).
4) In addition, insert a single determination label into the tube (would normally be mounted on the pin with each beetle).
5) Shut the lid on the tube tightly.
6) All individuals of other ground beetle types (those not on the site-specific list) must be pinned/pointed for identification.
7) Record the data.
8) After pinning, designate no more than 1000 specimens per domain to send to taxonomists.
    a) Ideally at least one specimen from each species or morphospecies from each site is sent to taxonomists.
    b) Specimen identifications that are uncertain should be heavily favored for sending to taxonomic experts.
    c) It is ideal to send the same specimens to the taxonomist as have been sent for DNA barcoding (see RD[06]).
    d) A damaged specimen that is still largely intact is fine to send. However, a specimen with its head missing, for instance, should not be sent to the taxonomist for identification.
9)

9.1.1 Processing Bycatch Samples in Ethanol

1) Transfer bycatch from each sample to as few 50 mL centrifuge tubes as possible while ensuring that all specimens are fully covered with 95% ethanol. This will be a variable amount of ethanol depending on the volume of animals collected.
2) Vertebrate bycatch that does not fit in 50 mL centrifuge tubes should be kept in 8oz wide mouth straight sided plastic or glass jars.

Figure 29. A bycatch sample stored in a 50 mL centrifuge tube.

Figure 30. The amount of ethanol in a bycatch sample will vary by sample size.

3) Insert three individually cut locality labels (date filled in) into each bycatch 50 mL centrifuge tube.
4) Label the tube with permanent marker with the collection code (Domain.Plot.Trap.Date).
5) Store processed samples in ethanol (optimally at -20 °C). If there is no access to -20 °C, they may be stored at 4 °C. If there is no access to 4 °C they may be stored temporarily (for less than one year) at room temperature in the flammables cabinets.

10.1.1 Mounting Specimens and Preparing Samples for Genetic Analysis

Detailed instructions for mounting specimens and preparing samples for genetic analyses are provided in RD [06]. Pinning/pointing involves mounting specimens either directly on a pin or on a small paper triangle on a pin. A subset of mounted specimens are shipped to taxonomic experts for identification.

11.1.1 After beetles have been identified to species or morphospecies at the lab, a subset of beetles will be submitted to a lab for DNA barcoding. The process of DNA barcoding involves removing a leg to submit as a tissue sample and requires a matching physical voucher specimen. The voucher specimen must be both pinned/pointed and photographed.Sample Preservation

1) Store prepared, pinned or pointed ground beetle specimens 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) The fumigant box must be firmly secured in the corner of the Schmitt box or unit tray with pins.

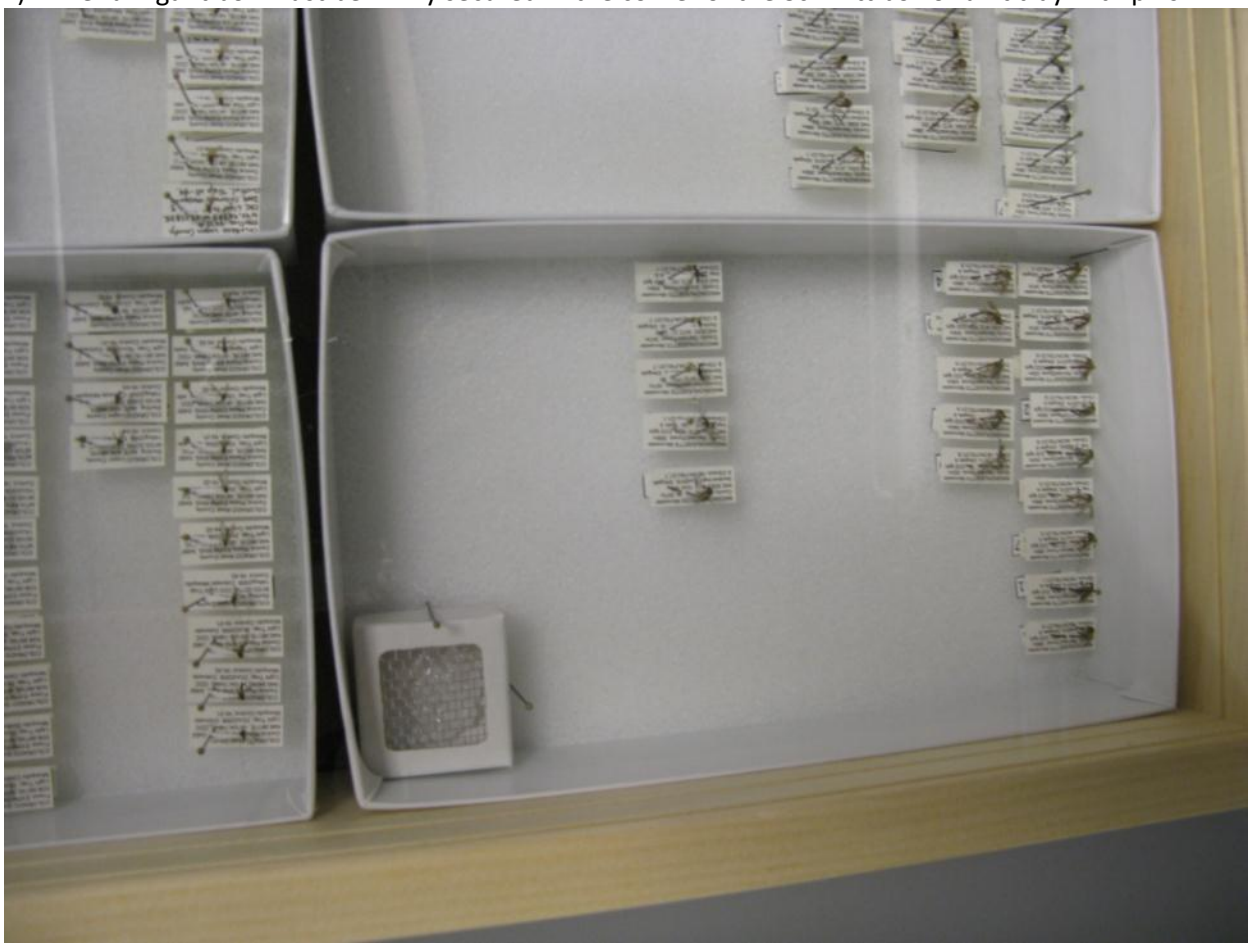

Figure 31. Fumigant boxes should be firmly secured with pins.

10.5 Sample Shipping

Samples to be barcoded and/or identified by expert taxonomists are shipped to external facilities.

1) Gather shipping materials (cardboard mailers, cardboard boxes, packing tape, etc.).
2) Hazardous Materials certification for shipping ethanol.
   a) Print SDS for Ethanol.
3) Print completed datasheet to include in shipment.

12.1.1 Taxonomists

1) Ship prepared dry (pinned/pointed) beetle specimens overnight to Taxonomists with a hard copy of the Insect Biodiversity Datasheets. Email a digital copy of the datasheet to the taxonomist.
2) Any large beetles (typically 1.5 cm or larger, but as required for smaller but heavy bodied specimens) must be brace-pinned on either side of the label to prevent them from moving during transit.
3) Once the mailers are prepared for shipping take a photo of each box (with specimens visible) for tracking purposes. Upload these photos to the same location as the Insect Biodiversity Datasheets.
4) Cut a cardboard insert to size to place on top of specimens (resting on pins). Make a packing tape tab to easily pull off the cardboard insert (otherwise it can become wedged in the box and difficult to remove).
5) Do NOT include moth crystals in shipments.

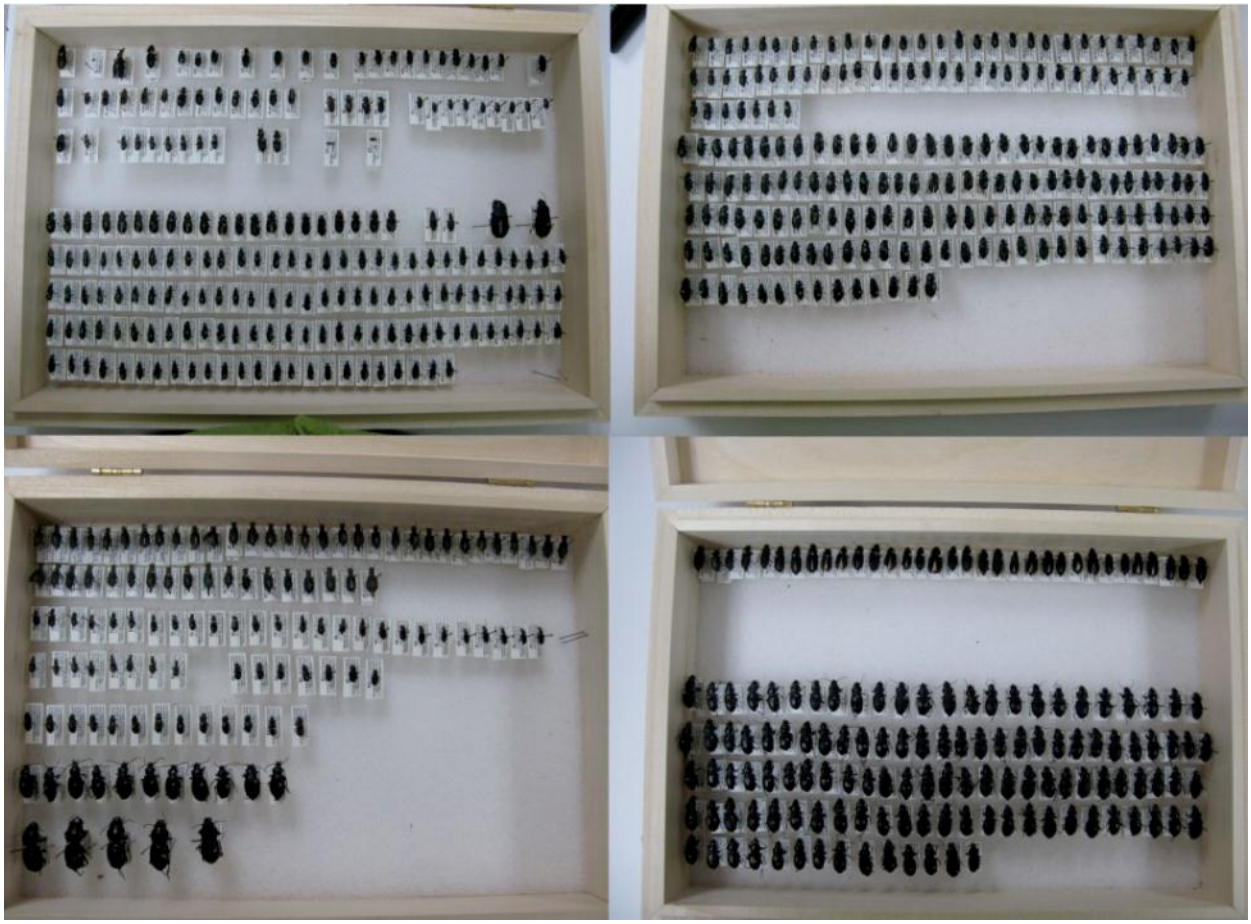

Figure 32. Specimens prepared to be sent to a taxonomist.

Figure 33. Large beetles should be brace-pinned during shipping.

6) Examine specimens before sealing the box.
   a) Make sure all specimens are appropriately labeled.
   b) Ensure that all of the specimens are firmly glued to their points by tapping the box to test.
   c) A specimen dissociated from its locality label is worthless.

7) 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 34. A mailer placed inside a larger box for shipping.

Figure 35. Mailers should be adequately padded within the shipping box.

8) Clearly label outer boxes with 'Fragile' and 'Dead Insects for Scientific Study'.
9) Include taxonomists sheets with the unique specimen numbers listed in the shipment.
   a) Taxonomists will return sheets to each domain with a Genus and species name and Authority for each specimen.

b) Taxonomists will also return the specimens with determination labels indicating their identification.
c) Record this information for each specimen in the database.

13.1.1 Genetic Analytical Laboratories

1) Ship 96-well microplates dry overnight at ambient temperature to external facilities with a hard copy of the Insect Biodiversity Datasheet.
2) E-mail a digital copy of the datasheet upon shipment.

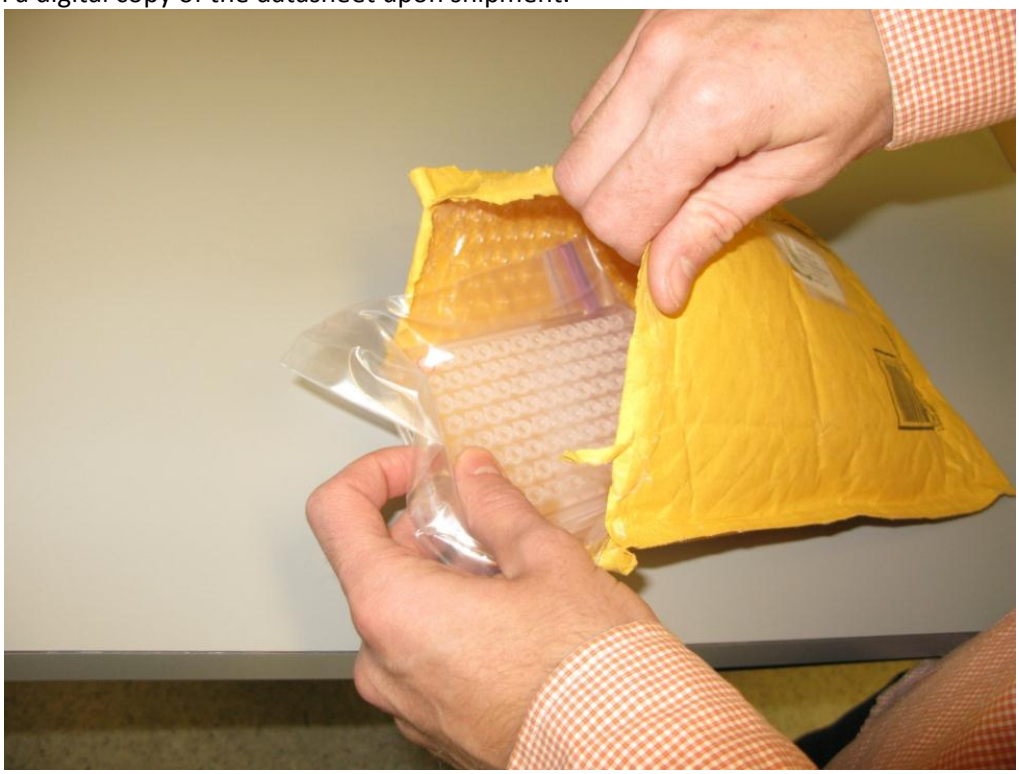

Figure 36. A 96-well microplate prepared for shipping.

14.1.1 Archives

Archives are voucher collections of a variety of materials that represent many different kinds of storage (e.g. dry in cabinets, -80 °C freezers, room temperature specimens in ethanol). Each of these differing kinds of facilities should receive a hard and digital copy of the Insect Biodiversity Datasheet.

1) Ship prepared dry material in cardboard mailers packed in outer boxes with ample packing material to ensure that the specimens are not jostled.
2) Label outer boxes clearly with 'Fragile' and 'Dead Insects for Scientific Study'.
3) Keep at least three specimens of each species collected in a domain in the teaching collection at that domain. All other specimens should be shipped to the Archive. For more details about the teaching collection, see the Common Insect Lab Protocols RD [06].

4) Mail vials of ground beetles (those on the common site-specific list, to reduce pinning) and bycatch in ethanol to archive facilities. Shipping samples in ethanol (e.g., pitfall bycatch) may require additional permits and shipping labels.

10.6 Data Handling

All information from field and lab datasheets must be entered into the digital datasheets as soon as possible after data collection. Data collected on paper datasheets must be transcribed into the NEON database. Scan and save paper datasheets for reference.

All data from the image processing must also be uploaded to the NEON server daily.

Ensure that all metadata from the paper datasheets are entered into the digital datasheets. 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 analyses.

10.7 Equipment Maintenance, Cleaning, and Storage

1) Empty, wash out, and dry waste receptacles (e.g. ethanol, PG).
2) Return all flammables to the appropriate cabinets.
3) Pack equipment and consumables for subsequent field work and store neatly.

Appendix A Field and Lab Datasheet Information

Field	Description
domain	number of the NEON domain
locality	full site name as specified in site_ids
collector	full names of personnel that conducted the ground beetle sampling
plotID	plot ID (4-character site code _XXX)
trapID	pitfall trap number
eventDate	date that the sample was collected (YYYYMMDD)
sample_collected	indication of whether the sample was collected, Yes or No?
lid_disturbed	whether the lid on the pitfall trap was disturbed during the sampling bout, Yes or No
cup_disturbed	whether the cup of the pitfall trap was disturbed during the sampling bout, Yes or No
fluid_level	an indication of the status of the fluid level in the trap when it was collected, circle Hi (above line) , Low (below line) or Ok
trap_reset	was the trap reset? Yes or no
etOH_change_date	date (YYYYMMDD)that the ethanol rinse was conducted
etOH_change_time	time (24 h format, HHMM) the ethanol rinse was conducted
sorter_name	full name of the technician who sorted the sample
sorting_date	date (YYYYMMDD) the sample was sorted
remarks	notes written by field technicians about sampling
facility_name	the name of the facility where taxonomy was performed
sample_rcvd_date	date (YYYYMMDD) the facility received the pinned beetle samples sent from a NEON domain lab
catalogID	the label on the specimen pin applied at the NEON domain lab. This is a linking variable that can be used to fill in the domain, site, plot, and sampling date
scientificName	name of a ground beetle species identified by taxonomist and noted on a determination label
tax_send_date	the date (YYYYMMDD) identified pinned specimens were mailed
sent_to	location to which identified pinned specimens were sent out
rcvd_tax_date	date (YYYYMMDD) samples were returned to the domain lab from taxonomists
location_rcvd_tax	the taxonomist/institution that identified and returned the samples
catalogID	the label on the pin applied at the NEON domain lab. This is a linking variable that can be used to fill in the domain, site, plot, and sampling date
scientificName	species ID provided by the taxonomist
barcoded	was the individual selected for barcoding?

Appendix B Ground Beetle Field Datasheet

NEON Ground Beetle Pitfall Sampling ***Page __ of __***

Locality: __________ Collector: __________

								Ethanol change date/time		Lab sorting	
Plot ID	Trap Number	Sample collected?	eventDate (YYYY/MM/DD)	Lid disturbed?	Cup disturbed?	Fluid level	Trap reset?	eventDate (YYYY/MM/DD)	eventTime (24 HR)	sorter name	sorting date (YYYY/MM/DD)
	1	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	2	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	3	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	4	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
Remarks*											
	1	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	2	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	3	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	4	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
Remarks*											
	1	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	2	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	3	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	4	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
Remarks*											
	1	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	2	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	3	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	4	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
Remarks*											
	1	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	2	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	3	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	4	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
Remarks*											
	1	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	2	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	3	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	4	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
Remarks*											
	1	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	2	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	3	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	4	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
Remarks*											
	1	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	2	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	3	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	4	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
Remarks*											
	1	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	2	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	3	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	4	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
Remarks*											
	1	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	2	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	3	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
	4	Y / N		Y / N	Y / N	Hi/Low/Ok	Y / N				
Remarks*											

* Unless remarks specify a particular trap, copy note for all traps within a plot on the digital datasheet