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

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1 DESCRIPTION

1.1 Purpose

The primary purpose of this document is to provide change controlled version of Observatory protocols and procedures for Plot Establishment. This document provides the content for training and field-based materials for NEON staff and contractors. Content changes (i.e. changes in particular tasks or safety practices) occur via this change controlled document, not through field manuals or training materials.

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

1.2 Scope

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

- General safety practices
- Site-specific safety practices
- General equipment maintenance

It does identify procedure-specific safety hazards and associated 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.000909	TOS Science Design for Ground Beetle Abundance and Diversity
AD [07]	NEON.DOC.001100	TOS Protocol and Procedure: Ground Beetle and Mosquito Specimen Processing
AD [08]	NEON.DOC.005003	NEON Level 0 Data Products Catalog
AD [09]	NEON.DOC.001580	Datasheets for TOS Protocol and Procedure: Ground Beetle Sampling
AD [10]	NEON.DOC.001271	TOS Protocol for Manual Data Entry
AD [11]	NEON.DOC.001400	NEON Raw Data Ingest Workbook for TOS Ground Beetle Abundance and Diversity

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.014051	Field Audit Plan
RD [04]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan

2.3 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

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. Relatively common in many habitats, ground beetles form well-defined gradients in species richness across North America and have been used as indicators of environmental change because 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 (AD[06]).

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 [04]).

4 PROTOCOL

Ground beetle sampling involves preparation for sampling (SOP A: Preparing for Sampling), collection of ground beetles using pitfall traps (SOP B: Field Sampling), processing samples in the lab (SOP C: Laboratory Processing and Analyses) to identify and record individuals by species, sending a subset to

external facilities for identification and DNA barcode analysis (SOP D: Sample Shipment) and data handling (SOP E: Data Entry and Verification).

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. A cover protects the bowls from weather (e.g., dilution from rain, drying from sun).

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 on the 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 labeled “B” for beetles and are shown farther inside the plot for figure clarity. Plots for pitfall sampling will be identified prior to the field season.

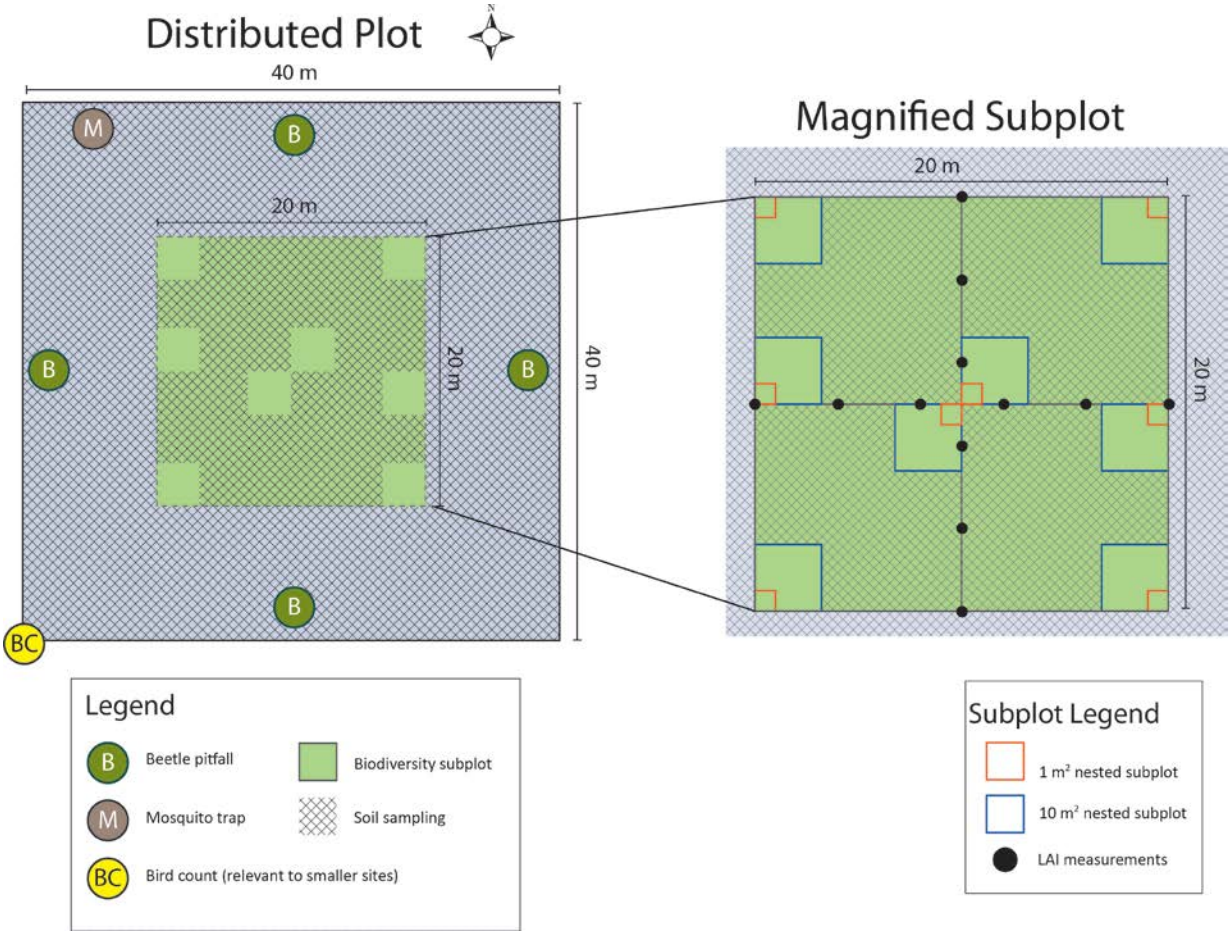

Figure 1. Standard plot layouts

5 QUALITY ASSURANCE AND CONTROL

The procedures associated with this protocol will be audited according to the Field Audit Plan (RD[03]). Additional quality assurance will be performed on data collected via these procedures according to the Data and Data Product Quality Assurance and Control Plan (RD[04]).

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[03]. 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.

When unexpected field conditions require deviations from the field protocols outlined in this document, contingent decisions outlined in table should be followed in the interest of maintaining data quality. Table 1 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 pitfall sampling

Sampling Delay	Required Action	Adverse Outcome for Data Quality?	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 weeks	Collect traps asap; note duration & cause; resume standardized sampling (2 weeks)	YES. Data not comparable to standard collection events; integrity of specimen DNA suspect	May not be readily able to obtain DNA barcodes; affects Abundance & Diversity measurements

5.1 Pitfall Fluid Shortage

Comparability between samples relies on sufficient preserving solution in each pitfall cup. Bring more preserving fluid out to the field than you anticipate using. 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, you may reuse recently collected PG to reset traps (in cases of need, note on field datasheet if this measure is taken). If the trap dries out and specimens are not preserved, the entire sample is compromised.

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 (AD[03]).

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 motor skill for handling individual specimens and pinning/pointing.

8 TRAINING REQUIREMENTS

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

Training for field techs will include attempted analysis of datasheets at 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 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).

9 SAMPLE FREQUENCY AND TIMING

9.1 Sampling Frequency and Timing

Pitfall traps will be checked, emptied, and reset every 14 days.

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	16	38	24	15-Apr	17-Sep
3,4,7,9,11,14,17,20	16	38	25	15-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 low temperature being > 4 °C (as determined by domain staff), 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. 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 consistently every 2

weeks for the entire field season. Sampling is estimated to require 2 technicians, at 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.

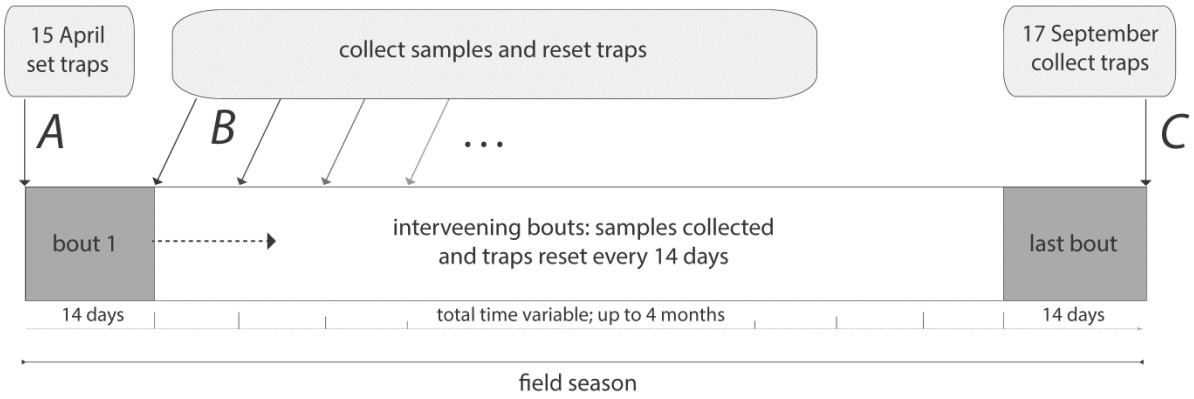

Figure 2. Schedule for pitfall trap deployment and servicing during a sampling season. Trapping occurs continuously throughout the growing season and traps are checked every 14 days. The length of the growing season varies among sites, but will begin no earlier than 15 April and end no later than 17 September.

9.4 Sampling Timing Parameters

Sampling will occur on the same day of the week, every 2 weeks, within 12 hours of the previous time sampled.

10 STANDARD OPERATING PROCEDURES

SOP A: Preparing for Sampling

Sample labels required for sampling

Four different types of labels are required and described 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 Lab Protocol for Beetles and Mosquitoes (AD[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 Lab Protocol for Beetles and Mosquitoes (AD[07]). Vials that contain multiple members of a single species (e.g., common species with enough representatives pinned so that additional individuals can be stored in vials) also require a determination label.
3. INDIVIDUAL ID LABELS: For sample tracking purposes every identified individual requires an individual ID label. These are labels that go on pinned/pointed specimens that are used to label and track specimens from a given sample plot from a given sampling period. Instructions for generating individual ID labels are provided in the Lab Protocol for Beetles and Mosquitoes (AD[07]).
4. MICROPLATE LABELS: 96 well microplates will have designated numbers on labels when they arrive from BOLD.

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 in (AD[09]).
4. Prepare locality labels (Instructions for generating and cutting these labels are described in the Lab Protocol for Beetles and Mosquitoes (AD[07]).
5. Fully charge all electronic equipment (e.g., PDA, GPS unit).

Preparations for the first sampling period:

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. Locate other trap materials including 1.5 cm PVC sections to hold up cover and filter squares (10 cm) of mesh organdy
3. Review/prepare your -specific checklist of materials needed for pitfall sampling. Ahead of going into the field:
    a. 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).
    b. Prepare one liter bottles of propylene glycol:water solution (500 mL PG : 500 mL water). A minimum of 6 one liter bottles of this solution is required to fill 40 pitfall traps with 150 mL of preserving fluid.
    c. In fume hood, prepare 95% ethanol for the field by pouring it into 1 L wide mouth Nalgene bottles.

Field Equipment and Materials

Table 3: Equipment list, materials and supplies required for the beetle sampling procedure at one site for one sampling day.

Maximo Item No.	Item Description	Purpose	Quantity	Habitat-Specific	Special Handling
Required	Pitfall trap locality labels	Labeling samples	3 per trap; 3*40=120	N	N
Required	Reusable ice packs	Keeping samples cool	2	N	N
Required	Chests coolers	Keeping samples cool	1	N	N
Required	Trowel or soil knife	Digging pitfall holes	2	N	N
Required	Permanent marker (fine black sharpie or equivalent)	Labeling	4	N	N
MX103582	Pitfall trap bowls (16 oz deli containers)	Trapping beetles	80 (in ground)	N	N
MX103484	Pitfall covers (Lexan squares)	Covering traps	40 (in ground)	N	N
Required	Plastic nails for pitfall covers (spikes 6-8 inches long, ¾ inch diameter)	Holding covers	200	N	N
Required	Propylene glycol (PG)	Preserving	75 mL per trap	N	N

Maximo Item No.	Item Description	Purpose	Quantity	Habitat-Specific	Special Handling
		samples			
Required	water	Diluting PG	75 mL per trap	N	N
Required	Ethanol 95%	Preserving samples	2L	N	Y
Required	Archival, ethanol-safe pens (Pigma brand, size 01)	Labeling	4	N	N
Required	Scissors	Cutting	2	N	N
Required	Jewelers forceps, fine point	Handling insects	2	N	N
Required	Whirl-Pak bags, 13 oz	Holding samples	40	N	N
Required	300 µm filter mesh organdy pre-cut (10 cm square)	Filtering pitfall samples	40	N	N
Required	Hard-bottomed container of appx. 10 cm width (e.g., Tupperware)	Holding samples	10	N	N
Required	Modified wide mouth Nalgene bottles (bottom portion of bottle cut off)	Filtering pitfall samples	3	N	N
Required	Modified wide mouth Nalgene lids as filter tops (top cut out of lid, filter screws onto bottle	Filtering pitfall samples	3	N	N
Required	Ethanol squeeze bottle	Dispensing ethanol	2	N	N
Required	Water squeeze bottle	Dispensing water	2	N	N
Required	Carry-all craft tote bag or other field pack	Carrying gear	2	N	N
Required	Specimen cups	temporary pitfall waste storage; temporary ethanol storage during transfer from Whirl-Pak to petri dish	3	N	N
Required	1-gallon reclosable freezer bags	Sample storage	12	N	N

Maximo Item No.	Item Description	Purpose	Quantity	Habitat-Specific	Special Handling
Required	Ethanol waste wide mouth bottle (1000 mL Nalgene)	Waste storage	2	N	N
Required	PG waste wide mouth bottle (4000 mL jug)	Waste storage	2	N	N
Optional	Nitrile gloves	Protecting hands	1 pair	N	N
Optional	Handwipes	Cleaning		N	N
Required	1000 mL wide mouth Nalgene bottles filled with ethanol	Ethanol storage	2	N	N
Required	1000 mL wide mouth Nalgene bottle filled with water	Water storage	3	N	N
Required	1000 mL wide mouth Nalgene bottle filled with PG	PG storage	3	N	N
Optional	Duct tape	As needed	1 roll	N	N
Optional	Flagging	As needed	1 roll	N	N
Required	First-aid kit	As needed	1	N	N
Optional	Camera	As needed	1	N	N
Required	GPS/Trimble	As needed	1	N	N
	PDA (not applicable in 2014)	Recording data	1	N	N
Required	Datasheets	Recording data	10	N	N
Required	Transfer pipette	Removing ethanol from petri dish and Whirl-Pak during transfer	1	N	N

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

Initial Deployment of Pitfall Traps

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

Initial trap deployment in field:

1. Locate the first plot where sampling is to occur.
2. 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.
3. Dig a small hole with a soil knife to accommodate the pitfall bowl (**Figure 3**). 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 3. Digging a hole for the pitfall bowl.

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.
5. 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 and accumulate in the top bowl only). Also ensure that there is no gap between the bowls and the ground. The

cups should fit snugly into the hole. The top bowl is the only part of the trap where specimens should be collected for the sample.

6. Use the pre-drawn line to fill the top bowl with 150 mL of PG:water solution. Initially fill all traps to equal volume. See contingency section (5) above if you have failed to bring an adequate amount of PG to fill pitfalls.
7. Position cover with four plastic nails and plastic spacers (**Figure 4-6**) so that it is visually level and 1.5 cm above the surface of the bowls (**Figure 6**). Optional step: label the top of the cover with N, S, E or W to indicate the trap location within the plot. This may facilitate matching locality labels with traps during subsequent visits. The purposes of the lid include: 1) preventing vertebrate bycatch while allowing ground beetles to enter, 2) shading the trap to lower fluid temperature, thus reducing evaporative loss and decomposition rate, and 3) preventing precipitation accumulation in the trap, thus reducing dilution of the preserving fluid.

Cover deployment will vary based on local topography and vegetation cover. Keep the purposes of the cover in mind while installing the cover.

Figure 4. 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 5: Installing pitfall lid with spacers. Note lower position of cup.

Figure 6. Fastening down pitfall cover. Note the spacer below the cover.

8. Moving clockwise, the second trap is 20 m east, the third is 20 m south, and the fourth is 20 m west 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).

SOP B: Field Sampling

Collecting Samples from the Trap

Upon arrival at the trap

1. Write the date and the trap ID (i.e., N, S, E or W)on three locality labels using an archival pen.
2. Place three copies of locality labels into the bowl (**Figure 7**; see Appendix D in the Lab Protocol for Beetles and Mosquitoes for instructions on generating these labels). 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.

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

Collecting Insects From Each Trap

Figure 8. A pitfall trap containing insect specimens.

1. Pick up the top bowl containing the sample.
2. 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.
3. In the unlikely event that you find live vertebrate bycatch in the trap, attempt to remove and release the animal.
4. Filter the trap contents using a 300 µm mesh filter and a modified Nalgene bottle with a screw cap lid (**Figures 9-12**). 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 water (**Figure 14**). Rinse above the (temporary) waste container so rinse water/PG is efficiently 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 15**).

Figure 15. Rinsing sample with 95% ethanol.

8. Place insects and filter in a Whirl-Pak bag **(Figure 16)** and cover with approximately 100 mL of 95% ethanol (**Figure 17**). 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. 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 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 20**). Store the Whirl-Pak samples in the shade (e.g., in a backpack) until you return to the field vehicle when samples are transferred into coolers.

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., date of collection, any issues with the trap or sample) in the PDA or paper datasheet (Ground Beetle Field Datasheet contained in (AD[09]). 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).

Resetting the Pitfall

1. Reset pitfall trap. 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.
2. Position cover with four plastic nails so that it is visually level and 1.5 cm above the surface of the bowls.

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.

Sample preservation

1. Place all 4 Whirl-Pak bags from a single plot are into a ziploc bag and label the outside of the bag with date and plot ID (includes site) before leaving the plot. You may choose to pre-label these bags in the lab.
2. Place bags into hard-bottom carrying device (e.g., an airtight plastic Tupperware container) ensuring that the Whirl-Pak bag openings are upward.
3. 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.
4. Change ethanol within 1 day of collection (see ethanol rinse in laboratory procedure).

Field Equipment and Materials

Please see Table 3 above for relevant equipment.

SOP C: Laboratory Processing and Analyses

Sample processing timing – ethanol rinse

The ethanol that each sample is stored in must be changed within 1 day after collection (ideally <24 hours). Vertebrate bycatch must be removed and reported no later than the ethanol rinse (details below). Ideally, invertebrate bycatch will also be removed during this rinse (if time allows). Record the presence of vertebrate and invertebrate bycatch and the date of the ethanol change on the paper field datasheet. These data should also be transcribed onto the field data spreadsheet, found in (AD[11]).

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 to FSU scientists at headquarters must occur within four months of the end of the field season. The specimen identifications must be verified and domain voucher collection updated before the beginning of the next field season. See Appendix E for taxonomic identification aids and the Lab Protocol for Beetles and Mosquitoes (AD[07]) for additional information regarding DNA barcode submission, the identification verification process and voucher collection maintenance.

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. [Recommended but optional step] Put on nitrile gloves.
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 with ethanol and inspect the filter cup to ensure none of the sample is left on the mesh. Filter cups in the lab can be reused.

Figure 22. Filtering diluted ethanol off sample into a specimen cup (a temporary waste container).

3. Transfer waste ethanol into ethanol waste containers either by filtering directly over waste containers or using specimen cups as temporary waste containers.

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

4. Remove all vertebrate bycatch and record associated data on the vertebrate bycatch datasheet (AD [09]). Store vertebrates separately (see below for more details).
5. If sorting is concurrent with the ethanol rinse, skip down to the sorting section below. Otherwise, the following steps include all invertebrates captured in the pitfall trap.
6. Transfer invertebrates 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.

7. 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.
8. Confirm that the appropriate labels are still in the Whirl-Pak bag.
9. Follow the above steps for each trap, one at a time. This ensures that specimens from different traps are not mixed.
10. Keep the 4 Whirl-Pak bags from 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 resealable bag and seal tightly until further processing (sorting, pinning, etc., detailed in the following steps).

Figure 24. Samples from one sampling bout stored in a sealed container. Label containers using lab tape and permanent markers.

11. Store samples in the freezer (-20 °C optimal) or refrigerator (4 °C) if freezer space is limited.

Vertebrate Bycatch

After removing vertebrate bycatch during the ethanol rinse, record the number of individuals and their identity to the finest taxonomic level possible (preferably species) on the vertebrate bycatch spreadsheet (AD [09]). Local and/or state permits should be consulted for reporting requirements of vertebrate bycatch. . Store vertebrate bycatch in 95% ethanol (refrigerator recommended) in separate containers from invertebrate bycatch. Include locality and determination labels in the containers and label the exterior of the vials with the collection code plotID.collectEventDate.taxonIDBycatch. If more than one of the same species is collected at the same site and day then a consecutive number can be added to the code (e.g., .1, .2, etc.). These vertebrate bycatch specimens will be deposited at the same archive facility as the non-vertebrate bycatch unless specified otherwise by the permitting agency.

Preparation for Sorting and Pinning

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.

Sorting Ground Beetles from Bycatch

Sorting, separating ground beetles from other invertebrates in the pitfall sample, 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. Record the sorter's initials on the paper field datasheet to indicate that the sample has been sorted. The sorter's name also appears on the digital Beetle Sorting spreadsheet (called identifiedByPara). Record additional data collected during sorting in the Beetle Sorting spreadsheet.

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. Jostle the bag and use squirt bottle to add ethanol if necessary.
3. Pour them into a large Petri dish marked with a grid. Remove or add ethanol as needed using a transfer pipette and a secondary container for used ethanol (removal) or squirt bottle of ethanol (addition).
4. Inspect the filter for any small invertebrates that may be attached to it and remove the filter from the sample.

Figure 25. Preparing to sort a sample. Note: technicians will be pouring ethanol and samples from a Whirl-Pak bag and not from a jar.

5. Sort beetle samples to select all adephagan beetles using a dissecting microscope.

   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 easily 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.

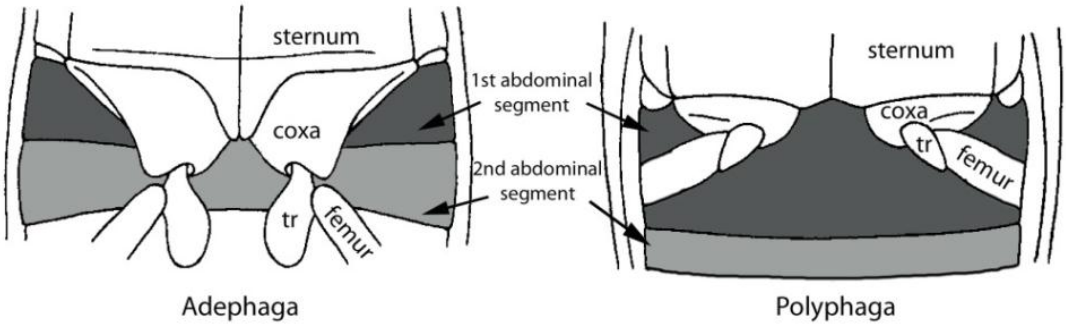

Figure 26. Hind leg articulation in Adephaga and Polyphaga.

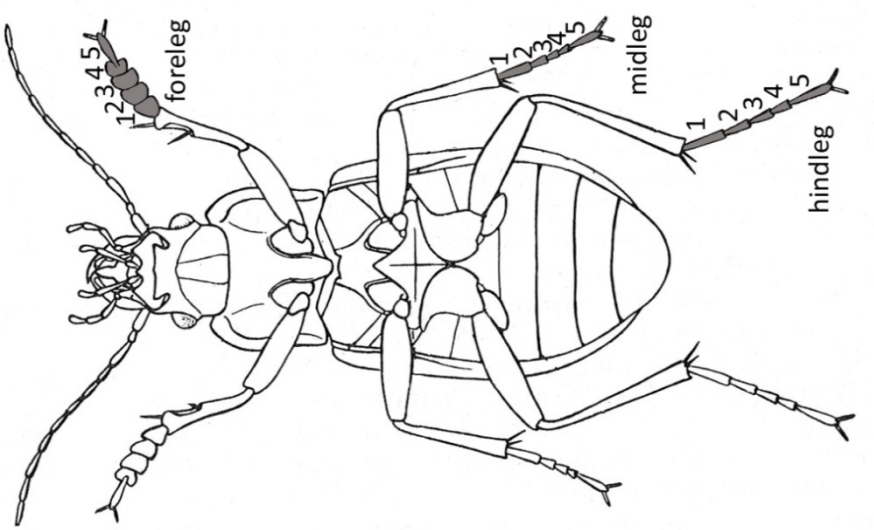

Figure 27. Tarsal formula of adephagan beetles.

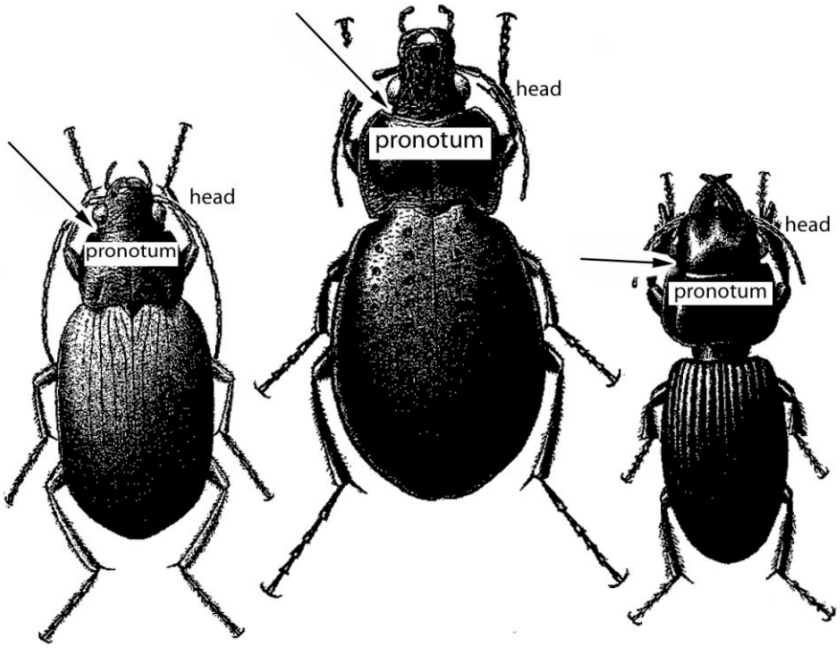

Figure 28. Three ground beetle specimens with head and pronotum labeled.

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 centrifuge tubes are required to hold the sample, add at least 1 locality label to each tube.

Processing Bycatch Samples in Ethanol

1. After removing the ground beetles from each sample, transfer invertebrate bycatch into 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. 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).
2. Pool the bycatch from all 4 pitfall traps at a plot together into a single vial or multiple vials if necessary.
3. Vertebrates should already have been removed but if any additional vertebrate bycatch is encountered at this stage, it should be identified and stored separately.

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.

4. Insert three individually cut locality labels (date filled in) into each bycatch 50 mL centrifuge tube.
5. Label the tube with an adhesive label or permanent marker with the collection code (plotID.collectEventDate).
6. 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.

Additional Sorting Instructions for Common Reliably Identified Ground Beetles

Many beetles will be pinned/pointed, however in the interest of time and storage efficiency, not all specimens will be pinned/pointed. For a select group of common and easily identified ground beetles, a maximum of 20 individuals per species will be (pinned/pointed) from each site per year. These will be selected at the end of the sorting process. The identity of abundant and easily distinguishable beetles will vary among sites and sites-specific lists are provided (Appendix D).

1. For those species listed as common and reliably identified, 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).
2. In addition, insert a single determination label into the tube (would normally be mounted on the pin with each beetle)

3. Also label the outside of the tube follows: plotID.collectEventDate.taxonID (e.g., CPER.002.20130811.paselo). Printing onto adhesive-backed labels is recommended, but vials can also be written on using permanent markers.
4. Shut the lid on the tube tightly so no ethanol leaks out (can result in smeared labels).
5. Record the data in the Beetle Sorting spreadsheet.
6. Up to 20 individuals per species will be selected for pinning/pointing (see section below).

Pinning and Pointing Large and Small Ground Beetles

Pinning/pointing involves mounting specimens either directly on a pin or on a small paper triangle on a pin. Detailed instructions are provided in in the Lab Protocol for Beetles and Mosquitoes (AD[07]). Note that all ground beetles not listed on the Common Reliably Identified Ground Beetle Species List (Appendix D) will be pinned or pointed (depending on size). For those that are listed, select 20 individuals per site per year of each common reliably identified species (on the list) for pinning/pointing. Select these individuals to represent different plots, sampling bouts and morphological variation.

During pinning/pointing, create locality labels and mount them on the pin below each specimen. After all specimens have been pinned/pointed and grouped into species/morphospecies, create and attach determination labels and IndividualID labels for every specimen and fill out the Domain Lab ID and Pinning datasheet (found in AD[11]). Most of the data for the datasheet can be found on specimen labels.

Ground beetles are mounted on pins with locality labels prior to identification by technicians (to morphospecies or species determinations based on the teaching collection). As an alternative to pinning all the beetles from a single site before any identification, it is acceptable to process samples in batches (e.g., 50-100 individuals). In this case, first sort ground beetles from bycatch for a group of samples, then pin/point that group of samples. However, to avoid confusion and improve data quality all traps from a single plot should be sorted and pinned together and all traps from a single site should be sorted and pinned before moving on to another site within each domain.

Identify Beetles to Species or Morphospecies

Identification of ground beetles generally occurs after all of the ground beetles collected at a site during the season have been pinned/pointed (except those most common and reliably identified beetles on the site-specific lists).). Various tools are available for aiding species identifications (Appendix E).

If a species designation cannot be made based on the teaching collection or other identification resources, assign a morphospecies name to that type of ground beetle. A morphospecies is a temporary designation for an individual or group of beetles that are all of the same group (ideally that group is a species, but all that is designated by a morphospecies is that all these individuals look to be the same). As a general rule, split groups that look similar but not identical into different morphospecies. It is easy to lump them together later, but difficult to later split them into multiple species. Ground beetle

morphospecies should be prioritized for identification by taxonomic experts and/or DNA barcoding so that species designations can be assigned to them in the teaching collection at the domain lab. Morphospecies are designated with a domain number and a unique letter. For example, "D15morphA" would be the first morphospecies from domain 15. Each morphospecies is recorded and described on the morphospecies datasheet along with the year and name of the person who designated it. If, a taxonomist is able to identify the specie s of some individuals of a morphospecies, the identity of all the other individuals of that morphospecies should be updated.

DNA barcoding

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. A detailed description of insect DNA barcoding protocols are provided in the Lab Protocol for Beetles and Mosquitoes (AD[07]).

Photographing

Similar to pointing, all ground beetles that are submitted for DNA barcoding must be photographed. Detailed instructions for photographing pinned/pointed insects are provided in the Lab Protocol for Beetles and Mosquitoes (AD[07]) and will not be repeated here.

Sample preservation

1. Prepared, 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. The fumigant box must be firmly secured in the corner of the Schmitt box or unit tray with pins.

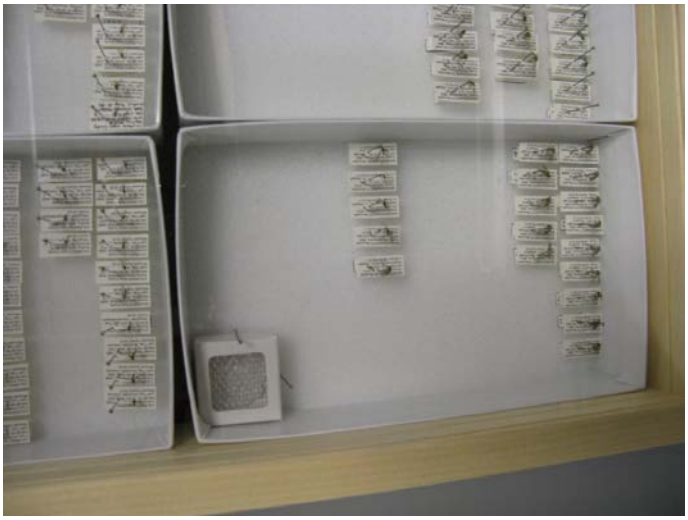

Figure 31. Fumigant boxes should be firmly secured with pins.

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. Label ethanol preserved specimens in 50 mL tubes (collection code and date) and in cool storage.
4. Store pinned material in the appropriate Schmitt box or Cornell cabinet. If there are not enough unit trays that contain specimens to fill a Cornell drawer, add empty unit tray/s so that filled trays are unable to slide around.
5. Keep the teaching collection inside a Cornell cabinet with a fumigant box of moth crystals anchored in the corner. Check and add more moth crystals as needed.
6. Pack equipment and consumables for subsequent field work and store neatly.

Laboratory Equipment and Materials

Table 4: Equipment list, Laboratory processing and analyses (quantity per sampling event)

Maximo Item No.	Item Description	Purpose	Quantity	Habitat-Specific	Special Handling
Required	Ethanol	Sample preservation		No	Yes
Required	Plastic petri dishes	sorting specimens under microscope	2	No	No
Required	Permanent markers	labeling	5	No	No
Required	Archival, ethanol-safe pens (Pigma brand, size 01)	Labeling	5	No	No
Required	50 mL non-sterile centrifuge tubes	Sample storage	Variable	No	No
Required	Specimen cups - bottoms	Temporary storage	5	No	No
Required	8 oz. plastic jars	Sample storage	50	No	No
Required	Specimen cups - lids	Temporary storage	5	No	No
Required	Jewelers forceps	Handling insects	3	No	No
Required	Microscope	Viewing insects	2	No	No
Required	Shipping supplies (boxes, padded envelopes)	Shipping	Variable	No	No
Required	Cardboard specimen mailers	Shipping	Variable	No	No

Maximo Item No.	Item Description	Purpose	Quantity	Habitat-Specific	Special Handling
Required	Scissors	cutting	2	No	No
Required	Secondary containment bins	holding liquids	1	No	No
Optional	Nitrile gloves	Protecting hands	Variable	No	No
Required	Waste drum (for ethanol and PG)	Waste disposal	1	No	No
Required	Tupperware	temporary storage of each bout of Whirl-Paks	10	No	No
Required	LED lights	Illuminating microscope	3	No	No
Optional	Adhesive-backed, paper	for labeling of sample vials		No	No

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

SOP D: Sample Shipment

Samples to be barcoded and/or identified by expert taxonomists are shipped to external facilities. Other samples will be shipped directly to archiving facilities.

Preparation

1. Gather shipping materials (cardboard mailers, cardboard boxes, packing tape, etc.).
2. If shipping samples in ethanol, you must have a Hazardous Materials certification and follow all applicable requirements and shipping laws.
3. If shipping to taxonomists, print completed Taxonomy Shipping datasheet (found in AD[11]) to include in shipment.

Taxonomists

The number of ground beetle specimens sent to taxonomists will vary based on the number collected and the annual budget (e.g., 350 per domain may be typical). At least one specimen from each species or morphospecies from each site (but ideally several individuals) are sent to taxonomists. Specimen identifications that are uncertain should be heavily favored for sending to taxonomists. Select these individuals to represent different plots, sampling bouts and morphological variation. Any species that are not present in the teaching collection or are represented by fewer than 10 individuals in the teaching collection should be sent to taxonomists. Each year, for each ground beetle species send up to 10 representatives, to taxonomists. In general, send the same specimens to the taxonomist as have been sent for DNA barcoding (this is especially important for new species). 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 for identification.

Use the Domain Lab ID and Pinning spreadsheet to create a “pick list” of specimens to be shipped to taxonomists. This can be done by sorting the sheet by scientificName and siteID.

1. Ship prepared dry (pinned/pointed) beetle specimens to Taxonomists with a hard copy of the Taxonomy Shipping datasheet (found in AD[11]). Email a digital copy of Taxonomy Shipping datasheet to the taxonomy lab.
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 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.

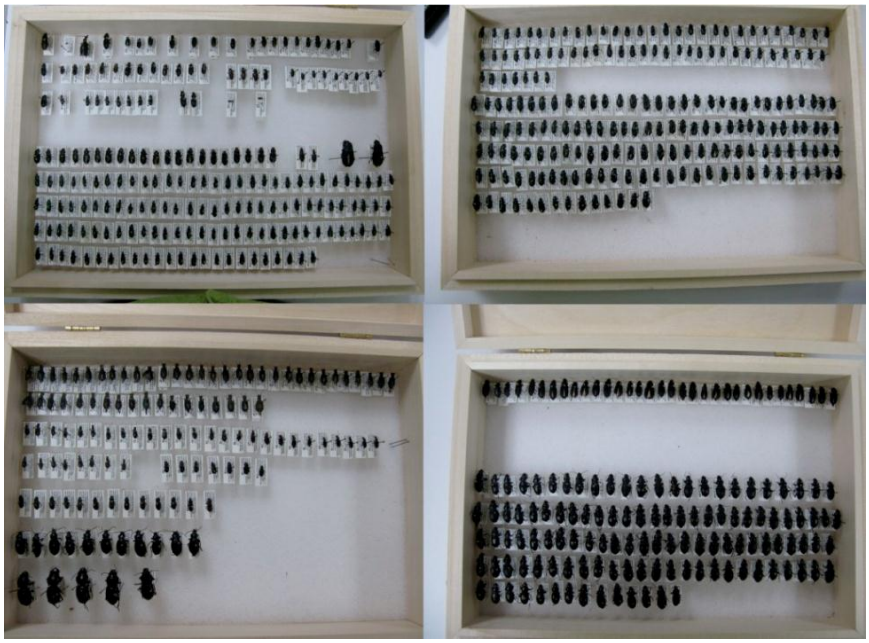

Figure 32. Specimens prepared to be sent to a taxonomist.

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

6. Examine specimens before sealing the box. Make sure all specimens are appropriately labeled. Ensure that all of the specimens are firmly glued to their points by tapping the box to test. 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. Outer boxes should be clearly labeled with 'Fragile' and 'Dead Insects for Scientific Study'.

Taxonomists will return the specimens with determination labels indicating their identification. These specimens will subsequently be used to update the domain lab teaching collection (described in the Lab Protocol for Beetles and Mosquitoes (AD[07]).

Genetic Analytical Laboratories

Information about shipping 96-well microplates containing tissue samples for DNA barcode analysis is provided in the Lab Protocol for Beetles and Mosquitoes (AD[07]).

Archives

Archives are voucher collections of a variety of materials that represent many different kinds of storage (e.g. dry in cabinets, -ultralow (-80 °C) freezers, room temperature specimens in ethanol). Each of these differing kinds of facilities should receive a digital copy of related datasheets (found in AD[11]).

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 Lab Protocol for Beetles and Mosquitoes (AD[07]).
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.

SOP E: Data Entry and Verification

Data Handling

All information from field and lab datasheets must be entered into the digital datasheets within 14 days as soon as possible after data collection (found in AD[11]). Data from paper datasheets should be transcribed into the NEON CI digital system. Follow QA/QC procedures for ensuring accurate transcription of data (AD[10]). Include notes on all deviations from procedures. Save paper datasheets for reference.

Ensure that all metadata from the paper datasheets are entered into the digital datasheets (found in AD[11]). 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.

Datasheets

Field datasheets

1. Upon return from the field, field data should be promptly and carefully transcribed into the Field Data spreadsheet (if PDAs are not available) 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[10]). 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 Field Data datasheet. Notes that apply to an entire plot should be copied to each row for that plot (one for each trap).
2. Some steps that take place after field collection (e.g., ethanol rinse, sorting) are also noted on the paper datasheet.
3. Scan datasheets and save in PDF file format.
4. Save paper copy of datasheets.

Morphospecies datasheet

1. Promptly and carefully transcribe data the digital datasheet.
2. Scan datasheets and save in PDF file format.
3. Save paper copy of datasheets.

Refer to the TOS Protocol for Manual Data Entry (AD[10]) for instructions regarding digital data entry.

Image Data

All data from the image processing must also be uploaded to the CI dropbox daily. *Note*: temporary image storage locations are currently being determined with NEON CYI and IT. Instructions on folder locations for image storage for 2014 will be provided in a JIRA ticket. Copy RAW images from the camera flash card to the NEON servers for storage and analysis.

a) Create the following folder and sub-folder, if they do not exist already: "Beetle_RAW_images → YYYY (where YYYY=the current year)

b) Instructions for creating a list of image names can be found in the Lab Protocol for Beetles and Mosquitoes (AD[07]).

After successfully copying all of the images to the NEON servers, image files on the camera flash memory may be erased if space on the memory card is limited.

11 REFERENCES

N/A

APPENDIX A QUICK REFERENCES FOR GROUND BEETLE SAMPLING

Quick Reference: Getting Ready for Field Sampling

STEP 1 – Upload waypoints onto GPS and get maps.

STEP 2 – Print locality labels.

STEP 3 – Prepare chemicals.

STEP 4 – Include extras of everything: pitfall trap parts, chemicals, and storage containers.

Properly installed pitfall trap has lip of *top bowl flush* with ground.

Improperly installed trap. Notice how the lip is above the ground surface.

Properly installed pitfall trap has:

- Lip of top bowl flush with ground.
- No gap between top and bottom bowl.
- No gap between bottom bowl and ground.
- Minimal disturbance of surrounding vegetation.
- Spacers that provide a 1.5 cm gap between trap cover and ground.

Adjust height of lid as needed to ensure that:

- Ground beetles can enter.
- Vertebrates are excluded.
- Precipitation cannot get into traps (and dilute PG solution).
- Lid provides shade, slowing evaporation and decomposition of captured specimens.

Quick Reference: Collecting Insects from Trap

STEP 1 – Write date and trap ID on three locality labels.

STEP 2 – Put three locality labels in top bowl.

STEP 3 – Remove top bowl.

STEP 4 – Filter contents over temporary waste container.

STEP 5 – Rinse sample through filter with distilled water.

STEP 6 – Rinse sample through filter with ethanol (95%).

STEP 7 – Transfer **ALL** organisms and locality labels to whirlpak bag.

STEP 8 – Rinse filter cup with ethanol over whirlpak bag to ensure no organisms are left behind.

STEP 9 – Place filter mesh into whirlpak bag (with organisms and locality labels).

STEP 10 – Record all metadata and any irregularities on data sheet.

Rinsing filter with ethanol over whirlpak to ensure no organisms are left behind.

Quick Reference: Processing Beetle Specimens

Ethanol Rinse (within 24 hours of sample collection)

STEP 1 – Filter contents of whirlpak bag (specimens, labels, and filter mesh) and discard waste ethanol.

STEP 2 – Remove vertebrate bycatch, record on vertebrate bycatch datasheet, and store in a separate vial with a locality and determination label.

STEP 3 – Pour contents (specimens, labels, and filter mesh) back into same whirlpak bag.

STEP 4 – Squirt filter device with 95% ethanol over whirlpak bag.

STEP 5 – Fill whirlpak with enough ethanol to completely cover all organisms.

STEP 6 – Seal bag, leaving as little air space as possible.

STEP 7 –Record date and time of ethanol rinse on data sheet.

STEP 8 – Put all bags from same sampling bout in airtight container.

STEP 9 – Store specimens in freezer (-20°) or refrigerator (4°C).

Sorting Ground Beetles from Bycatch

STEP1 – Pour contents of whirlpak bag into Petri dish with grid

STEP 2 – Check that no organisms are stuck in whirlpak bag or on filter.

STEP 3 – Place all ground beetles (and locality label) into temporary holding cup.

STEP 4 – Transfer ground beetles (and locality label) into 50 mL centrifuge tube(s) and cover with ethanol.

STEP 5 – Put bycatch (and locality labels) into 50 mL centrifuge tube(s) and cover with ethanol.

STEP 6 – Label centrifuge tube lids: Domain.Plot.Trap.Date and store in freezer (-20°C) or refrigerator (4°C).

APPENDIX B CHECKLISTS FOR GROUND BEETLE SAMPLING

Avoid looking like this guy.

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

Gather supplies and prepare chemicals at least one day before sampling bout.

Checklist: Preparing for Field Sampling

Locality labels: Be sure to...

- ☑ Print labels with correct month and locations.
- ☑ Cut labels into strips.

Equipment: Do you have...

- ☑ Trap replacement parts.
- ☑ Ice packs in the cooler.
- ☑ Extra solutions (PG and ethanol).
- ☑ Distilled water.
- ☑ Map and coordinates uploaded onto GPS.
- ☑ Ground Beetle Data Sheets.

Checklist: Collecting Quality Beetle Specimens

Before heading into the field: Make sure you have...

- ☑ All supplies (and extra)
- ☑ Replacement parts for traps
- ☑ Ground Beetle Field Data Sheets

Sample collection: Be sure to...

- ☑ Use temporary container to catch PG solution.
- ☑ Double check that your actual location matches the one on the locality label.
- ☑ Write collection date and trap ID (N,S,E,W) on locality label.
- ☑ Remove twigs, leaves, and debris from catch.
- ☑ Rinse sample; first with water and then with ethanol.
- ☑ Rinse filter device with ethanol to ensure all beetles and bycatch are transferred to whirlpak bag.
- ☑ Check whirlpak bag for locality labels and filter mesh.
- ☑ Record all metadata on data sheet (e.g., plot ID, date, field staff, etc.).
- ☑ Describe any irregularities or deviations from procedure that may impact data, e.g., trap damaged by bear, trap flooded with rainwater, or cover missing.

Before leaving trap: Check that...

- ☑ Top bowl is flush with ground.
- ☑ There is no gap between bottom and top bowls.
- ☑ Lid spacers are in place
- ☑ There is a 1.5 cm gap between lid and ground.

Transporting samples: Make sure...

- ☑ Whirlpak and ziploc bags are sealed and upright.
- ☑ Cooler is out of direct sunlight and away from extreme temperatures.
- ☑ Cooler is secured in vehicle so it does not tip over while driving.
- ☑ Promptly transfer specimens to laboratory upon returning from the field.

Checklist: Processing Specimens in the Lab

Ethanol Rinse: Check that…

- ☑ Ethanol rinse is completed within 1 day of sample collection.
- ☑ No insects were lost during filtering.
- ☑ Date and time of rinse is recorded on correct line of Ground Beetle Data Sheet.
- ☑ Locality labels are with specimens (in whirlpak bag).
- ☑ Specimens are stored in -20°C freezer or 4°C refrigerator.

Separating Ground Beetles from Bycatch: Be sure to…

- ☑ Only work with one whirlpak bag at a time.
- ☑ Examine whirlpak bags, filter mesh, and Petri dish under microscope for tiny organisms.
- ☑ Ask for second opinion, use reference collection, and refer to species/photo lists if you're unsure whether or not 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 data and trap number) as needed.
- ☑ Put a locality label (with collection date and trap number) in each centrifuge tube and/or jar.
- ☑ Completely cover organisms in centrifuge tube with ethanol and use multiple tubes as needed.
- ☑ Record sorter's name and date of sort on correct line of Ground Beetle Data Sheet.

APPENDIX C CHANGE NOTES

The following change shave been made between Rev C_DRAFT and Rev D of this protocol:

- Datasheets have been updated to match related ATBDs
- Recording the date of various protocol steps has changed slightly, when possible time has been removed
- Trap labeling within a plot has changed from numerals (1-4) to cardinal directions (N,S,E,W)
- Vertebrate bycatch instructions have been clarified in terms of sample processing, data and archiving

APPENDIX D COMMON RELIABLY IDENTIFIED GROUND BEETLE SPECIES

Note: Lists of common reliable identified ground beetle species will be available after the first year of sampling within each domain.

Domain 1

Table 5. D1 abundant, easily identifiable ground beetle species listed by site

Domain	Site	Species
1	HARV	*Carabus nemoralis nemoralis*

Domain 3

Table 6. D3 abundant, easily identifiable ground beetle species listed by site

Domain	Site	Species
3	OSBS	*Pasimachus sublaevis*
3	JERC	*Pasimachus sublaevis* *Tetragonoderus intersectus*
3	DSNY	*Pasimachus sublaevis* *Selenophorus fossulatus* *Tetragonoderus laevigatus*

Domain 10

Table 7. D10 abundant, easily identifiable ground beetle species listed by site

Domain	Site	Species
10	CPER	*Cicindela punctulata punctulata* *Cyclotrachelus torvus torvus* *Pasimachus elongatus*
10	STER	*Cicindela punctulata punctulata* *Cyclotrachelus torvus torvus* *Harpalus amputatus amputatus* *Harpalus pennsylvanicus* *Harpalus reversus* *Poecilus scitulus*
10	RMNP	*Carabus taedatus agassii*

APPENDIX E INSECT TAXONOMIC IDENTIFICATION AIDS

These instructions detail the various taxonomic identification aids available to domain technicians when identifying ground beetles. Here we describe how to use various aids and how to maintain and update them from year to year.

Most of the ground beetle and mosquito specimens collected by NEON at a domain are to be archived at external facilities; however, up to 10 individuals of each ground beetle species are stored at each domain support facility in order to aid technicians with species identifications. If a species is present at multiple sites, representatives from each site should be present in the collection. This permanent collection, referred to as the 'teaching collection', is collected during site characterization and maintained and updated throughout NEON operations. Each domain support facility will also be provided with a set of files that will serve as a local NEON field guide to species.

Each year, after samples have been barcoded, sequences validated, and identifications revised, technicians are responsible for updating their teaching collection and NEON field guide. Remaining specimens are shipped to an archive facility.

Use specimens that have been identified by taxonomists when selecting specimens to include in the teaching collection. Add specimens to the teaching collection such that each species is represented by up to 10 individuals. Select specimens to add to the teaching collection that represent the range of morphological variation present in the species.

If not all the members of a morphospecies group were sent to taxonomists, update the determination labels on individuals of a morphospecies that were not sent based on identifications by taxonomists. After adding updated determination labels and adding relevant specimens to the teaching collection, add any species that may be missing to local NEON field guide using photos from specimens added to the teaching collection. Add a description of morphological characters that will aid in identification where available from a taxonomic guide or internet source. Be sure to cite any references.

Keys

One or more of these keys may be useful aids in identifying beetles in a given domain.

General

- 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

Regional

- 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