TOS PROTOCOL AND PROCEDURE: GROUND BEETLE SAMPLING

PREPARED BY	ORGANIZATION	DATE
Katherine LeVan	FSU	04/08/2015
Natalie Robinson	FSU	12/05/2014
David Hoekman	FSU	12/13/2013
Kali Blevins	FSU	12/13/2013

APPROVALS	ORGANIZATION	APPROVAL DATE
Mike Stewart	PSE	04/21/2015
Andrea Thorpe	FSU	04/27/2015

RELEASED BY	ORGANIZATION	RELEASE DATE
Judy Salazar	CM	05/04/2015

See configuration management system for approval history.

© 2014 NEON Inc. All rights reserved.

The National Ecological Observatory Network is a project solely funded by the National Science Foundation and managed under cooperative agreement by NEON, Inc. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
-	04/26/2011	ECO-00160	Initial Draft Release
A_DRAFT	10/03/2011	ECO-00280	Update to new document numbers and template
B_DRAFT	06/25/2015	ECO-00443	Adjusted for known issues from 2011 prototype and revised for Domain 3 specific information
C_DRAFT	01/10/2014	ECO-01138	Thorough review with many small changes in terms of timing, sampling details, data handling/processing.
D	03/26/2014	ECO-01674	Production release, template change, and other changes as detailed in Appendix C
E	04/10/2014	ECO-01792	Updated Appendix D with site-specific information
F	12/05/2014		Migration to new protocol template Substantial revision, including: - Clarification to address JIRA tickets and provide more detailed instruction (throughout) - Moved information to appendices and supplementing the information (e.g., Appendix G) - Exported information to (e.g., shipping instructions), and imported information from (e.g., pinning/pointing instructions), related document RD[06] to ease the use of multiple protocols for technicians. - Added tolerances for placement of pitfall traps in plots - Added information for dealing with disturbed and/or empty traps - Added information on handling vertebrate bycatch and morphospecies
G	03/17/2015	ECO-02749	Re-imported shipping information in SOP I; added corresponding info and tables (e.g., table 9) throughout
H	05/04/2015	ECO-02890	Clarification that sampling will not exceed 4 months.

			Language requiring that gloves be worn by anyone touching vertebrate by-catch with their hands. Also, technicians performing cervical dislocation must have received prior training. Fixing an error in the figures in response to FOPS-2034.

TABLE OF CONTENTS

1 OVERVIEW 1

1.1 Background 1

1.2 Scope 1

1.2.1 NEON Science Requirements and Data Products 1

1.3 Acknowledgments 2

2 RELATED DOCUMENTS AND ACRONYMS 3

2.1 Applicable Documents 3

2.2 Reference Documents 3

2.3 Acronyms 3

2.4 Definitions 3

3 METHOD 4

4 SAMPLING SCHEDULE 6

4.1 Sampling Frequency and Timing 6

4.2 Criteria for Determining Onset and Cessation of Sampling 6

4.3 Timing for Laboratory Processing and Analysis 6

4.4 Sampling Timing Contingencies 7

5 SAFETY 8

6 PERSONNEL RESOURCES 9

6.1 Equipment 9

6.2 Training Requirements 22

6.3 Specialized Skills 22

6.4 Estimated Time 22

7 STANDARD OPERATING PROCEDURES 23

SOP A PREPARING FOR SAMPLING 23

SOP B INITIAL DEPLOYMENT OF PITFALL TRAPS 24

SOP C FIELD SAMPLING 28

SOP D LABORATORY PROCESSING – ETHANOL RINSE AND SORTING OF VERTEBRATE BYCATCH 36

SOP E LABORATORY PROCESSING – SORTING INVERTEBRATE BYCATCH 43

SOP F LABORATORY PROCESSING - SORTING GROUND BEETLES 49

SOP G LABORATORY PROCESSING – PINNING AND POINTING GROUND BEETLES AND SAMPLE PRESERVATION 55

SOP H DATA ENTRY AND VERIFICATION 69

SOP I SAMPLE SHIPMENT 71

8 REFERENCES 77

APPENDIX A DATASHEETS 78

APPENDIX B QUICK REFERENCES 79

APPENDIX C REMINDERS AND CHECKLISTS 82

APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING 85

APPENDIX E SITE SPECIFIC COMMON RELIABLY IDENTIFIED GROUND BEETLE SPECIES 86

APPENDIX F INSECT TAXONOMIC IDENTIFICATION AIDS 88

APPENDIX G GUIDE TO DISTINGUISHING THE FAMILY CARABIDAE 89

APPENDIX H INTERMEDIATE AND FINAL SAMPLE IDS PER SAMPLE TYPE 94

APPENDIX I LOCALITY LABELS 95

APPENDIX J DETERMINATION LABELS 106

APPENDIX K INDIVIDUALID LABELS 111

LIST OF TABLES AND FIGURES

Table 1. Contingent decisions 7
Table 2. Equipment list - Preparation 9
Table 3. Equipment list – Initial deployment of pitfall traps 10
Table 4. Equipment list – Sampling beetles at one site for one sampling day 11
Table 5. Equipment list – Laboratory sorting of vertebrate bycatch for one sampling event 14
Table 6. Equipment list – Laboratory sorting of bycatch and carabids 16
Table 7. Equipment list – Laboratory pinning and pointing of carabids 18
Table 8. Equipment list - Insect labels 20
Table 9. Equipment list - Shipping to taxonomic facility 21
Table 10. Summary of beetle mounting criteria 56
Table 11. Datasheets associated with this protocol. 78
Table 12. Estimated dates of historical temperature thresholds 85
Table 13. D01 abundant, easily identifiable ground beetle species. 86
Table 14. D02 abundant, easily identifiable ground beetle species 86
Table 15. D03 abundant, easily identifiable ground beetle species 86

Table 16. D10 abundant, easily identifiable ground beetle species 86
Table 17. Sample IDs for various types of samples generated during beetle sorting 94
Table 18. Examples of site name abbreviations for locality labels 96
Table 19. Number of labels needed for the difference activities requiring insect locality labels. 104

Figure 1. Standard beetle plot layout 5
Figure 2. Schedule for pitfall trap deployment and collection 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 (see APPENDIX D for domain-specific estimated dates for onset and cessation of sampling). 6
Figure 3. Digging a hole for the pitfall container. 24
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. 26
Figure 5. Installing pitfall lid with spacers. Note lower position of container. 26
Figure 6. Fastening down pitfall cover. Note the spacer below the cover. 27
Figure 7. Example label format 28
Figure 8. Adding locality labels to trap 29
Figure 9. 300 µm mesh filter fitted to the top of a Nalgene bottle 30
Figure 10. Preparing to filter out specimens from collecting fluid. 30
Figure 11. Filtering specimens from trap. The temporary waste container is below the filter (not visible). 31
Figure 12. Beetle specimens after collecting fluid has been poured off. 31
Figure 13. Rinsing sample with water. 32
Figure 14. Rinsing sample with 95% ethanol. 32
Figure 15. Placing sample in Whirl-Pak bag. 33
Figure 16. 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. 33
Figure 17. Checking filter for remaining insects. Place filter in bag with sample. 34
Figure 18. A packed sample. 34
Figure 19. Workflow of the ethanol rinse and vertebrate bycatch removal processing step. Activities are represented by blue boxes, decisions by grey triangles, and labels by yellow boxes 37
Figure 20. Filtering diluted ethanol off sample into a specimen cup (a temporary waste container). 38
Figure 21. Pouring sample through filter directly into larger waste container. 38
Figure 22. Samples from one sampling bout stored in a sealed container. Label containers using lab tape and permanent markers. 42
Figure 23. Workflow for processing invertebrate bycatch. Activities are represented by blue boxes, intermediate and final products by red boxes, decisions by grey triangles, and labels by yellow boxes. . 44
Figure 24. Bycatch samples stored in a 50 mL centrifuge tubes, the amount of ethanol in a bycatch sample will vary by sample size. 47

Figure 25. Workflow for processing carabid beetles. Activities are represented by blue boxes, intermediate and final products by red boxes, decisions by grey triangles, and labels by yellow boxes. . 49
Figure 26. Pinned (left) and pointed (right) beetle specimens 56
Figure 27. Materials for pointing including a schematic of a point showing where to place pin 57
Figure 28. A pinning block with a point and the various labels that will be affixed to a pinned or pointed specimen. This shows the heights at which points and various labels should be placed on a pin. 57
Figure 29. A ground beetle on its side in preparation for pointing 59
Figure 30. Examples of bending a point tip to maintain a level body position of the specimen 60
Figure 31. A well-pointed beetle specimen, with associated labels 60
Figure 32. A poorly pointed beetle specimen 61
Figure 33. Preparing to glue a specimen to a point by dipping the tip of the point in glue. 61
Figure 34. Diagram of ventral side of a beetle, with and X indicating where to place point 62
Figure 35. Attaching a specimen to a point, and pointed specimens left to set. 62
Figure 36. Attaching a locality label below a pointed beetle 63
Figure 37. Ground beetle pinning and dorsal side schematic showing where to place the pin (marked by an "X") 64
Figure 38. Correctly and incorrectly pinned beetle specimens 65
Figure 39. Attaching a locality label below a pinned ground beetle 65
Figure 40. A leg being inserted back into its socket, using forceps and a dab of glue 66
Figure 41. Proper orientation of mounted specimens in unit trays within a Cornell drawer. 67
Figure 42. Fumigant box filled with moth crystals and secured in the corner of a unit tray with pins. 67
Figure 43. Specimens prepared for shipping 73
Figure 44. Large beetle that is brace-pinned for shipment 73
Figure 45. Cardboard insert that serves as extra protection for pinned/pointed specimens, and helps fills dead space between specimens and mailer lid. 75
Figure 46. A mailer placed inside a larger box for shipping 75
Figure 47. Mailer within a shipping box, with adequate padding to keep mailer from moving in transit . 76
Figure 48. Example shipping manifest for the taxonomic ID facility 77
Figure 49. Hind leg articulation in Adephaga and Polyphaga. 89
Figure 50. Tarsal formula of adephagan beetles. 89
Figure 51. Four ground beetle specimens with head and pronotum labeled (first three) and showing the metallic coloration that is common in tiger beetles. 90
Figure 52. Lateral view of a beetle in the family Gyrinidae, showing dorsal and ventral portions of the compound eye. 90
Figure 53. Dorsal view of beetle in the family Gyrinidae showing mid and hind legs adapted for swimming 90
Figure 54. Dorsal view of a beetle in the family Dytiscidae, showing the oval shaped body and long hairs on the hind legs. 91

Figure 55. Diagram and photo of a haliplid beetle, showing the large coxal plates that are characteristic for this family. 91
Figure 56. Diagram and photo of a noterid beetle, showing the noterid plate between the second and third pairs of legs, on the ventral side. 92
Figure 57. Diagram and photo of a rhysodid beetle, showing the 'neck' where the head and pronotum meet, and the characteristically deeply grooved head, pronotum, and elytra. 93
Figure 58. Example label for pitfall trap sample. Size shown is larger than size of actual label 95
Figure 59. Example of spreadsheets containing information for locality labels, including the "plotID" field for locality labels that will be used in the field (A) and the "Day" and "Trap Code" fields for labels that will be used to pin/point/store insect specimens (B). 98
Figure 60. Dimensions for creating a locality label template in Microsoft Word 99
Figure 61. Selecting recipients from a Microsoft Excel file during Mail Merge 100
Figure 62. Example of a strip of labels, dashed lines indicate where the labels should be cut (when/if they are cut into individual labels) 104
Figure 63. Example determination and morphospecies ID labels; size shown is larger than size of actual labels. 106
Figure 64. Spreadsheet containing information for determination labels 107
Figure 65. Dimensions for creating a determination label template in Microsoft Word 108
Figure 66. Selecting recipients from a Microsoft Excel file during a Mail Merge 109
Figure 67. IndividualID label for a beetle specimen 111

1 OVERVIEW

1.1 Background

The purpose of the ground beetle abundance and diversity sampling design is to capture inter- and intra-annual variation of the ground beetle (*Coleoptera: Carabidae*) community in the NEON purview. Ground beetles were chosen as a focal terrestrial invertebrate taxon for several reasons, including that they: are easy to sample and well known taxonomically, are relatively common in many habitats, form well-defined species richness gradients across North America, and are useful as indicators of environmental change due to their sensitivity to habitat disturbance. In addition, ground beetles can strongly influence trophic structure, both because they are generally predacious (thereby influencing prey populations) and because they are consumed by other predators (thereby influencing predator populations). As such, changes in ground beetle populations may alter proportions of various other guilds in a community. A full justification for the inclusion of ground beetle sampling in the NEON framework is provided in the TOS Science Design for Ground Beetle Abundance and Diversity (AD[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 the traps. The animals that fall into the trap become preserved by a liquid mixture of DNA-safe preservative in the bottom of the trap. Animals that are collected in these traps but are not ground beetles are termed “bycatch.” In addition, this protocol describes laboratory processing and storage of the collected animals. Information regarding the shipment of specimens or samples to contracted experts for morphological identifications, and for Genetic Analytical Laboratories for DNA sequencing is provided in TOS Protocol and Procedure: Ground Beetle and Mosquito Specimen Processing (RD[06]).

1.2 Scope

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

1.2.1 NEON Science Requirements and Data Products

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

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

1.3 Acknowledgments

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

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

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

AD[01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.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.014051	Field Audit Plan
AD[08]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.001580	Datasheets for TOS Protocol and Procedure: Ground Beetle Sampling
RD[06]	NEON.DOC.001100	TOS Protocol and Procedure: Ground Beetle and Mosquito Specimen Processing
RD[07]	NEON.DOC.001400	NEON Raw Data Ingest Workbook for TOS Ground Beetle Abundance and Diversity
RD[08]	NEON.DOC.001953	Pitfall trap spacers assembly instructions
RD[09]	NEON.DOC.001818	Pitfall trap cover assembly instructions
RD[10]	NEON.DOC.001816	Filter cloth assembly instructions
RD[11]	NEON.DOC.001814	Filter cup assembly instructions

2.3 Acronyms

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

2.4 Definitions

None given.

3 METHOD

Ground beetle sampling, as detailed here, involves: preparation for sampling (**SOP A**); pitfall trap deployment (**SOP B**) and the collection of ground beetles (**SOP C**); processing samples in the lab to sort target taxa (ground beetles) from invertebrate and vertebrate bycatch (**SOP D - SOP F**) and then identify individuals to species and properly preserve specimens (**SOP G**); data entry and verification (**SOP H**); instructions for shipping specimens to a taxonomist (**SOP I**). Additional ground beetle-related data will be gathered when tissues from a subset of specimens are DNA barcoded , with details for these procedures provided in the TOS Protocol and Procedure: Ground Beetle and Mosquito Specimen Processing (RD[06]).

The pitfall trap design consists of two 16 oz deli containers (7 cm deep with an 11 cm diameter, 540 mL total volume) nestled within one another. One container ensures that the hole stays in the ground and enables efficient collection and resetting of the trap. This lower container has holes drilled into it so that any excess moisture can drain rather than build up and cause the top container to float. The second container has lines at the 150 and 250 mL marks and contains the preserving fluid. This top container is picked up and changed during sampling. A cover protects the container 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 are 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 with the cardinal direction of the trap location (e.g., 'N' for the north trap). Plots for pitfall sampling will be identified prior to the field season.

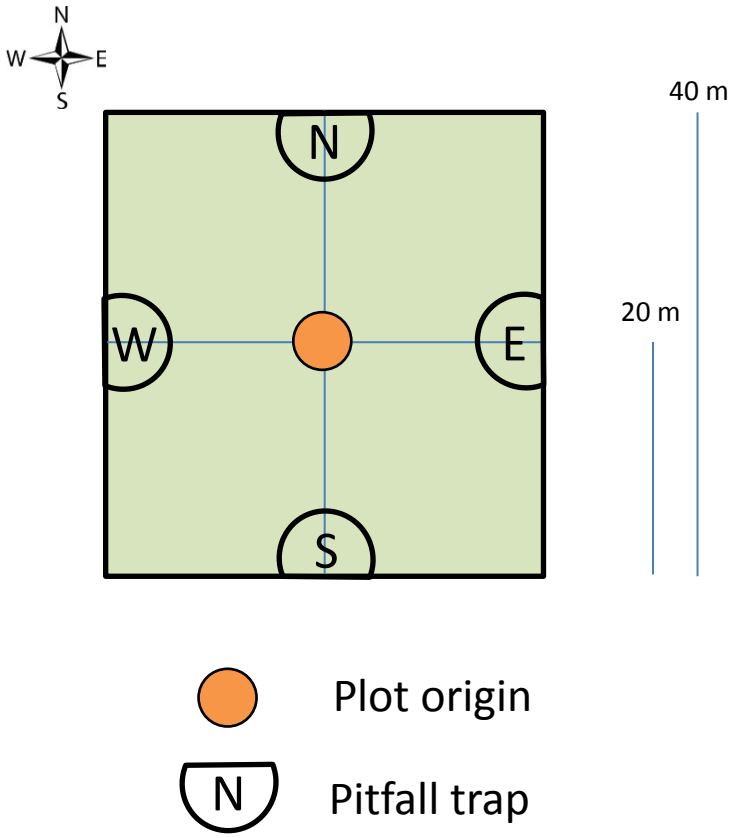

Figure 1. Standard beetle plot layout

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

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

The procedures described in this protocol will be audited according to the Field Audit Plan (AD[07]). 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 (AD[08]).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

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

4.2 Criteria for Determining Onset and Cessation of Sampling

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). Sampling should end when the 10-day running average low temperature is < 4 °C (see **Figure 2** for schematic of ground beetle sampling throughout a season). Estimated dates for onset and cessation of sampling are provided, per domain, in **APPENDIX D**.

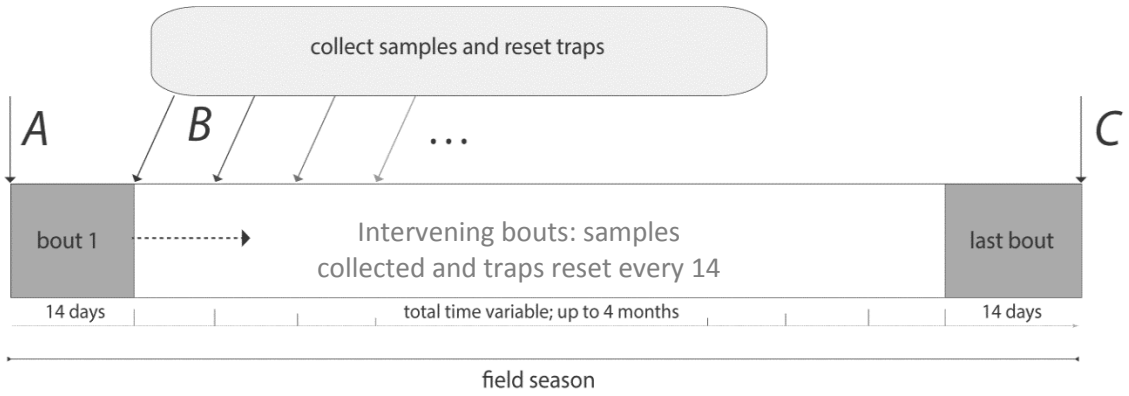

Figure 2. Schedule for pitfall trap deployment and collection during a sampling season. Trapping occurs continuously throughout the growing season and traps are checked every 14 days. The length of the growing season varies among sites (see APPENDIX D for domain-specific estimated dates for onset and cessation of sampling).

4.3 Timing for Laboratory Processing and Analysis

Following the collection of each pitfall trap, its contents must undergo an ethanol rinse (see **SOP D**) within 24 hours. Additional processing (e.g., sorting (**SOP D, SOP E, SOP F**), pinning and identifying (**SOP G**)) may be conducted at a later date, when there is sufficient time and personnel for such activities.

These activities may occur several months after sample collection, although all beetle processing must be completed within four months of the end of the field season.

4.4 Sampling Timing Contingencies

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

Table 1. Contingent decisions

Delay/Situation	Action	Outcome for Data Products
Collection delayed by 1 day[1]	Collect traps asap. Do not reset trap until all traps can be reset simultaneously (e.g., onset of *next* sampling bout); note duration & cause; resume standardized sampling (2 weeks)	Data not comparable to standard collection events. Cannot calculate diversity indices if trap collections are not comparable.
Collection delayed by 2 or more days	Collect traps asap. Do not reset trap until all traps can be reset simultaneously (e.g., onset of *next* sampling bout); note duration & cause; resume standardized sampling (2 weeks)	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

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

5 SAFETY

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

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

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

6 PERSONNEL RESOURCES

6.1 Equipment

The following equipment (**Table 2 - Table 9**) is needed to implement the procedures in this document. Equipment lists are organized by task. They do not include standard field and laboratory supplies such as charging stations, first aid kits, drying ovens, ultra-low refrigerators, etc.

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

Table 2. Equipment list - Preparation

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

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

R/S=Required/Suggested

Table 3. Equipment list – Initial deployment of pitfall traps

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items					
MX103582	R	Deli container, 16 oz.	Pitfall trap container	80	N
EB07710000	R	Lexan square with pre-drilled holes	Pitfall trap cover	40	N
MX100318	R	Measuring tape, 100 m	Locate trap installation points	1	N
MX104742	R	Laser Rangefinder, 0.5yd accuracy, 1500m range	Locate trap installation points	1	N
MX103881	R	Plastic spike	Secure pitfall trap covers	200	N
EG07890000	R	PVC, ½" schedule 40, 1.75 cm length	Pitfall trap spacers	160	N
	R	Trowel or soil knife	Dig holes for traps	2	N

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
MX103582	R	Deli container, 16 oz.	Pitfall trap container	80	N
Consumable items					
MX100585	R	Bottle with 50:50 PG:DI solution, 1 L wide-mouth HDPE	Preserve samples	6	N
MX103940	S	Flagging tape	Flag location of trap	1 roll	N
Resources					
RD[05]	R	Field datasheet	Record data		

R/S=Required/Suggested

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

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

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

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
	R	Forceps	Push filter into Whirlpak bag during rinse	1	N
Consumable items					
MX100585	R	Bottle with 50:50 PG:DI solution, 1 L wide-mouth HDPE	Preserve samples	6	N
MX100585	R	Bottle with deionized water, 1 L wide-mouth HDPE	Rinse samples	6	N
MX100665	R	Bottle with ethanol, 1 L wide-mouth HDPE	Rinse samples	2	N
EB07690000	R	Filter cloth	Rinse samples	40	N
MX103940	S	Flagging tape	Reflag location of trap	1 roll	N
MX104741	R	Jug with ethanol, 1 gal narrow mouth	Store/transport extra ethanol	2 L	N
	S	Nitrile gloves, powderless	Protect hands	1 pair	N
MX104422	R	Permanent marker, archival ethanol-safe	Record TrapID and date on locality labels	4	N
	R	Permanent marker, fine tip	Record plotID and date on resealable plastic bag	4	N

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
MX100592	R	Resealable plastic bag, 1 gal	Contain Whirl-Pak bags from each plot	12	N
MX100686	R	Whirl-Pak bags, 13 oz	Contain samples	40	N
Resources					
RD[05]	R	Field datasheet from trap deployment	Record data	10	N
	R	Locality label	Label samples; 3 per trap	120	N

R/S=Required/Suggested

Table 5. Equipment list – Laboratory sorting of vertebrate bycatch for one sampling event

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items					
EB07680000	R	Filter assembly	Rinse samples	1	N
MX104728	R	Specimen cup	Chemical waste containment during rinse	1	N

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Consumable items					
MX100213	R	Ethanol, 190 proof (95%)	Rinse samples	Variable	Y
MX108277	R	Falcon tubes, 50 mL	Contain samples	Variable	N
EB07690000	R	Filter cloth	Rinse samples	Variable	N
	S	Nitrile gloves, powderless	Protect hands	Variable	N
MX104422	R	Permanent marker, archival ethanol-safe	Record sample information on labels	1	N
MX100686	R	Whirl-Pak bags, 13 oz	Contain samples	Variable	N
Resources					
	R	Determination label	Label samples	Variable	N
RD[05]	R	Sorting datasheet	Record data	1	N

R/S=Required/Suggested

Table 6. Equipment list – Laboratory sorting of bycatch and carabids

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

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
MX100213	R	Ethanol, 190 proof (95%)	Preserve samples		Y
MX108277	R	Falcon tubes, 50 mL	Contain samples	Variable	N
	R	Jar, 16 oz. wide mouth jars	Contain samples	50	N
MX103249	R	Label paper, ethanol-safe	Create sampleID labels		N
	S	Nitrile gloves, powderless	Protect hands	Variable	N
	R	Permanent marker	Label sample containers	5	N
MX104422	R	Permanent marker, archival ethanol-safe	Record sampleID number on sampleID labels	5	N
MX103145	R	Transfer pipette	Remove ethanol from petri dish and Whirl-Pak during transfer	1	N
	R	Tube or container	Contain bycatch while sorting		N
	R	Tube or container	Contain carabids while sorting		N
Resources					
RD[05]	R	Morphospecies datasheet	Record data		

R/S=Required/Suggested

Table 7. Equipment list – Laboratory pinning and pointing of carabids

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items					
MX100270	R	Dual gooseneck light	Illuminate specimens under microscope	1	N
	R	Forceps	Manipulate insects	3	N
MX100720	R	Insect pinning block	Position point and labels on pin	1	N
	R	Microscope	Aid in pinning/pointing specimens	2	N
MX104422	R	Permanent marker, archival ethanol-safe	Record sample information on labels	5	N
MX102811	R	Point punch	Create points	1	N
Consumable items					
MX104417	S	Balsa wood	Hold specimens or pins/points and attach points/labels to pins	4	N
	S	Copy paper, white	Aid in pinning/pointing specimens under microscope	1	N
MX102810	R	Fumigant box	Contain moth crystals within Cornell cabinet	25	N
MX100682	R	Insect pin, No 3	Mount insects	10	N

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
MX100582	R	Moth crystals	Prevent dermestids	1 lb, ~6.5 g per box	Y
MX100595	R	Multi-purpose glue	Attach specimens to points	2	N
MX104421	R	Schmitt box	Store mounted specimens	Variable	N
MX104505	R	Strathmore paper	Paper for points	1 sheet	N
MX104981		Yellow cardstock	Print IndividualID labels		
Resources					
	R	Determination label	Label samples	1 per specimen	N
	R	Locality label	Label samples	1 per specimen	N
RD[05]	R	Pinning datasheet	Record data	1	N

R/S=Required/Suggested

Table 8. Equipment list - Insect labels

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

Table 9. Equipment list - Shipping to taxonomic facility

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

6.2 Training Requirements

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

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

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

6.3 Specialized Skills

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

6.4 Estimated Time

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

Sampling is estimated to require 2 technicians: 12 min per trap x 40 traps = 480 min = 4 hours per person each day of sampling; 30 min per plot x 10 plots = 300 min = 5 total hours each day of driving; and 15 min x 10 plots = 150 min = 2.5 total hours each day of hiking. Exact drive and hike times will vary by site, and should be considered when deciding whether 1 or 2 teams of 2 technicians are required for sampling.

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

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

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

A.2 Prior to a sampling bout:

1. Prepare bench and fume hood space in the lab for the preparation of field materials (e.g., ethanol, PG). Ensure that space in freezer, refrigerator or flammable materials cabinet is available for samples. Coordinate with potentially conflicting activities (e.g. soil sieving).
2. Assemble field equipment at least one day prior to field sampling.
3. Print blank datasheets (see Ground Beetle Field Datasheet in (RD[05]).
4. Prepare locality labels (see **APPENDIX I**).
5. Charge all electronic equipment (e.g., PDA, GPS unit).
6. Prepare one liter bottles of propylene glycol:deionized water solution (500 mL PG:500 mL water). A minimum of six 1 L bottles of this solution is required to fill 40 pitfall traps with 150 mL of preserving fluid. Be sure to prepare more of this solution than you think you will need in the field to ensure that there will still be enough to set or reset pitfall traps in the case of accidental spillages, etc.
7. In fume hood, prepare 95% ethanol for the field by pouring it into 1 gal narrow mouth and 1 L wide mouth bottles.
8. Prepare deionized water for the field by pouring it into 1 L wide mouth bottles.

SOP B Initial Deployment of Pitfall Traps

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

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

1. Locate the first plot where sampling is to occur.
2. Determine the first trap location by moving 20 m north of the center plot marker. Use a Rangefinder and measuring tape to verify that your position is correct, and orient yourself between the corner markers, in the middle of the northern edge of the plot. If there is a natural feature (e.g., a rock or stream) in the target trap location, move ±2m to the east, west, or south of the targeted location. *You may not place the trap outside of the plot boundaries, due to permitting constraints.*
3. Dig a small hole with a soil knife to accommodate the pitfall trap (**Figure 3**). Start small because it is much easier to enlarge the hole than fill in the edges to maintain a tight fit. Outline the container in the dirt and then dig just inside the line to ensure a snug fit. The exact tools most useful for this step will vary based on soil type, roots, rocks, etc.

Figure 3. Digging a hole for the pitfall container.

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 container (with holes) into the hole. Slide the top container into bottom container. **Ensure that the lip of the top container is flush with the ground** so no lip is sticking up above the ground surface (this ensures that insects fall into the trap rather than walk around it, and accumulate in the top container only). Also ensure that there is no gap between the containers and the ground. The containers should fit snugly into the hole.

6. Use the pre-drawn line to fill the top container with 150 mL of PG:water solution. See contingency section (**4.4**) above if you have failed to bring an adequate amount of PG to fill pitfalls. If it is not possible to fill all traps to the 150 mL volume (e.g., spilled in field), revisit traps with additional preserving fluid in the following days. If re-visitation is not possible, and the amount of PG was insufficient to preserve specimens captured during the bout, the data from this trap are lost for this sampling bout.

    a. Note - comparability between samples relies on sufficient preserving solution in each pitfall trap. Bring more preserving fluid out to the field than you anticipate using.

7. Position cover with four plastic spikes and PVC spacers (**Figure 4-Figure 5**) so that it is visually level and 1.5 cm above the surface of the containers (**Figure 6**).

    a. 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

8. Trap cover deployment will vary based on local topography and vegetation cover. Keep the following purposes of the cover in mind while installing the cover:
    a. preventing vertebrate bycatch while allowing ground beetles to enter,
    b. shading the trap to lower fluid temperature, thus reducing evaporative loss and decomposition rate, and
    c. preventing precipitation accumulation in the trap, thus reducing dilution of the preserving fluid.

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.

Figure 5. Installing pitfall lid with spacers. Note lower position of container.

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

d. 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).

e. For each trap, record the date in the setDate field of the PDA or on the field datasheet.

SOP C Field Sampling

C.1 Collecting samples from the trap

Upon arrival at the trap:

1. Record data on the same datasheet as used when setting the traps (see Ground Beetle Field Datasheet in (RD[05]), or find the trap activity record on the PDA.
2. Note the relative fluid level remaining in the container (high, low, or ok) in the 'fluidLevel' field, and whether the lid and/or upper container are missing or have been disturbed (in the 'lidStatus' and 'cupStatus' fields). If the upper container is missing and the bottom container does not obviously contain beetles or fluid, skip to 'Resetting the Pitfall Trap'. Otherwise, even if the trap appears empty, continue to the next steps to collect a sample.
3. On three locality labels, write: 1) the day (on which the trap collection is occurring) in the space preceding the month and year on the fourth line of the label; 2) the trap ID (i.e., N, S, E or W) and collect date (in the format YYYYMMDD) on the last line of the label (see **Figure 7**). Use an archival ethanol-safe pen, and record the date (in the collectDate field) and sampleID (plotID.trapID.collectDate) on the field datasheet or in the PDA.

GEORGIA Baker County. Jones
Ecological Res Ctr. 47m
N31.2034 W84.4631 Pitfall trap
16 Jul2014. RNelson
JERC_406. N· 20140716

Figure 7. Example label format

4. Place three copies of locality labels into the container, even if it appears to contain no specimens (**Figure 8**). The labels can be treated in the same way as the trap contents (e.g., rinsed along with specimens in the trap) in the subsequent steps without harm.

Figure 8. Adding locality labels to trap

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

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

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

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

9. Filter the trap contents using a filter cloth and a filter cup **(Figure 9**).

Figure 9. 300 µm mesh filter fitted to the top of a Nalgene bottle

10. Hold the filter above the waste container and pour the PG solution through the filter so that the sample is collected on the filter and the fluid does not spill onto the ground (**Figure 10-Figure 12**).

Figure 10. Preparing to filter out specimens from collecting fluid.

Figure 11. Filtering specimens from trap. The temporary waste container is below the filter (not visible).

Figure 12. Beetle specimens after collecting fluid has been poured off.

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

12. Rinse the sample (in the filter cup) using deionized water (**Figure 13**). Rinse above the waste container so rinse water/PG is efficiently collected instead of spilling onto the ground.

Figure 13. Rinsing sample with water.

13. Rinse sample in filter cup with 95% ethanol over a separate ethanol waste container, or directly over the Whirl-Pak bag in which the sample will be stored until processing in the lab (**Figure 14**).

Figure 14. Rinsing sample with 95% ethanol.

14. Place insects in a Whirl-Pak bag (**Figure 15**), rinse the filter contents into the same Whirl-Pak bag (**Figure 16**), and add additional ethanol until the contents of the bag are covered with approximately 100 mL of 95% ethanol. Finally, remove the filter from the filter cup and place the filter in the Whirl-Pak (**Figure 17**), verifying that the filter cup is free from all sample materials before using it to process another sample. Use forceps to gently push the filter into the Whirl-Pak without tearing the Whirl-Pak.

Figure 15. Placing sample in Whirl-Pak bag.

Figure 16. 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 17. Checking filter for remaining insects. Place filter in bag with sample.

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

Figure 18. A packed sample.

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

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

18. Store the Whirl-Pak samples in the shade (e.g., in a backpack) until you return to the field vehicle (see **C.4** for additional instructions).

C.2 Resetting the Pitfall

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

3. Record that the trap was reset, in the PDA or on the field datasheet.

C.3 When collecting the final sample of the field season:

1. Return all trap components to the lab.

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

3. Indicate that the final trap processed was not reset, in the PDA or on the field datasheet.

C.4 Sample preservation

1. Place all 4 Whirl-Pak bags from a single plot into a resealable plastic bag and label the outside of the bag with date and plot ID (includes siteID) before leaving the plot. You may choose to pre-label these bags in the lab.

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

3. Store samples in a cooler (with ice packs lining the bottom) in the field vehicle to prevent exposure to direct sunlight or extremely high temperatures during the remainder of the field work. When transporting coolers back to the lab avoid exposure to heat (e.g., direct sun) and wind to the extent possible.

4. Change ethanol within 1 day of collection (see **SOP D**).

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

D.1 Sample processing timing

The ethanol in which each sample is stored must be changed within 1 day of collection (ideally <24 hours). All vertebrate bycatch must be removed and sorted, and threatened or endangered species reported. Invertebrate bycatch is also removed during this rinse (if time allows). For each trap, record the date of the ethanol change on the sorting datasheet (RD[05]).

Following the rinse, specimens can be stored in ethanol for several months. Final processing, including identification and pinning, as well as submission of samples to taxonomists and/or DNA barcoding facilities (see RD[06]) must occur within four months of the end of the field season. The specimen identifications must be verified and domain voucher collection updated (see RD[06]) before the beginning of the next field season. See **APPENDIX F** for taxonomic identification resources. Quick reference guides and checklists for processing beetle specimens in the lab can be found in **APPENDIX B - APPENDIX C**.

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

> *Example 1*: Fifteen leopard frogs are caught in HARV_001 during bout 1. A problem ticket must be issued to the Insect Ecologist prior to the next sampling bout.
>
> *Example 2*: Seven five leopard frogs are caught in HARV_001 during sampling bout 1, two more are caught in HARV_001 during sampling bout 3, and ten more are caught in HARV_001 during sampling bout 7. A problem ticket must be issued to the Insect Ecologist prior to the sampling bout 8.
>
> *Example 3:* One hundred red-backed salamanders are caught in HARV pitfall traps, but by-catch never exceeds 15 salamanders in a single plot. No problem ticket is required.
>
> *Example 4*: Eight leopard frogs and 8 shrews are caught in HARV_001 pitfall traps. No problem ticket is required.

D.2 Ethanol rinse and removal of vertebrate bycatch

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

Figure 19 illustrates the steps for etOH rinse and removal of vertebrate bycatch, with detailed instructions in the following text.

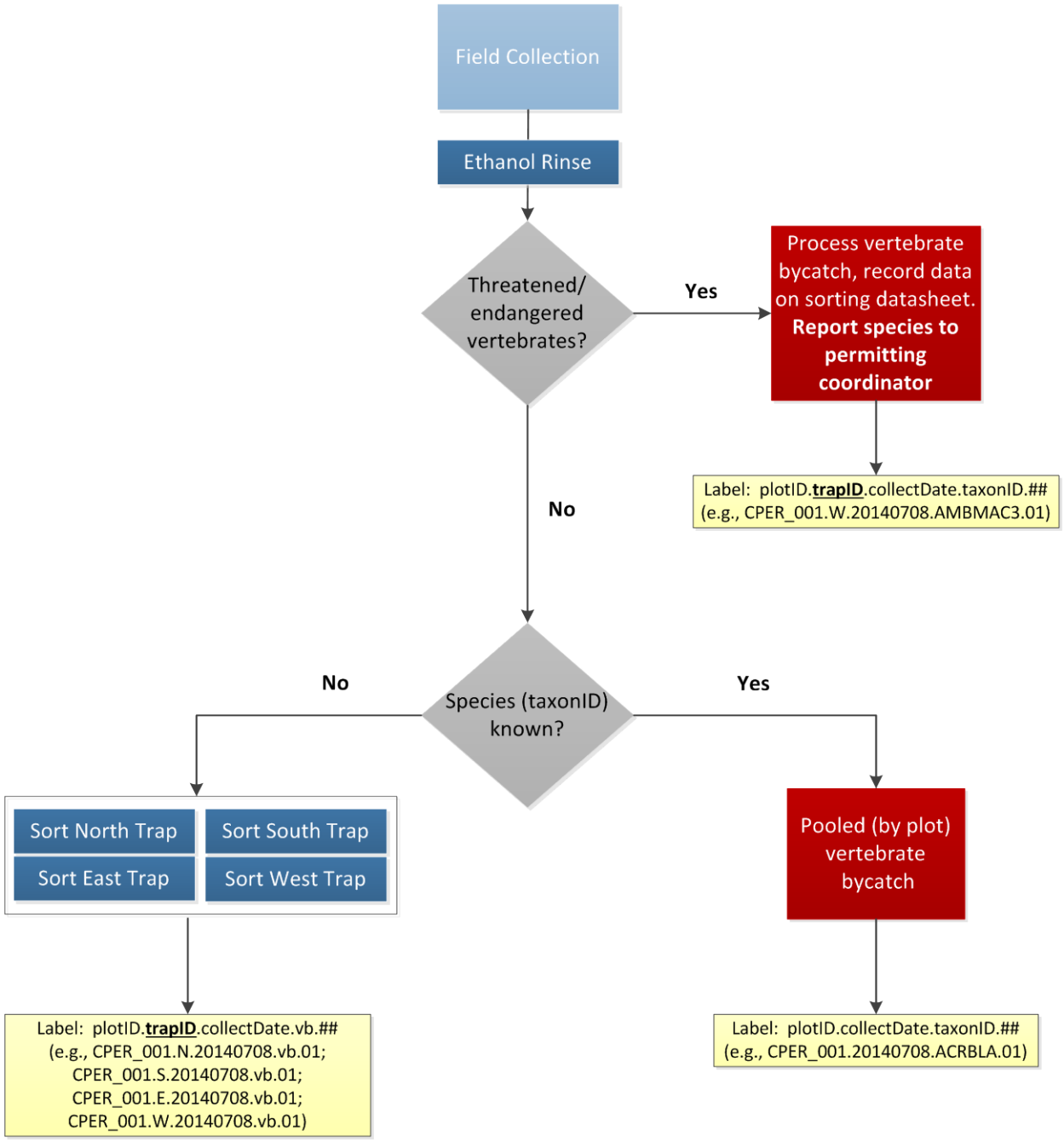

Figure 19. Workflow of the ethanol rinse and vertebrate bycatch removal processing step. Activities are represented by blue boxes, decisions by grey triangles, and labels by yellow boxes

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

2. For each trap, pour the sample (including the specimens and the initial ethanol added to the Whirl-Pak in the field) through a filter cloth and into an ethanol waste container (**Figure 20**). Remove the used filter cloth from the Whirl-Pak and rinse, with 95% ethanol, over the new filter cloth until there is nothing visible on the filter cloth (**Figure 21**). Place the filter cloth back into the original Whirl-Pak, keeping the original filter with the sample in case small beetles and bycatch are stuck to it.

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

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

3. Remove all vertebrate bycatch and record associated data on the sorting datasheet (RD [05]) (**local and/or state permits should be consulted for reporting requirements of vertebrate bycatch**). Do not enter sorting data into the webUI until all fields have been filled out on the sorting datasheet (RD[05]), which may not occur until beetles have been fully sorted and pinned/pointed, and identifications confirmed. In this way, records that need to be updated with new identifications and/or final sample IDs can be updated prior to transcription to the webUI.

4. Process vertebrate bycatch in one of two ways, depending on whether the identity of the species is known:

    a. For each individual taxon in a given plot :

        i. If the species identity is known:

            A. Pool all individuals (from the same plot) of the taxon into as few 50mL tubes as are required

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

            C. Include a locality and determination label in each tube, and insert a label into each tube on which the following sample ID is recorded (on an ethanol safe label and using an archival ethanol-safe pen): plotID. collectDate.taxonID.tubeNumber (where tubeNumber is two digits, starts with 01, and increases for as many tubes as contain the same taxon).

> Example: Two tubes are required to hold a vertebrate bycatch species for the plot. SampleIDs for these tubes are plotID.collectDate.taxonID.01 and plotID.collectDate.taxonID.02.
>
> Note – sampleIDs are automatically generated by the webUI when the Sample Type is 'vertebrate bycatch', there is an entry in the 'Taxon ID' field, and the box for 'This is a pooled sample' is checked.

D. Only one record is required per tube of pooled specimens. For each tube:

I. Record "N" in the trapID field

II. Insert the "N" between the plotID and collect date in the sampleID and record this in the "intermediate (**associated**) sampleID" field (format: plotID.N.collectDate.taxonID.tubeNumber) (see **APPENDIX H**).

III. Record the sample ID generated in step **a.i.C** in the 'final sample ID (**sampleID**)' field (see **APPENDIX H**).

IV. The identity of the individuals in the tube, to the finest taxonomic level possible (preferably species), in the 'Sci. Name OR taxonID'.

V. Record the number of individuals in the tube, in the 'Individual Count' field

VI. If it can be determined whether Carabid beetles are present in the rest of the pitfall sample, record 'Y' or 'N' in the 'Carabids (**targetTaxa**) Present' field; otherwise leave this field blank

VII. Record the etOH rinse date in the 'etOH change date' field

VIII. All other data that can be filled out (e.g., sample type, date of identification, etc.)on the sorting datasheet

ii. If the species identity is unknown:

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

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

C. Include a locality label in each tube, and insert a label into each tube on which the following sample ID is recorded (on an ethanol safe label and using an archival ethanol-safe pen): plotID.trapID.collectDate.vb.tubeNumber (where vb stands for 'vertebrate bycatch' and the tubeNumber is two digits, starts at 01, and increases for as many tubes as contain unknown vertebrate bycatch) in the trap.

> Example: One tube is required to hold a particular species of unknown vertebrate bycatch for trap S, and two tubes are required to hold a different unknown vertebrate bycatch species for trap S. SampleIDs for these tubes are: plotID.S.collectDate.vb.01; plotID.S.collectDate.vb.02; plotID.S.collectDate.vb.03.
>
> Note – sampleIDs are automatically generated by the web user interface (webUI) when the sample Type is 'vertebrate bycatch' and the Taxon ID field contains *no*value

u

D. For each tube, record on the sorting datasheet:

I. The sample ID in **BOTH** the 'Intermediate (**associated**) Sample ID' and 'final sample ID (**sampleID**)' fields (see **APPENDIX H**).

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

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

IV. If it can be determined whether Carabid beetles are present in the rest of the pitfall sample, record 'Y' or 'N' in the 'Carabids (**targetTaxa**) Present' field; otherwise leave this field blank.

V. The etOH rinse date in the 'etOH change date' field

VI. All other data that can be filled out (e.g., sample type, date of identification, etc.)on the sorting datasheet

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

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

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

7. Confirm that the appropriate labels (including that with the etOH rinse date) are still in the Whirl-Pak bag.

8. Keep the Whirl-Pak bags from all traps within a plot together in a small bag and put each sampling bout (all the plots from a single date) into a labeled airtight plastic container or re-sealable bag (**Figure 22**) and seal tightly until further processing (sorting, pinning, etc., detailed in the following steps).

Figure 22. Samples from one sampling bout stored in a sealed container. Label containers using lab tape and permanent markers.

9. Store processed samples at room temperature in the flammables cabinets unless there is ample space in refrigerators (4°C) or freezers (-20°C). Information regarding the shipment of vertebrate bycatch to archive facilities is in RD[06].

SOP E Laboratory Processing – Sorting Invertebrate Bycatch

E.1 Preparation for Sorting

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

2. Secure access to:

    a. Propylene glycol and ethanol waste storage.

    b. Sink for washing materials.

    c. At least one dissecting microscope

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

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

E.2 Sorting Invertebrate Bycatch

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

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

Steps for sorting invertebrate bycatch are detailed in **Figure 23**, with instructions in the following text.

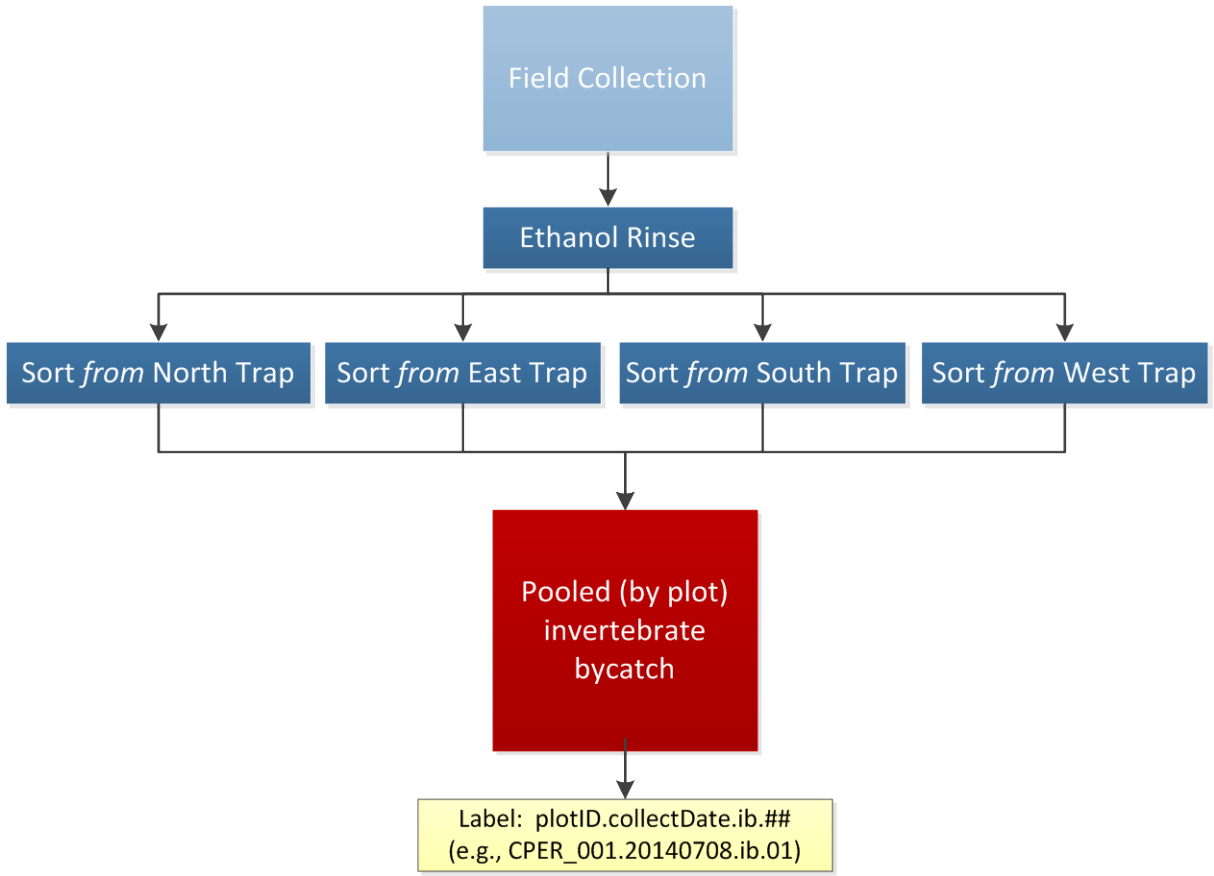

Figure 23. Workflow for processing invertebrate bycatch. Activities are represented by blue boxes, intermediate and final products by red boxes, decisions by grey triangles, and labels by yellow boxes.

1. Sort each sample individually, from one trap at a time.
2. Suspend specimens in ethanol in the Whirl-Pak bag so that they are uniformly floating throughout. Jostle the bag and use wash bottle to add 95% ethanol if necessary.
3. Pour specimens into a large Petri dish marked with a grid (hereafter referred to as the sorting dish). Remove or add 95% ethanol as needed to keep specimens submerged in the sorting dish, using a transfer pipette and a secondary container for used ethanol (removal) or wash bottle of ethanol (addition).
4. Remove the filter cloth from the Whirl-Pak and inspect it for any small invertebrates that may be attached. Rinse invertebrates into the sorting dish, with 95% ethanol, and then discard the filter.

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

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

7. If, at the end of sorting, no carabids have been found in the sample, mark 'N' on the sorting datasheet for 'Carabids (targetTaxa) Present'.

8. Record the presence of invertebrate bycatch at the trap-level (circle 'invert bycatch' in the sampleType field), and fill in the 'processingDate', 'recordedBy', and 'etOH Change Date' fields. Neither counts nor taxonomic identifications are expected for invertebrate bycatch specimens.

9. When all samples from a plot have been fully sorted, pool invertebrate bycatch by the plotID and collectDate.

    a. Transfer all invertebrate bycatch specimens from the four traps within a plot into as few 50 mL tube as are required, leaving ½ inch empty at top of tube.

        i. Be sure to move the locality labels into the new storage tubes (e.g., if the invertebrate bycatch from traps N and S fit into one 50mL storage tube, place the locality labels for both traps into the new storage tube).

        ii. If multiple tubes are required from the same trap, generate additional locality labels and place a locality label in each tube.

    b. Insert a new sampleID into each tube: plotID.collectDate.ib.tubeNumber (where tubeNumber is two digits and starts at 01.

> Example: Two tubes are required to hold invertebrate bycatch for a plot, one with specimens from traps N and S, and the other with specimens from traps E and W. SampleIDs for these tubes are plotID.collectDate.ib.01 and plotID.collectDate.ib.02.
>
> Note – sampleIDs are automatically generated by the webUI when the Sample Type is 'invertebrate bycatch'.

c. If the number pooled tubes = 1 **AND** carabids are present in all traps **or** absent from all traps:

   i. Only one record is required for all traps

   ii. Record "N" in the trapID field

   iii. Record the sample ID in **BOTH** the "intermediate (**associated**) sampleID" and "final sampleID (**sampleID**)" fields (see **APPENDIX H**).

   iv. Circle Y/N in the "targetTaxaPresent" field to indicate the presence or absence of carabids in the pooled samples.

d. If the number pooled tubes >1 **OR** carabids are present in some but not all traps:

   i. A sorting record is required for each trap

   ii. Record the sample ID for the tube into which a traps contents were transferred in **BOTH** the "intermediate (**associated**) sampleID" and "final sampleID (**sampleID**)" fields (see **APPENDIX H**).

   iii. Circle Y/N in "targetTaxaPresent" field of each record, to indicate the presence or absence of carabids in each trap

> Example: If the invertebrate bycatch from traps N and S are stored in one tube, enter the sample ID generated in step (9.b) in the 'intermediate (associated) sampleID' and 'final sampleID (sampleID)' fields for the records for both the N and S traps.
>
> This will result in multiple records with the same value in the 'Intermediate (**associated**) Sample ID' and 'final sample ID (**sampleID**)' fields, but different values in the trapID and (potentially) targetTaxaPresent fields. This is OK.

e. Fill each tube with 95% ethanol, with at least ½ inch of ethanol above the fully submerged specimen(s). Split the specimens into multiple tubes if necessary to ensure that there is ½ inch or more of ethanol above the specimens in each tube (**Figure 24**).

f. 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).

Figure 24. Bycatch samples stored in a 50 mL centrifuge tubes, the amount of ethanol in a bycatch sample will vary by sample size.

10. In the rare instance that invertebrate bycatch has *not* been pooled by plotID and collectDate:

    a. Fill out all trap-level metadata (e.g., processingDate, etOH Change Date, etc.) as above.

    b. Insert a new sampleID label into each tube: plotID.trapID.collectDate.ib.tubeNumber (where tubeNumber is two digits and starts with 01, then increases for as many tubes as contain the invertebrate bycatch for the trap).

> Example: Two tubes are required to hold invertebrate bycatch for a trap N. SampleIDs for these tubes are plotID.N.collectDate.ib.01 and plotID.N.collectDate.ib.02.
>
> Note – sampleIDs are automatically generated by the webUI when the Sample Type is 'invertebrate bycatch' and the box for 'Not a pooled sample' is checked.

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

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

e. Record the sample ID in **BOTH** the 'Intermediate (**associated**) Sample ID' and 'final sample ID (**sampleID**)' fields of each record, and circle "Y" or "N" in the targetTaxaPresent field (see **APPENDIX H**).

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

SOP F Laboratory Processing - Sorting Ground beetles

Carabid beetles will be sorted, and then pinned or pointed (instructions in **SOP G**), or stored in 95% ethanol. The final destination of these specimens will include: 1) a domain's teaching collection; 2) a DNA barcode facility (just a tissue sample); 3) an archive facility. This protocol provides instructions for beetle processing through the pinning or pointing stage. Instructions for additional processing steps (e.g., sending specimens to various facilities, creating teaching collections, etc.) are found in RD[06].

Carabid beetles should be sorted in concurrence with the sorting of invertebrate bycatch, as described in **SOP E**. Sorting should occur on a trap-by-trap basis so that specimens from individual traps are kept separate through the entire sorting process. The general process of sorting carabid beetles is illustrated in **Figure 25**, with detailed instructions in the following text.

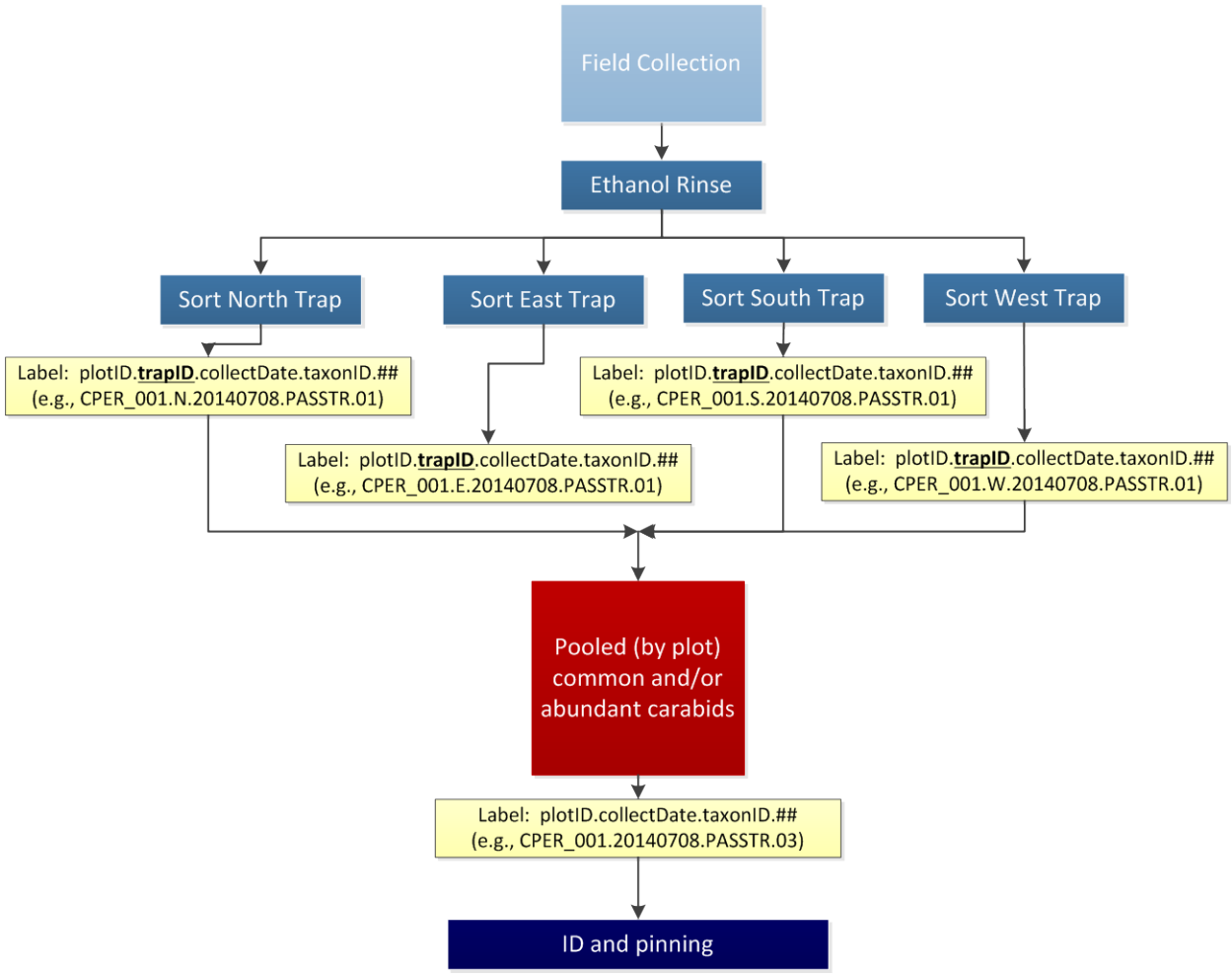

Figure 25. Workflow for processing carabid beetles. Activities are represented by blue boxes, intermediate and final products by red boxes, decisions by grey triangles, and labels by yellow boxes.

1. For each individual taxon in a given plot:

    a. For species for which the identity is known (including but not limited to those listed as common and reliably identified (**APPENDIX E**) OR the identity is unknown (morphospecies – see section D.8 for morphospecies identification guidelines) but > 20 individuals are present:

        i. Place all individuals of a given taxon, and from the same trap, into as few 50 mL tubes as required.

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

        iii. Include a locality and determination label in each tube.

        iv. For species whose identities are known:

            A. Insert a label into each tube on which the following sample ID is recorded (recorded on ethanol safe paper and using an archival ethanol-safe pen): plotID.trapID.collectDate.taxonID.tubeNumber (where tubeNumber is two digits, starts with 01, and increases for as many tubes as contain the same taxon).

> Example: Two tubes are required to hold a particular carabid species for trap W. SampleIDs for these tubes are plotID.W.collectDate.taxonID.01 and plotID.W.collectDate.taxonID.02.
>
> Note – sampleIDs are automatically generated by the webUI when the Sample Type is 'common carabid', there is an entry in the 'Taxon ID' field.

        v. For species whose identities are unknown and are abundant (>20 individuals) :

            A. *Note- Morphospecies that are abundant (>20 individuals) are treated as common species (sampleType = 'common carabid') even though they may not be listed as common or reliably identified. This allows for proper sample tracking and reduces the number of individuals that must be pinned*

B. Insert a label into each tube on which the following sample ID is recorded (recorded on ethanol safe paper and using an archival ethanol-safe pen): plotID.trapID.collectDate.cc.tubeNumber (where 'cc' stands for 'common carabid' and the tube number is two digits, starts at 01, and increases for as many tubes as contain morphospecies in the trap).

> Example: Two tubes are required to hold MorphA and two tubes are required to hold MorphB in trap E. SampleIDs for these tubes are plotID.E.collectDate.cc.01, plotID.E.collectDate.cc.02, plotID.E.collectDate.cc.03, and plotID.E.collectDate.cc.04.
>
> Note – This sampleID is automatically generated by the webUI when the Sample Type is 'common carabid' and the Taxon ID field contains *no* value.

vi. For each tube, record on the sorting datasheet:

A. The sample ID generated in step iv.A or v.B in the 'Intermediate (**associated**) Sample ID' field (see **APPENDIX H**).

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

C. 'Y' in the 'Carabids (**targetTaxa**) Present' field

D. 'common carabid' in the sampleType field

E. The etOH rinse date in the 'etOH change date' field

F. The identity of the individuals in the tube, to the finest taxonomic level possible (preferably species), in the 'Sci. Name OR taxonID' or 'morphospecies ID' field (see section D.8 for instructions on morphospecies naming conventions).

G. All other data that can be filled out (e.g., date of identification, etc.)

b. If the species is unknown and there are $\leq$ 20 individuals present:

i. Place all individuals from a single *trap* into as few 50mL tubes as are required.

ii. Fill each tube with enough 95% ethanol that there is at least 1/2 inch of ethanol above the fully submerged specimen(s). If there are too many specimens to allow for 1/2 inch of ethanol above the level of the specimens,

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

iii. Include a locality label in each tube.

iv. Insert a label into each tube on which the following sample ID is recorded (on an ethanol safe label and using an archival ethanol-safe pen): plotID.trapID. collectDate.oc.tubeNumber (where 'oc' stands for 'other carabid' and the tube number is two digits, starts at 0.1 and increases for as many tubes as contain the unidentified carabids)

> Example: Two tubes are required to hold all unknown carabids in trap N. SampleIDs for these tubes are plotID.N.collectDate.oc.01 and plotID.N.collectDate.oc.02.
>
> Note – sampleIDs are automatically generated by the webUI when the Sample Type is 'other carabid'.

v. For each tube, record on the sorting datasheet:

A. The sample ID generated in step b.iv in the 'Intermediate (**associated**) Sample ID' field (see **APPENDIX H**).

B. 'Y' in the 'Carabids (**targetTaxa**) Present' field

C. 'other carabid' in the sampleType field

D. The etOH rinse date in the 'etOH change date' field

E. The morphospecies ID (see section D.8 for instructions on morphospecies naming conventions) in the 'morphospecies ID' field

F. All other data that can be filled out (e.g., processingDate, etc.)

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

2. When all samples from a plot have been fully sorted, pool individuals of the same species by the plotID and collectDate:

a. Transfer all individuals of the same species (from all four traps in the plot) into as few 50 mL tubes as are required. Be sure to move the locality labels into the new storage tubes

i. Example: If the individuals from traps N and S fit into one 50mL storage tube, place the locality labels for traps N and S into the new storage tube.

b. Insert a new sampleID label (recorded on ethanol safe paper and using an archival ethanol-safe pen) into the tube with: plotID.collectDate.taxonID.tubeNumber (where tubeNumber is two digits and starts with 01, then increases for as many tubes as contain the individuals of that taxon for the plot).

c. For each record, write the sample ID from step (2.b) in 'final sample ID (**sampleID**)' field of the sorting datasheet (see **APPENDIX H**).

i. Example: If the specimens from traps N and S are stored in one tube, enter the sample ID generated in step (2.b) in the 'final sample ID (**sampleID**)' fields for the records for both the N and S traps.

ii. Note – This will result in multiple records with the same value in 'final sample ID (**sampleID**)' fields, but different values in the trapID field. This is OK.

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

3. 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).

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

F.1 Equipment maintenance, cleaning, and storage

1. After all samples have been sorted:

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

b. Return all flammables to the appropriate cabinets.

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

F.2 Identify ground 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 or pointed (except those most common and reliably identified beetles on the site-specific lists and large groups (>20) of individuals of the same, unknown, species encountered during sorting). Various tools are available for aiding species identifications (e.g., see **APPENDIX F**).

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).

- 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.
- As a general rule, split groups that look similar but not identical into different morphospecies, focusing on features like: size, color, proportions and the shape of the pronotum. 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, who will return identified specimens that should then be added to the teaching collection at the domain lab.
- Morphospecies IDs include: domainID, the year of capture, and the word "Morph" followed by one or more unique letters. For example, "D15.2014.MorphA" would be the first morphospecies from domain 15 that was captured in 2014.
- The letter at the end of the morphospecies ID (e.g., "A") should *never* be repeated for any other morphospecies than that for which it was originally designated, in a given year.If more than 26 morphospecies are encountered in a given domain in a given year, the 27th morphospecies ID should include two letters at the end (e.g., the 27th morphospecies in domain 15, for 2014, would be "D15.2014.MorphAA")
- If it is unclear whether a newly captured specimen is the same as individuals of a previously assigned morphospecies ID, a new morphospecies ID should be assigned (it is better to have the same species designated as different morphospecies than to have multiple different species designated as the same morphospecies).

Record and describe each morphospecies on the morphospecies datasheet, along with the year and name of the person who designated it. If a taxonomist is able to identify some individuals of a morphospecies to species, the identity of all the other individuals of that morphospecies should be updated on both the sorting and ID and pinning datasheets (or in the respective web user interfaces (webUIs)).

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

G.1 Pinning and pointing large and small ground beetles

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

1. Up to 100 specimens for ground beetles not listed on the Common Reliably Identified Ground Beetle Species List (**APPENDIX E**), including morphospecies. When 100 specimens have been pinned or pointed, the remaining specimens should be pooled into 50 mL tubes and a new record generated on the sorting datasheet in accordance with **SOP F** (e.g., process the remaining specimens as you would if you were processing them during sorting).

2. Up to 20 individuals per species per site per year of common and easily identified ground beetles.

   Note – In order to ensure that temporal, spatial, and morphological variation is accounted for in the selection of beetles to be pinned and pointed, individuals of common and reliably identified species will be selected after all sorting has been completed for the year for all bouts in all plots at the site.

Data for each pinned or pointed specimen should be recorded on the ID and Pinning datasheet (RD[05]) or in a Pin Activity of the web user interface (webUI). Most of the data for the datasheet can be found on labels in the tubes from which the specimens are obtained, or in the record in the sorting datasheet that corresponds to this tube. This includes data for the 'intermediate (**associated**) sample ID' field, which is the sample ID from the label inside of the tube from which the specimen is obtained.

*Note – The counts per tube for records on the sorting datasheet need to be updated when individuals from tubes containing a taxonID or 'cc' in the 'final sampleID (**sampleID**)' are pinned. This is not required if the 'final sampleID (**sampleID**)' contains 'oc', because no counts were initially provided on the sorting datasheet.*

Example: Two individuals were removed from a tube with the following information recorded on the sorting datasheet:

Intermediate (**associated**) sample ID: CPER_001.N.20140506.cc.01
Final sample ID (**sampleID**): CPER_001.20140506.cc.01
Individual count: 8

After these individuals have been pinned or pointed, the sorting datasheet record for the tube must be updated with an **individual count** of 6.

Note- If there are multiple sorting datasheet records with a final sample ID of CPER_001.20140506.cc.01, *update the individual count in **only one** of these records, it does not matter which.*

G.2 Determine whether to pin or point the specimen

1. Generally specimens 5 mm and greater are pinned, and specimens smaller than 5 mm are pointed (**Table 10**; **Figure 26**).

2. Pointing is a good default option for any specimen you are concerned will be destroyed by pinning (**Table 10**; **Figure 26**).

Table 10. Summary of beetle mounting criteria

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

Figure 26. Pinned (left) and pointed (right) beetle specimens

G.3 Prepare points (if pointing)

1. Prepare a batch of points prior to beginning pointing specimens.
2. Using 100% rag cotton paper (Bristol or equivalent), prepare ~40 triangles using the point punch.
3. Over a balsa wood surface, for each point, press a No. 3 archival quality pin into the point at the center of the point's base (see the "X" in **Figure 27**). Ensure that the point is at a 90 degree angle from the pin and neither angled up nor down.

Figure 27. Materials for pointing including a schematic of a point showing where to place pin

4. Use a pinning block (**Figure 28**) to push the point up the pin until it is 15 mm from the top (non-pointed end) of the pin. This leaves enough space above the insect for the pin to be handled, and below for labels. Additionally, specimens at a uniform height are easier to examine and compare with one another.

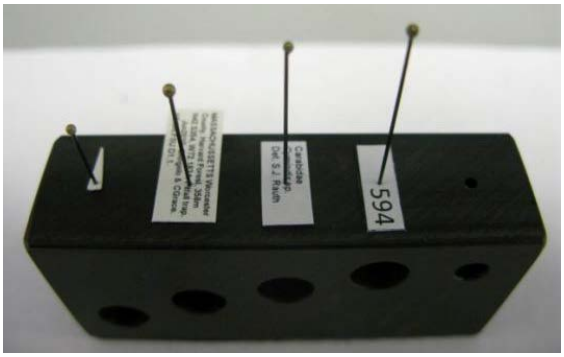

Figure 28. A pinning block with a point and the various labels that will be affixed to a pinned or pointed specimen. This shows the heights at which points and various labels should be placed on a pin.

G.4 Prepare labels

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

2. See **APPENDIX J** for instructions on creating determination labels. These are created for all mounted beetle specimens during field preparation and will be placed on the pin below the locality label located below each specimen. Ground beetles that have not yet been identified, however, may be mounted on pins with locality labels but no determination labels.

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

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

G.5 Mount Specimens

1. For pointed beetle specimens:

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

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

    c. Use forceps to put the beetle specimen on its left side on a slip of plain white paper, so that its head is facing the right and its legs are toward the edge of the bench (**Figure 29**)

Figure 29. A ground beetle on its side in preparation for pointing

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

e. Examine the slope of the ground beetle's thorax to determine if the tip of the point needs to be bent to ensure that the specimen will lie flat on the point. Use forceps to bend the point if needed (**Figure 30**).

   i. **NOTE**: The sides of a beetle's thorax can slope inward to differing degrees, therefore, the tip of the point can be bent down with forceps to accommodate the size and shape of each specimen. For example, a specimen that has a flat-bottomed thorax can be mounted on a flat (unbent) point. A specimen on which the sides of the thorax are vertical must have the tip of the point bent downward at a right angle. See **Figure 30** for thorax slope scenarios and **Figure 31** - **Figure 32** for examples of well-pointed and poorly-pointed specimens, respectively.

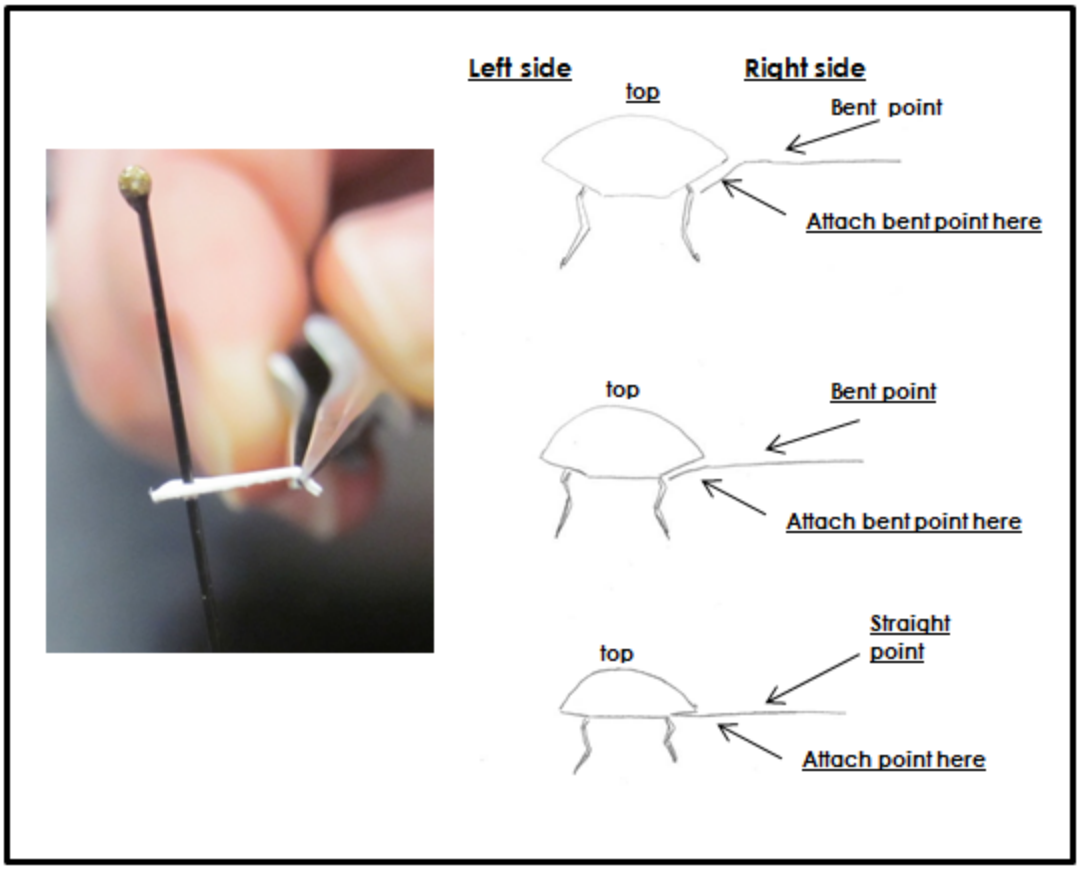

Figure 30. Examples of bending a point tip to maintain a level body position of the specimen

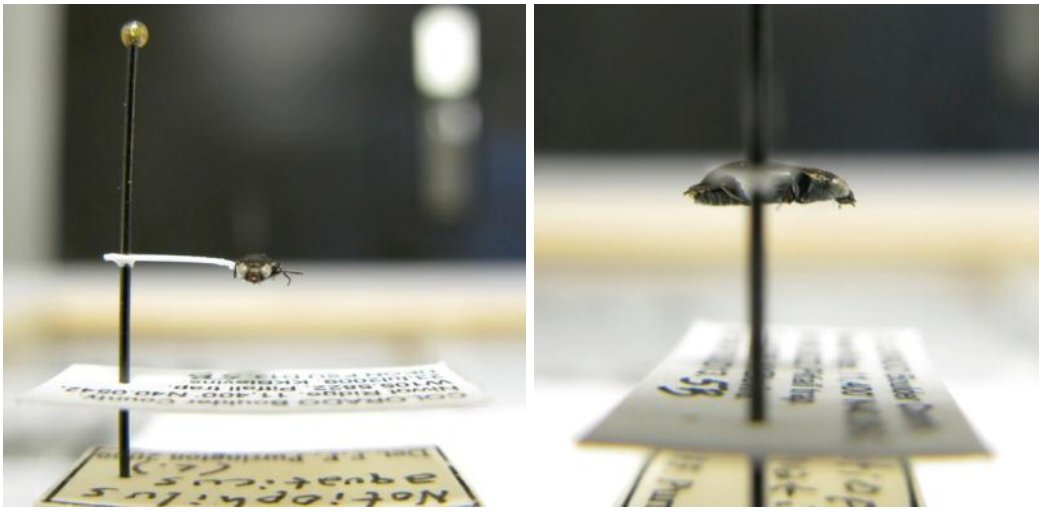

Figure 31. A well-pointed beetle specimen, with associated labels

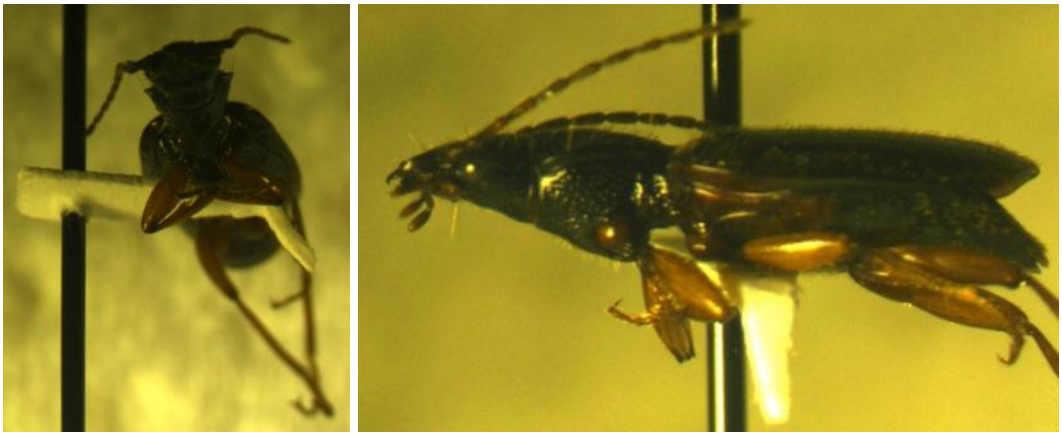

Figure 32. A poorly pointed beetle specimen

f. Take the prepared point and touch the tip of the point in a bit of glue that has been squeezed out onto a paper towel or piece of paper (**Figure 33**). Use the minimum amount of glue needed in order to attach the specimen firmly to the point.

Figure 33. Preparing to glue a specimen to a point by dipping the tip of the point in glue.

g. Under a microscope, zoom in on the target location for the tip of the point, this being on the **RIGHT** side (from a dorsal perspective) of the specimen's thorax and between the bases of the 2nd and 3rd pairs of legs (see "X" on **Figure 34**). Gently press the glue-covered tip of the point to the beetle's body (**Figure 35**), and wipe off excess glue in order to avoid obscuring any part of the beetle with large amounts of glue.

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

   ii. **Note:** Be sure to place the point on the *right* side of the specimen

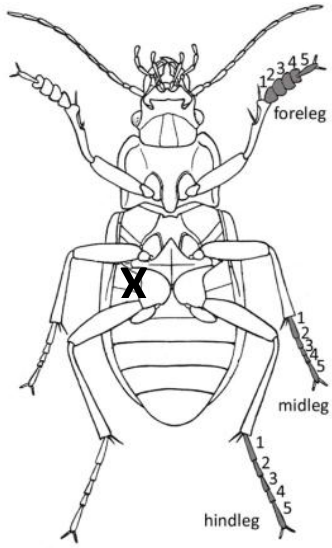

Figure 34. Diagram of ventral side of a beetle, with and X indicating where to place point

Figure 35. Attaching a specimen to a point, and pointed specimens left to set.

h. Wait for the glue to set slightly, so that the beetle stays relatively still when the pin is moved. Then lift and angle the pin so that the weight of the specimen is leaning directly into the point and the specimen is properly aligned on the point, as in **Figure 30 - Figure 31**.

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

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

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

correct height (**Figure 36**). Position the specimen perpendicular to the text on the label, with the back edge of the point slightly inset from the short edge of the label and the specimen centered between the two longer edges of the label (**Figure 36**).

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

Figure 36. Attaching a locality label below a pointed beetle.

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

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

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

2. For pinned beetle specimens:

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

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

c. Press your thumb and index finger together in order to form a “v”, which you will use to support the specimen as you insert the pin.

d. Place the specimen into the "v" and position it so that it is straight in your fingers and the legs are facing downward.

e. Insert the pin vertically through the top layer of the specimen's body (**Figure 37**).

   i. **Note: It is important to insert the pin in the correct location in the beetle's body**. A properly placed pin is positioned on the **RIGHT** side of the beetle (when viewed dorsally), halfway between the midline of the beetle's body and the edge where the elytra (wing shells) begin to round behind the thorax (see the "X" in **Figure 37**).

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

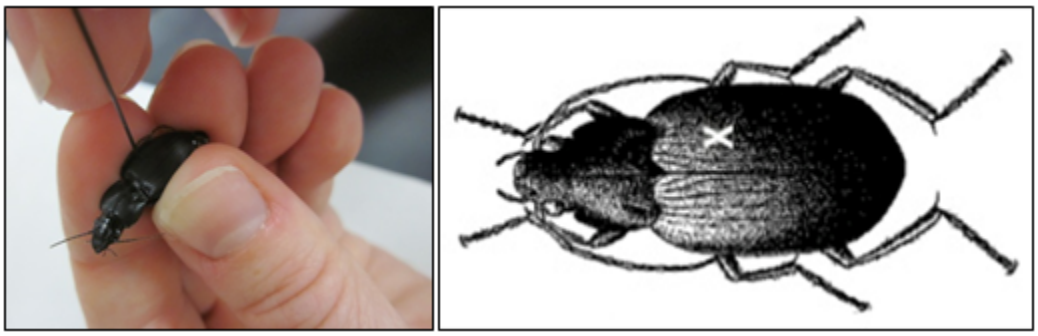

Figure 37. Ground beetle pinning and dorsal side schematic showing where to place the pin (marked by an "X")

f. Gently slide the pin through the body of the specimen, using your fingers to support the body as you push the pin through. Push the pin slowly and with constant pressure.

g. Pause and look at the specimen from the side BEFORE pushing the pin all of the way through.

h. Ensure that the pin is at a right angle to the beetle's body and push the specimen up the pin until its top surface is 15 mm from the top of the pin. This leaves enough space above the specimen for the pin to handled and below for labels. Additionally, specimens at a uniform height are easier to examine and compare with one another. See **Figure 38** for examples of well-pinned and poorly-pinned specimens.

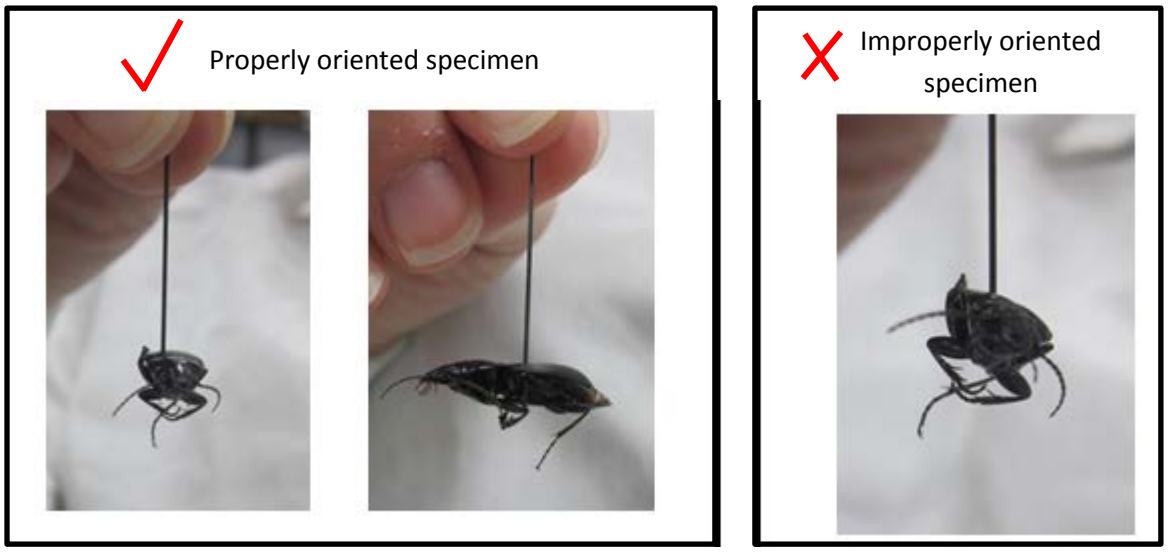

Figure 38. Correctly and incorrectly pinned beetle specimens

i. Slide the locality label (instructions in **APPENDIX I**) onto the pin below the specimen, and use the second-deepest hole in the pinning block to position the label at the correct height (**Figure 39**). Position the specimen in the middle of the locality label and with the head pointing to the left side. The specimen should be lined up with the long axis of the body parallel to the print on the label (**Figure 39**).

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

Figure 39. Attaching a locality label below a pinned ground beetle

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

k. Attach the insect individual ID label (instructions in **APPENDIX K**) below the determination label using the fourth-deepest (most shallow) hole. Orient the label in the same manner as the locality label.

G.6 Loss of Body Parts

1. If a part of a specimen, such as a leg, falls off during pinning, save it and re-attach it to the beetle (provided you are certain which specimen the body part belongs to; **Figure 40**). To do this:

    a. Hold the part being reattached with forceps.

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

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

Figure 40. A leg being inserted back into its socket, using forceps and a dab of glue

G.7 Sample Preservation

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

2. After a specimen has been pinned or pointed, and any glue has dried, it should be placed in a box or tray so that locality labels are oriented in the same direction as those of the other specimens in the box or tray (**Figure 41**).

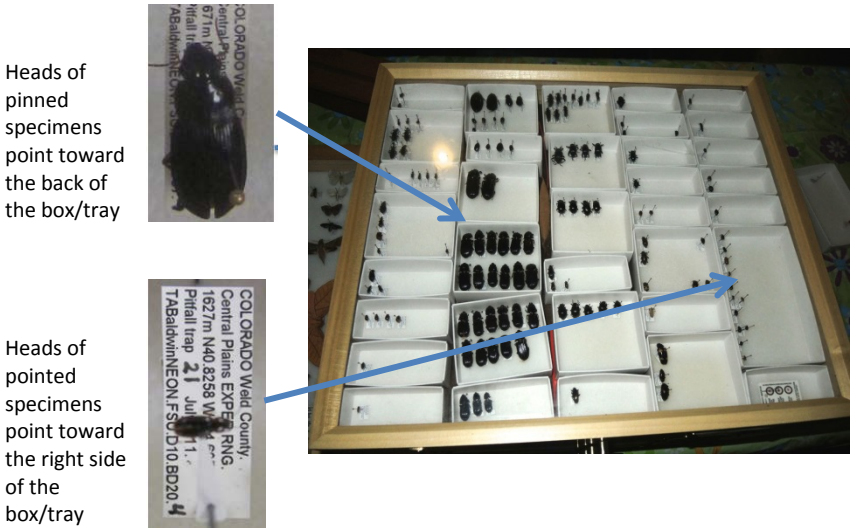

Figure 41. Proper orientation of mounted specimens in unit trays within a Cornell drawer.

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

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

5. A fumigant box should be secured in the corner of one of the unit trays with pins, so that it does not slide around and damage specimens (**Figure 42**).

Figure 42. Fumigant box filled with moth crystals and secured in the corner of a unit tray with pins.

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

SOP H Data Entry and Verification

Field data collected on paper datasheets should be digitally transcribed within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

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

H.1 Field datasheets

1. At the end of each field day or as soon as possible after data collection (14 day limit), according to instructions in the NEON Protocol: manual data transcription (AD[04]), field data should be promptly and carefully transcribed into the webUI (if PDAs are not available). Notes that apply to an entire plot should be recorded for each plot (e.g., 'mowing disturbed all traps at the plot).
2. Scan datasheets and save in PDF file format.
3. Save paper copy of datasheets.

H.2 Sorting and ID & Pinning datasheets

1. Data from sorting datasheets should be carefully transcribed *after* all beetle processing (including ID'ing and pinning) is complete and all data are recorded and/or updated (e.g., new records have been generated for beetles that were pooled after enough specimens had been pinned, final sampleIDs have been generated, counts have been updated when beetles were removed from tubes for pinning/pointing) on the datasheet.
2. Data from ID and Pinning datasheets should be promptly and carefully transcribed into the webUI.
3. Scan datasheets and save in PDF file format.
4. Save paper copy of datasheets.

H.3 Morphospecies datasheet

1. Promptly and carefully transcribe data into the beetleMorphospeciesTracking datasheet, in NEON sharepoint.
2. Scan datasheets and save in PDF file format.

3. Save paper copy of datasheets.

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

SOP I Sample Shipment

Select beetle specimens will be shipped to a taxonomic facility for professional identification to the species level and/or verification of identifications made at the domain facility. Those not selected for shipment to a taxonomist should continue to be stored in the domain facility, many of them in a teaching collection, until they can be shipped to a long term storage facility (see RD[06]). Specimens are to be shipped to taxonomists only after they have been pinned or pointed, and identified as thoroughly as possible by domain parataxonomists. Specimens **MUST**, however, be sent out *no later than* the 1st of February in the year following the field season in which the specimens were collected. This is due to the time required for the taxonomist to receive and process samples before sending them back, and the need for domain lab teaching collections to be updated and identification verifications completed prior to processing the next years' specimens.

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

I.1 Choosing specimens for shipment

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

1. At least five (if available), and up to 20% of all captured individuals of species that have not been identified to species by domain staff
2. At least five (if available), and up to 20% of all captured individuals of species rarely encountered throughout the field season. Rarely encountered individuals are those for which there are ≤ 50 individuals captured, combined and across all plots, over the course of the field season
3. Up to 10 specimens from species that are not represented in the teaching collection
4. Additional specimens from species that are represented by fewer than 10 individuals in the teaching collection (enough individuals to bring the total in the teaching collection to 10)
5. At least five, and up to 10% individuals of species that have been identified in the domain facility, are commonly encountered, and for which 10 individuals are already present in the teaching collection

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

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

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

I.2 Preparing for shipment

1. No hazardous materials or chemical substances will be included in this shipment. The fumigant box containing moth crystals (paradichlorobenzene) shall be **REMOVED** prior to shipment.

2. Supplies/Containers**:** See **Table 9** for a complete list of shipping materials.

3. Timelines: Beetles must be sent no later than the 1st of February in the year following collection, although more expedited shipment is recommended as other steps in the ground beetle processing workflow (see RD[06]) are contingent upon the samples being returned to the domain facility. Prior to shipment, dried, pinned/pointed specimens may be stored in an air tight container with paradichlorobenzene (to be removed prior to shipping) without detriment to the integrity of the specimens.

I.3 Packaging specimens for shipment

1. Ship prepared dry (pinned/pointed) beetle specimens to the taxonomic ID facility with a hard copy of the Taxonomy Shipping Manifest (taxonomy in datasheet from RD[05]). Email a digital copy of Taxonomy Shipping Manifest to the taxonomy lab and NEON's CLA contact.
2. Place specimens in a mailer box so that locality labels are oriented in the same direction and the specimens are neatly aligned (**Figure 43**)

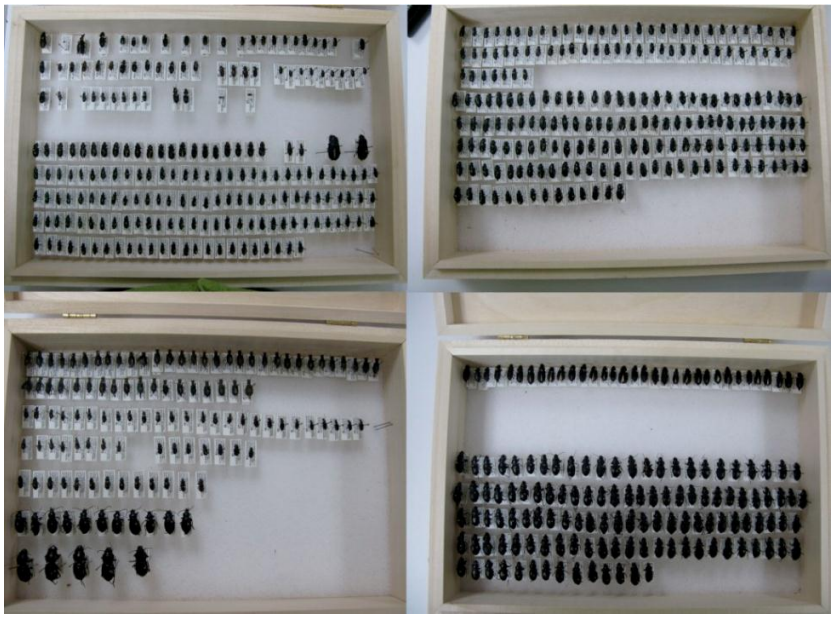

Figure 43. Specimens prepared for shipping

3. Large beetles (typically 1.5 cm or larger, but this step may also be required for smaller specimens with heavy bodies) must be brace-pinned on either side of the label to prevent them from moving during transit (**Figure 44**).

Figure 44. Large beetle that is brace-pinned for shipment

4. When the mailers are ready for shipping, take a photo of each box (with specimens visible) for tracking purposes (**Figure 43**). Save these photos to the domain's local drives for future reference.
5. **Do NOT include moth crystals in shipments.**
6. Examine specimens before sealing the box. Make sure all specimens are appropriately labeled. Ensure that all of the specimens are firmly glued to their points by gently tapping the box to test. A specimen dissociated from its locality label is worthless. Finally, make sure that all pins with specimens are as vertical as possible.
7. Place four pins (#3 size) in the four corners of the specimen mailer, and four more at the midpoint of each of the four sides. These pins will support the cover board (cardboard insert).
8. Cut a cardboard insert to size to place on top of specimens (resting on pins). This cover board should fit PRECISELY inside the box with a snug fit. The cover board will contact ALL pins in the box. Make a packing tape tab to easily pull off the cardboard insert (otherwise it can become wedged in the box and difficult to remove; **Figure 45**). The cardboard insert must fit exactly to the dimensions of the box because a slightly smaller insert will chatter over the pin heads and ultimately become a source of specimen breakage during shipment.
9. Fill in any additional head space between the cover board and the lid of the specimen mailer with packing or bubble-foam so tightly that it slightly bulges the top of the box when filled.
10. Secure the lid of the box with a rubber band, tape, or other method that will firmly push the lid downward onto the cover board. Gently break off the metal tabs that come as part of the box. The secondary securement backs up the metal tabs which are prone to break off after several uses. If they break during shipment, they are prone to rattling around among the specimens.

Figure 45. Cardboard insert that serves as extra protection for pinned/pointed specimens, and helps fills dead space between specimens and mailer lid.

11. Ship prepared dry material in cardboard mailers inside of larger cardboard boxes with ample packing material to ensure that the specimens are not jostled (**Figure 46** -**Figure 47**).

Figure 46. A mailer placed inside a larger box for shipping

Figure 47. Mailer within a shipping box, with adequate padding to keep mailer from moving in transit

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

I.4 Creating shipping inventory

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

The Taxonomy Shipping Manifest (**Figure 48)** lists every specimen in the sample shipment, in the identifier field (for which the entry will be individualID in the case of pinned beetles). Remarks may also be entered for every specimen; for example specimens that the domain facility would like returned in order to add them to the teaching collection may have "Return to NEON facility" in the remarks field. The other required information on the Taxonomy Shipping Manifest, sentTo (name of the facility to which the specimens are being sent), sentDate, and recordedBy, must only be entered once per Taxonomy Shipping Manifest. An electronic version of the Taxonomy Shipping Manifest is an Excel file that should be emailed to the taxonomic ID facility and the CLA contact as soon as possible after the samples have been shipped.

NEON Ground Beetle Pitfall Sampling: Shipping

sentTo: Insect ID facility of western America sentDate: 12/12/2014 recordedBy: nrobinson@neoninc.org

identifier (e.g., sampleID, individualID, plateID)	remarks (e.g., "Return to NEON facility")
NEON.BET.D10.001116	
NEON.BET.D10.001117	Return to NEON facility
NEON.BET.D10.001110	
NEON.BET.D10.001111	
NEON.BET.D10.001112	
NEON.BET.D10.001118	Return to NEON facility

Figure 48. Example shipping manifest for the taxonomic ID facility

I.5 Laboratory contact information and shipping/receipt days

See CLA's NEON intranet site.

I.6 Return of shipping materials

Taxonomists will return ONLY specimens that are to be DNA barcoded, added to the domain lab teaching collection, or for which a request was made in the remarks field of the Taxonomy Shipping Manifest (see RD[06] for additional information on DNA barcoding and teaching collection creation and maintenance). As such, include a return shipping label in the original shipment).

8 REFERENCES

N/A

APPENDIX A Datasheets

The following datasheets are associated with this protocol:

Table 11. Datasheets associated with this protocol.

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

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

APPENDIX B Quick References

Quick Reference: Getting Ready for Field Sampling

STEP 1 – Charge all electronic devices (e.g., GPS units and PDAs)

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

STEP 3 – Print locality labels

STEP 4 – Prepare chemicals

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

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

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

Properly installed pitfall trap has:

- Lip of top cup flush with ground
- No gap between top and bottom cups
- No gap between bottom cup and ground
- Minimal disturbance of surrounding vegetation

Adjust height of lid as needed, to ensure that:

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

Quick Reference: Collecting Insects from Trap

STEP 1 – Record date and trapID on three locality labels

STEP 2 – Place three locality labels in top container

STEP 3 – Remove top container

STEP 4 – Filter contents over waste container

STEP 5 – Rinse sample through filter with distilled water

STEP 6 – Rinse sample through filter with 95% ethanol

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

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

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

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

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

Quick Reference: Processing Trapped Specimens

Ethanol Rinse (within 24 hours of sample collection)

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

STEP 2 – Remove vertebrate bycatch, record relevant information on sorting datasheet or in webUI, and store vertebrate bycatch in 50 mL tubes with locality and determination labels. Fill tubes with enough 95% ethanol to submerge their contents.

STEP 3 – Place vertebrate bycatch in labeled 50 mL tubes, with locality and determination labels. Fill tubes with enough 95% ethanol to submerge their contents.

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

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

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

Sorting Ground Beetles from Invertebrate Bycatch

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

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

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

STEP 4 – Place invertebrate bycatch in labeled 50 mL tubes, with locality labels

STEP 5 – Sort common and reliably identified ground beetles into labeled 50 mL tubes, with a locality and a determination label

STEP 6 – Sort abundant morphospecies into labeled 50 mL tubes, with a locality and a determination label

STEP 7 – Place all other, unsorted, carabid beetles into labeled 50 mL tubes, with a locality and a determination label

STEP 8 – Fill all 50 mL tubes with enough 95% ethanol to submerge their contents, and store in the freezer (-20°C) or refrigerator (4°C)

APPENDIX C Reminders and Checklists

Avoid looking like this guy

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

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

Checklist: Preparing for Field Sampling

Locality labels: Be sure to…

- ☐ Print labels with correct location information
- ☐ Cut labels into strips

Equipment: Do you have…

- ☐ Trap replacement parts
- ☐ Ice packs in the cooler
- ☐ Extra chemicals (PG and 95% ethanol)
- ☐ Distilled water
- ☐ Map and coordinates uploaded onto GPS
- ☐ Ground beetle data sheets and PDA (if applicable)

Checklist: Collecting Quality Samples

Sample collection: Be sure to…

- ☐ Use waste containers to catch PG solution
- ☐ Double check that your actual location matches the one on the locality label
- ☐ Write collection date and trapID (N, S, E, W) on locality label
- ☐ Remove twigs, leaves and debris from trap
- ☐ Rinse the sample – first with distilled water, then with 95% ethanol
- ☐ Rinse the filter apparatus with 95% ethanol to ensure that all beetles and bycatch are transferred to the Whirl-Pak bag
- ☐ Check Whirl-Pak bag for locality labels and mesh filter
- ☐ Record all metadata (plotID, date, field staff, etc.) on the datasheet or in the PDA
- ☐ Record any irregularities or deviations from procedure that may impact data, e.g., trap damage, flooding in the area, alterations to the trap cover, etc.

Before leaving trap: Check that…

- ☐ Top cup is flush with ground
- ☐ There is no gap between the trap cups and the ground
- ☐ There is no gap between the bottom and top cups
- ☐ Lid spacers are in place
- ☐ There is a 1.5 cm gap between the lid and the ground

Transporting samples: Make sure…

- ☐ Whirl-Pak and Ziploc bags are sealed and upright
- ☐ Cooler is out of direct sunlight and away from extreme temperatures
- ☐ Cooler is secured in vehicle, so that if cannot tip over during driving
- ☐ Samples are transferred promptly to the laboratory upon return from the field

Checklist: Processing Specimens in the Lab

Ethanol rinse: Check that…

- ☐ Ethanol rinse is completed within 24 hours of sample collection
- ☐ No specimens were lost during filtering
- ☐ Date of rinse is recorded in the sorting datasheet or webUI
- ☐ Locality labels are with specimens (in Whirl-Pak bag)
- ☐ Specimens are stored in airtight containers so the ethanol does not leak out

Sorting beetles and bycatch: Be sure to…

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

APPENDIX D Estimated Dates for Onset and Cessation of Sampling

The dates in **Table 12** are based on historic records and are estimates for the start and stop dates of sampling. It is essential that domain staff monitor real-time conditions to determine when to start (and stop) sampling, as described in **Section 4** of this protocol.

Table 12. Estimated dates of historical temperature thresholds

Domain	Start Week	Stop Week (inclusive)	Total No. Weeks	Approx. Calendar Start Date	Approx. Calendar Stop Date
01	17	38	22	23-Apr	17-Sep
02	16	38	24	15-Apr	17-Sep
03,04,07,09, 11,14,17,20	16	38	25	15-Apr	17-Sep
05,10	20	38	19	14-May	17-Sep
06	18	38	20	30-Apr	17-Sep
08,15	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

APPENDIX E Site Specific Common Reliably Identified Ground Beetle Species

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

E.1 DOMAIN 1

Table 13. D01 abundant, easily identifiable ground beetle species.

Site	Species
HARV	*Synuchus impunctatus*

E.2 DOMAIN 2

Table 14. D02 abundant, easily identifiable ground beetle species

Site	Species
	Chlaenius aestivus

E.3 DOMAIN 3

Table 15. D03 abundant, easily identifiable ground beetle species

Site	Species
OSBS	*Pasimachus strenuus*
JERC	*Pasimachus strenuus* *Tetragonoderus intersectus*
DSNY	*Pasimachus strenuus* *Selenophorus fossulatus* *Tetragonoderus laevigatus*

E.4 DOMAIN 10

Table 16. D10 abundant, easily identifiable ground beetle species

Site	Species
CPER	*Cicindela punctulata punctulata* *Cratacanthus dubius* *Cyclotrachelus torvus torvus* *Euryderus grossus* *Pasimachus elongatus*
STER	*Amara carinata* *Cicindela punctulata punctulata* *Cratacanthus dubius*

	Cyclotrachelus torvus torvus *Harpalus amputatus amputatus* *Harpalus pennsylvanicus* *Harpalus reversus* *Poecilus scitulus*
RMNP	*Carabus taedatus agassii*

APPENDIX F Insect Taxonomic Identification Aids

In addition to the teaching collection (details, including instructions for creation and maintenance, in TOS Protocol and Procedure: Ground Beetle and Mosquito Specimen Processing (RD[06]), one or more of the following keys may be useful aids in identifying beetles in a given domain.

F.1 GENERAL KEYS

- Catalogue of Geadephaga (Coleoptera, Adephaga) of America, north of Mexico
  Bousquet Y. 2012. ZooKeys 245: 1–1722.
- The ground-beetles (Carabidae, excl. Cicindelinae) of Canada and Alaska, parts 1—6
  C.H. Lindroth. 1961. Opuscula Entomologica Supplementa XX, XXIV, XXIX, XXXIII, XXXIV, XXXV.
- Pearson, D.L., Knisley, C.B., and Kazilek, C.J. 2006. A Field Guide to the Tiger Beetles of the United States and Canada: Identification, Natural History, and Distribution of the Cicindelidae. Oxford University Press, New York, NY. Available from: http://www.amazon.com/Field-Beetles-United-States-Canada/dp/0195181565

Tiger Beetles of the United States (website: http://www.npwrc.usgs.gov/resource/distr/insects/tigb/index.htm). Coordinated by W. Wyatt Hoback and John J. Riggins

F.2 REGIONAL KEYS

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

APPENDIX G Guide to Distinguishing the Family Carabidae

The suborder Adephaga includes ground beetles and some aquatic groups. Adephagan beetles are easily identified by the manner in which the last pair of legs articulates with the beetle's underside (**Figure 49**), as well as the tarsal formula (5-5-5; **Figure 50**). 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.

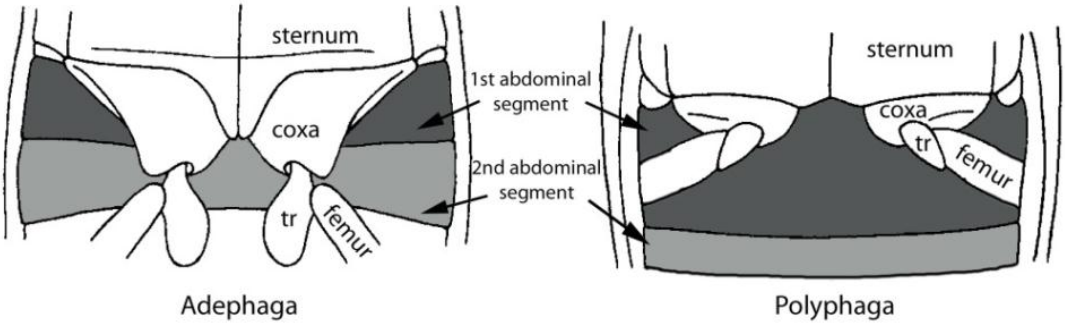

Figure 49. Hind leg articulation in Adephaga and Polyphaga.

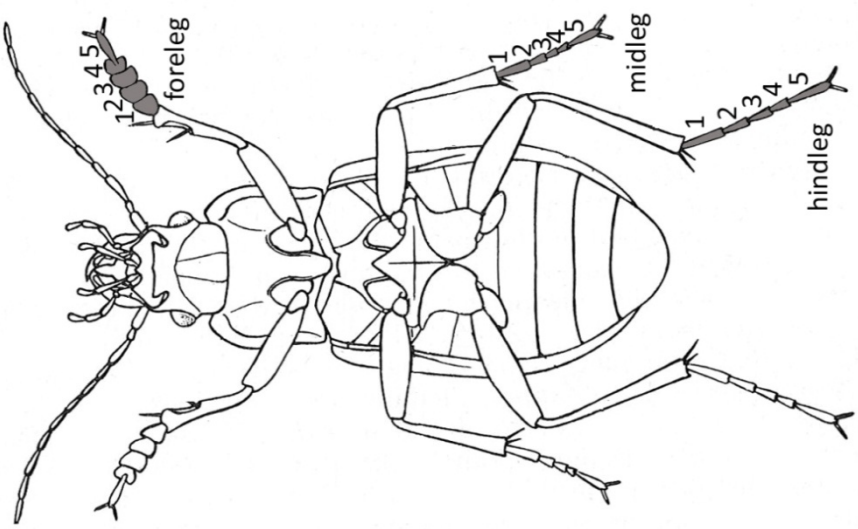

Figure 50. Tarsal formula of adephagan beetles.

Adephagan beetles of the family Carabidae are *usually* black and shiny, although they may also be metallically colored, and have large eyes, powerful legs, and large mandibles that fit their predatory life styles (**Figure 51**). Otherwise these beetles lack easily recognizable and distinct morphological features. However, several other families of adephagan beetles do have distinct morphological features, by which they can easily be identified as *not* carabids. Below are some

descriptions and illustrations of these identifying features (**Figure 52 - Figure 57**), which will allow for the identification of non-carabid adephagans, (a.k.a. invertebrate bycatch).

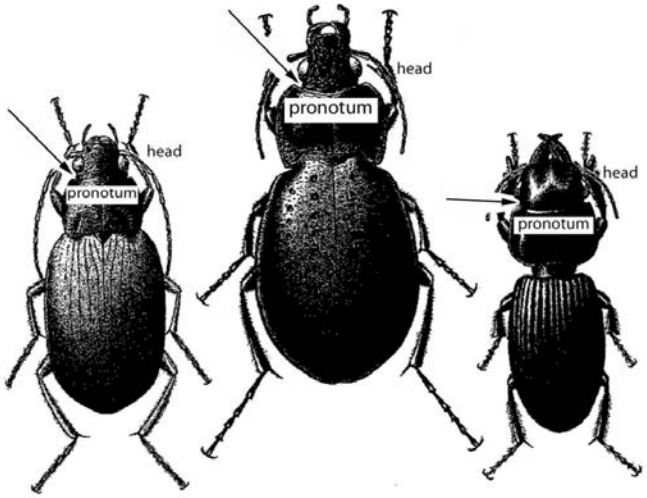

Image source:
http://www.myrmecos.net/2011/05/13/friday-beetle-blogging-a-six-spotted-tiger/

Figure 51. Four ground beetle specimens with head and pronotum labeled (first three) and showing the metallic coloration that is common in tiger beetles.

- Family Gyrinidae:
  Gyrinid beetles are most easily recognized for their divided compound eyes (**Figure 52**). Each of these beetles' eyes has a top (or dorsal) and bottom (or ventral) portion, such that their two eyes actually look like four. In addition, gyrinids have short, paddle-like mid and hind legs (**Figure 53**).

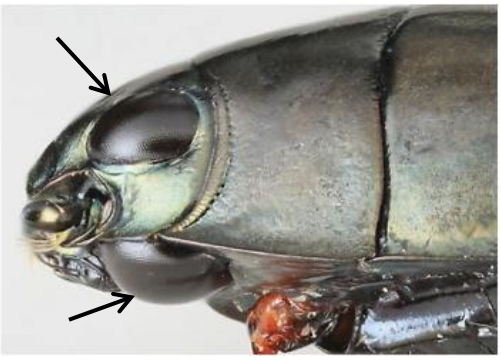

Figure 52. Lateral view of a beetle in the family Gyrinidae, showing dorsal and ventral portions of the compound eye.

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

Figure 53. Dorsal view of beetle in the family Gyrinidae showing mid and hind legs adapted for swimming

- Family Dytiscidae:
  Dytiscid beetles are recognizable by their oval bodies and the long hairs on their hind tibia (**Figure 54**), by which their hind legs become oar-like structures for movement through water.

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

Figure 54. Dorsal view of a beetle in the family Dytiscidae, showing the oval shaped body and long hairs on the hind legs.

- Family Haliplidae:

Haliplid beetles are most easily recognized by their unusually large hind coxal plates(**Figure 55**), which cover most of the ventral side of the abdomen (and actually obstruct the view of the adephagan-specific hind leg articulation described above).

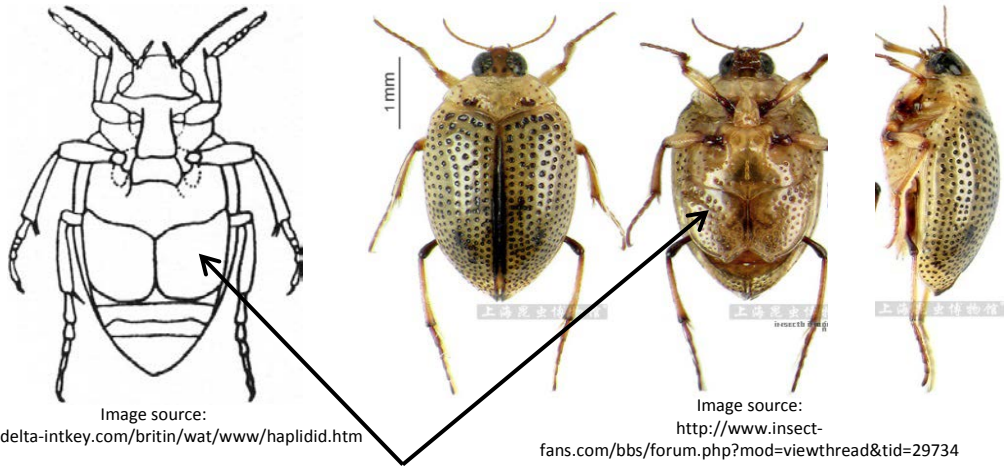

Image source:
http://delta-intkey.com/britin/wat/www/haplidid.htm

Image source:
http://www.insect-fans.com/bbs/forum.php?mod=viewthread&tid=29734

Large hind coxal plate of beetles in the family

Figure 55. Diagram and photo of a haliplid beetle, showing the large coxal plates that are characteristic for this family.

- Family Noteridae:

Noterid beetles have smooth, oval bodies, and range in color from light brown to dark, reddish brown. Their heads are small, and they have a distinct 'noterid' platform, or plate between the second and third sets of legs on their ventral side (**Figure 56**).

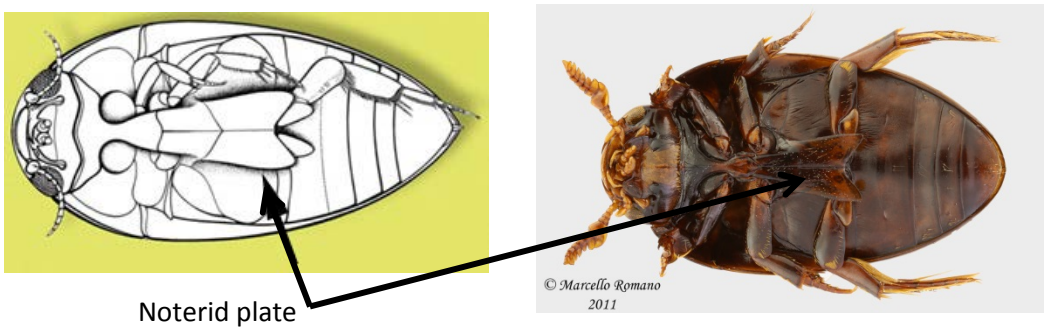

Image source: http://www.inbio.ac.cr/papers/coleoptera/NOTERIIN.html

Image source: http://www.entomologiitaliani.net/public/forum/phpBB3/viewtopic.php?t=19363

Figure 56. Diagram and photo of a noterid beetle, showing the noterid plate between the second and third pairs of legs, on the ventral side.

- Family Rhysodidae:

Rhysodid beetles have elongated bodies, beadlike antennae, protona that narrow to a 'neck' before joining with the head, and heads, protona, and elytra bearing deep longitudinal grooves (this being their most distinguishing feature; **Figure 57**).

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

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

Figure 57. Diagram and photo of a rhysodid beetle, showing the 'neck' where the head and pronotum meet, and the characteristically deeply grooved head, pronotum, and elytra.

APPENDIX H Intermediate and Final Sample IDs per sample type

Table 17 contains information on generating sampleIDs (intermediate and final) for different types of samples generated during beetle sorting. How sampleIDs are generated depends on the type of sample and its fate (e.g., how it will be stored in the long term).

Table 17. Sample IDs for various types of samples generated during beetle sorting

Sample Type	Fate of Sample	Intermediate (associated) sampleID	Final sampleID (sampleID)
Known vert. bycatch	Pooled (by plot) for storage	plotID.trapID.collectDate.taxonID.tubeNumber	plotID.collectDate.taxonID.tubeNumber[1]
Unknown vert. bycatch	Stored (by trap) until positively ID'ed	plotID.trapID.collectDate.vb.tubeNumber	plotID.trapID.collectDate.vb.tubeNumber[2]
Invert bycatch	Pooled (by plot) for storage	plotID.collectDate.ib.tubeNumber	plotID.collectDate.ib.tubeNumber[3]
Known carabid	Pooled (by plot) for storage OR pinned (≤ 20 per site per year)	plotID.trapID.collectDate.taxonID.tubeNumber	plotID.collectDate.taxonID.tubeNumber[1]
Unknown but abundant (≥ 20 individuals) carabid	Pooled (by plot) for storage	plotID.trapID.collectDate.cc.tubeNumber	plotID.collectDate.cc.tubeNumber[1,,3]
Unknown and less abundant carabid	Pinned	plotID.trapID.collectDate.oc.tubeNumber	NA[4]

[1] The tube number in the final sampleID may be different from that in the intermediate sampleID

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

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

[4] Individuals that are pinned will have an individualID, so no final sampleID is required

APPENDIX I Locality Labels

Locality labels are used to identify when and from where a sample was collected. They are used in the field to label traps, and in the laboratory to label whole samples and individual mounted specimens. Locality labels include particular information in order to be consistent with collection records from other global campaigns (see **Figure 58**; details to follow). At NEON we are using locality labels for samples containing ground beetles, mosquitoes and ticks.

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

GEORGIA Baker County. Jones
Ecological Res Ctr. 47m
N31.2034 W84.4631 Pitfall trap
Jul2014. RNelson
JERC_406.

Figure 58. Example label for pitfall trap sample. Size shown is larger than size of actual label.

I.1 COMPILE LABEL DATA

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

    a. Enter label data into a Microsoft Excel spreadsheet (**Figure 59**) with one column for each piece of information required for the locality label, as follows:

        i. Column Header = “State”, content = The state in which the sample was collected, **in all capital letters**

        ii. Column Header = “County”, content = The county in which the sample was collected, with the county name capitalized, followed by “County” (without quotes)

        iii. Column Header = “Period”, content = “.” (without quotes)

        iv. Column Header = “Site”, content = The abbreviated site name. Instructions for generating this abbreviated site name are as follows (with examples in **Table 18**):

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

                Environmental – Env

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

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

C. Generate the abbreviated site name with a period at the end (e.g., 'Jones Ecol Res Ctr.'; examples in **Table 18**)

D. If you are unsure of the abbreviated name, contact NEON Science for verification

Table 18. Examples of site name abbreviations for locality labels

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

v. Column Header = "Period", content = "." (without quotes)

vi. Column Header = “Elevation”, content = The elevation (in meters and to the nearest whole number) of the location at which the sample was collected. *This is provided by NEON Headquarters*

vii. Column Header = “Latitude”, content = The latitude (in decimal degrees and with four digits after the decimal) of the location at which the sample was collected. *This is provided by NEON Headquarters*

viii. Column Header = “Longitude”, content = The longitude (in decimal degrees and with four digits after the decimal) of the location at which the sample was collected. *This is provided by NEON Headquarters*

ix. Column Header = “Trap Type”, content = The type of trap used to collect the sample (Pitfall trap OR CO2 light trap OR drag OR flag (the last three corresponding to mosquitoes and ticks))

x. FOR LABELS FOR PINNING/POINTING/STORING INSECTS ONLY:

   A. Column Header = “Day”, content = The day on which the specimen was collected

xi. Column Header = “Month”, content = The month, abbreviated to three letters (e.g., Jun OR Sep), in which the sample/specimen:

   A. Will be collected, in the case of labels for field use

   B. Was collected, in the case of labels for pinning/pointing/storing insects

xii. Column Header = “Year”, content = The year in which the sample was collected, in numeric format

xiii. Column Header = “Period”, content = “.” (without quotes)

xiv. Column Header = “Collector”, content = The first initial and last name of the field operations manager, with no spaces and the initial and last name capitalized (e.g., RNelson)

xv. Column Header:

   A. “plotID”, for labels for field use, content = the plotID from which the sample will be collected, with a period at the end

B. "Trap Code", for labels for pinning/pointing/storing insects, content = plotID.trapID.collectDate, where collectDate is in the format YYYYMMDD.

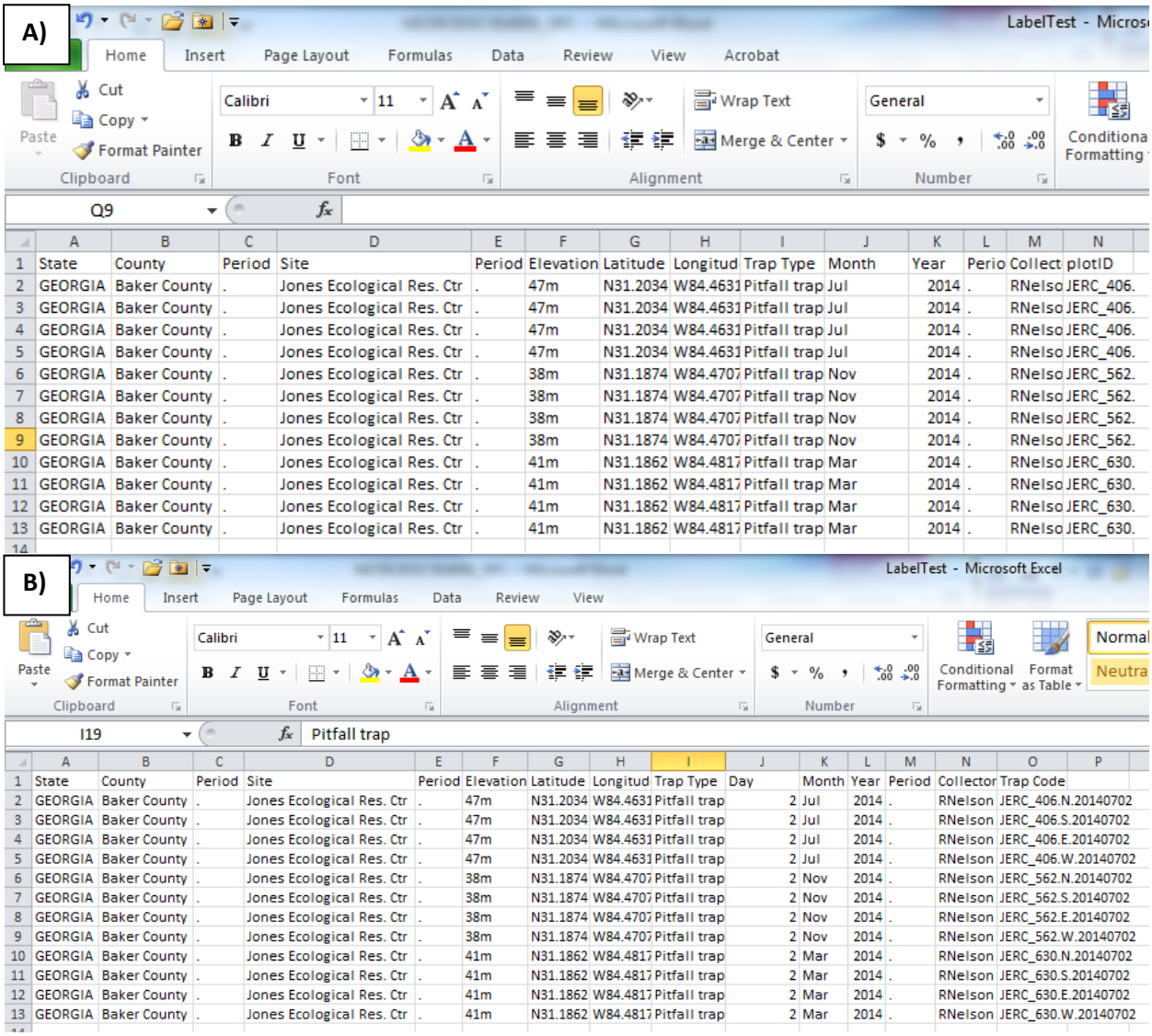

Figure 59. Example of spreadsheets containing information for locality labels, including the "plotID" field for locality labels that will be used in the field (A) and the "Day" and "Trap Code" fields for labels that will be used to pin/point/store insect specimens (B).

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

2. Name the sheet using a meaningful name such as 'BetLoc2014'. Save and close the file – if you do not close the file, the Mail Merge may not work correctly.

    a. *Note – it may be helpful to create one locality label document for labels to be used in the field, and another for labels to be used for pinning/pointing/storing insects. In such cases, name the files appropriately (e.g., 'BetFieldLoc2014' and 'BetPinLoc2014').*

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

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

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

2. In the **Labels** menu select **New Label** and enter information for the dimensions of the label in the "Label Details" box, exactly as in **Figure 60**. Give the template a name that makes it easy to locate when creating a new sheet of labels (e.g. 'insect determination labels').

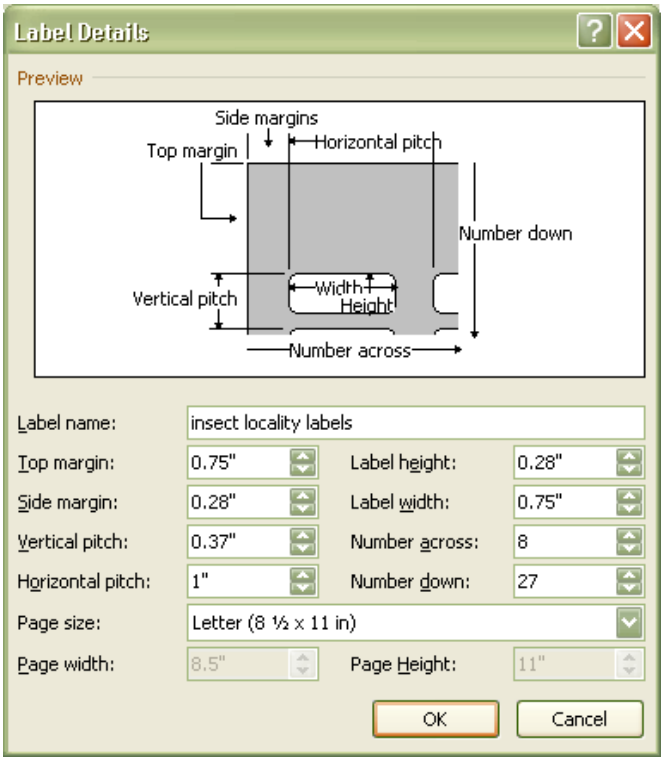

Figure 60. Dimensions for creating a locality label template in Microsoft Word

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

4. A "Select Table" pop-up window (**Figure 61**) will appear, showing a list of the sheets within the Excel workbook created in step (**I.1**). Select the worksheet containing the determination label information. Make sure to check the box that says 'First row of data contains column headers'.

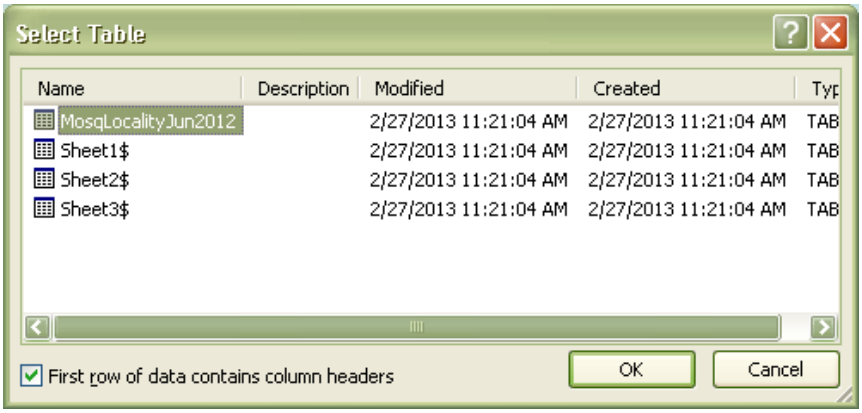

Figure 61. Selecting recipients from a Microsoft Excel file during Mail Merge

I.3 PERFORM FINAL FORMATTING IN MICROSOFT WORD.

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

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

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

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

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

    d. Highlight "Site" in the dropdown, then click "Insert"

    e. Highlight "Period" in the dropdown, then click "Insert"

    f. Highlight "Elevation" in the dropdown, then click "Insert"

g. Highlight “Latitude” in the dropdown, then click “Insert”

h. Highlight “Longitude” in the dropdown, then click “Insert”

i. Highlight “Trap Type” in the dropdown, then click “Insert”

j. FOR LABELS FOR PINNING/POINTING/STORING INSECTS ONLY :

   A. Highlight “Day” in the dropdown, then click “Insert”

k. Highlight “Month” in the dropdown, then click “Insert”

l. Highlight “Year” in the dropdown, then click “Insert”

m. Highlight “Period” in the dropdown, then click “Insert”

n. Highlight “Collector” in the dropdown, then click “Insert”

o. Highlight “plotID” or “Trap Code” in the dropdown, then click “Insert”

p. Click “Close” and you will see that the top left cell contains:

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

OR

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

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

3. Insert line breaks and spaces as follows:

   a. Place the cursor at the end of “«Species»” and hit the spacebar

   b. Place the cursor at the end of “«Species»” and hit the spacebar

   c. Place the cursor at the end of “«Species»” and hit the spacebar

   d. Place the cursor at the end of “«Species»” and hit the spacebar

e. Place the cursor at the end of "«Species»" and hit the spacebar

f. Place the cursor at the end of "«Species»" and hit the spacebar

g. Place the cursor at the end of "««Species»" and hit the spacebar

h. FOR LABELS FOR FIELD USE ONLY:

   A. Place the cursor in front of "«Day»" and hit the spacebar 8 times, in order to ensure that there is space to write in the day of the month

i. Place the cursor at the end of "«Species»" and hit the spacebar

j. Place the cursor at the end of "«Species»" and hit the spacebar

k. The top left label will now contain (notices the spaces, highlighted grey below):

«State» «County»«Period» «Site»«Period1» «Elevation» «Latitude» «Longitude» «Trap_Type»

«Month»«Year»«Period2» «Collector» «Plot_Code»

OR

«State» «County»«Period» «Site»«Period1» «Elevation» «Latitude» «Longitude» «Trap_Type»

«Month»«Month»«Year»«Period2» «Collector» «Plot_Code»

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

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

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

7. Confirm that the first row in each label starts with the state, the date information starts on a new line, and the plotID OR Trap Code starts on a new line.

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

9. Save the document. Use a meaningful name such as 'BetLoc2014'.

a. *Note – it may be helpful to create one locality label document for labels to be used in the field, and another for labels to be used for pinning/pointing/storing insects. In such cases, name the files appropriately (e.g., 'BetFieldLoc2014' and 'BetPinLoc2014').*

10. Copy and paste each label as needed, in order to generate additional locality labels for samples and/or specimens and/or storage containers. Group all labels for the same plotID and/or Trap Code within the same column.

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

11. Re-save the document after editing

I.4 PRINT LABELS

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

2. Determine how many labels are needed for each plot for the selected activity (see **Table 19**). If more than one page of labels per plot are required, print the document multiple times until enough labels are printed.

3. **NOTE:** The calculations and quality checks listed below for field work are based on current plans. If the schedule or frequency of trapping changes, the numbers will have to be recalculated using the following formula:

$$\mathbf{A * B * C = D;\ D/27 = E}$$

where

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

Table 19. Number of labels needed for the difference activities requiring insect locality labels.

Activity	Variables	Label Calculation	Label Sheet Calculation
Beetle field work	A = 3 labels/trap B = 4 traps/plot C = 2 trap collections per month** 10 plots/site	D = 3 labels per trap x 4 traps/plot x 2 collection events = 24 labels per plot E = 24/27 = .88 print 1 strip of labels per plot Print 1 set of labels (10 plots = 120 labels per bout)	Each label sheet contains 8 strips (columns). Using 1 strip per plot, for 10 plots you would need 2 sheets of labels (10 strips / 8 strips per sheet = 1.25 sheets). There are enough labels in each strip to complete 2 bouts (at the core site).
Beetle pinning	1 label/specimen or pooled sample	Print labels as needed. Refer to field datasheet.	

I.5 CUTTING LABELS

Cut labels into long columns using a snap-off blade knife and ruler. There should be no obstructions on the cutting mat or the ruler while doing this. Using a snap-off blade is essential to making clean cuts. Using scissors to make a cut of this length is highly prone to error.

1. Cut labels with as little white space on all sides of the text as possible (**Figure 62**).

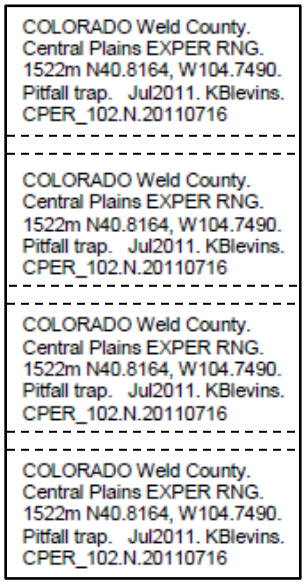

Figure 62. Example of a strip of labels, dashed lines indicate where the labels should be cut (when/if they are cut into individual labels)

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

APPENDIX J Determination Labels

Determination labels are used to label individual specimens (pinned, pointed, or stored individually in tubes or vials) or groups of pooled specimens of the same taxon with a species or morphospecies identification. These labels contain the following three lines of information: 1) The scientific name or morphospecies ID of the individual or group of individuals, 2) the first and middle initials and last name of the person who identified the individual or group of individuals (these following the letters "det."), and 3) the year in which the identification was made (see example in **Figure 63**).

Aedes triseriatus det. J. S. Smith 2013	D10.2013.MorphA det. J. S. Smith 2013

Figure 63. Example determination and morphospecies ID labels; size shown is larger than size of actual labels.

J.1 COMPILE DETERMINATION LABEL DATA

1. Compile the label data in a Microsoft Excel spreadsheet.
    a. Enter label data into a Microsoft Excel spreadsheet (**Figure 64**) with one column for each piece of information required for the determination label, as follows:
        i. Column Header = "Species", content = scientific name or morphospecies ID for the taxon.
            A. For scientific names: text needs to be italicized and with the first letter of the genus capitalized
            B. For morphospeciesIDs: text should not be italicized
        ii. Column Header = "det.", content =the characters "det." (with the period at the end and without quotes)
        iii. Column Header = "Identifier", content =First and middle initials and last name of the person who identified the individual or group of individuals
            A. Initials should be capitalized and followed by a period
            B. Last names should be capitalized
            C. All initials should be separated by a space, and there should be a space between the middle initial and the last name

iv. Column Header = "Year Identified", content =The year in which the identification was made, in numeric format (e.g., 2013)

	A	B	C	D
1	Species	det.	Identifier	Year Identified
2	*Aedes triseriatus*	det.	J. S. Smith	2013
3	*Aedes fulvus pallens*	det.	J. S. Smith	2013
4	*Anopheles perplexens*	det.	J. S. Smith	2013
5	*Cicindela punctulata punctulata*	det.	J. S. Smith	2013
6	*Clivina*	det.	J. S. Smith	2013
7	*Coquillettidia perturbans*	det.	J. S. Smith	2013
8	*Culex erraticus*	det.	J. S. Smith	2013
9	*Culex nigripalpus*	det.	J. S. Smith	2013
10	*Culiseta melanura*	det.	J. S. Smith	2013
11	D3morph_DA	det.	J. S. Smith	2013
12	D3morph_JB	det.	J. S. Smith	2013
13	D3morph_JC	det.	J. S. Smith	2013
14	*Mansonia dyari*	det.	J. S. Smith	2013
15	*Mansonia titillans*	det.	J. S. Smith	2013
16	*Pasimachus sublaevis*	det.	J. S. Smith	2013
17	*Pasimachus punctulatus*	det.	J. S. Smith	2013
18	*Psorophora ciliata*	det.	J. S. Smith	2013
19	*Scarites subterraneus*	det.	J. S. Smith	2013
20	*Tetracha carolina*	det.	J. S. Smith	2013
21	*Uranotaenia sapphirina*	det.	J. S. Smith	2013
22	*Uranotaenia lowii*	det.	J. S. Smith	2013

Figure 64. Spreadsheet containing information for determination labels

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

2. Name the sheet using a meaningful name such as 'BetDet2013'. Save and close the file – if you do not close the file, the Mail Merge may not work correctly.

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

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

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

2. Dimensions for these labels are the same as for locality labels, so the correct label may already exist in the list. If not, in the **Labels** menu select **New Label** and enter information

for the dimensions of the label in the "Label Details" box, exactly as in **Figure 65**. Give the template a name that makes it easy to locate when creating a new sheet of labels (e.g. 'insect determination labels').

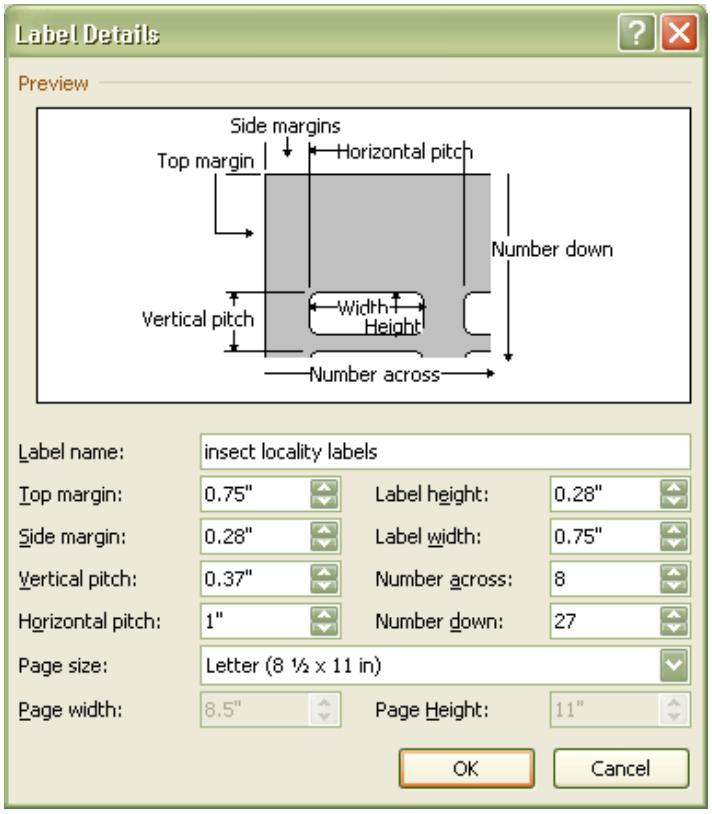

Figure 65. Dimensions for creating a determination label template in Microsoft Word

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

4. A "Select Table" pop-up window (**Figure 66**) will appear, showing a list of the sheets within the Excel workbook created in step (**J.1**). Select the worksheet containing the determination label information. Make sure to check the box that says 'First row of data contains column headers'.

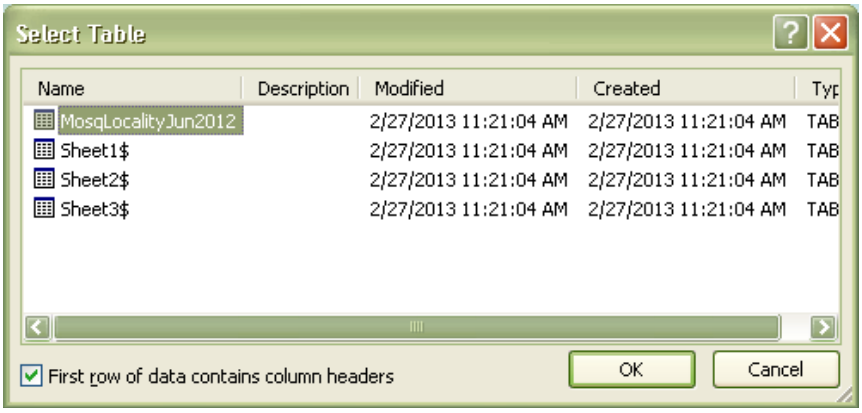

Figure 66. Selecting recipients from a Microsoft Excel file during a Mail Merge

J.3 PERFORM FINAL FORMATTING IN MICROSOFT WORD

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

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

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

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

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

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

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

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

3. Insert line breaks and spaces as follows:

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

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

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

d. The top left label will now contain:

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

4. Highlight the contents of the top left cell and set the font to Arial, font size 3.5.
5. *Note: Formatting such as italics does not get transferred from Microsoft Excel to Microsoft Word during a Mail Merge; italics will have to be applied to scientific names after the labels have been created*
6. After finishing the first cell, click **Update Labels** – this will copy formatting into remaining labels.
7. Preview the labels by clicking on **Preview Results**.
8. Confirm that the first row in each label has either a scientific name OR morphospeciesID, the second line has 'det.' and an identifier's name, and the third row has the identification year.
9. In the **Mail Merge** menu, select **Finish & Merge**, then **Edit Individual Documents**. A pop-up menu will appear. Choose the 'All' option. This will create a Microsoft Word document with the labels.
10. Save the document. Use a meaningful name such as 'BetDet2013'.
11. In the Microsoft Word document, italicize scientific names (but not morphospeciesIDs).
12. Copy and paste each label as needed, in order to generate the required number of labels for specimens of each taxon. Group all labels for the same taxon within the same column.
13. Re-save the document after editing

J.4 PRINT LABELS

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

APPENDIX K IndividualID Labels

individualID labels are used to label individual (pinned, pointed, or stored individually in tubes or vials) specimens from a specific location and sampling period. The label format is: MOD.DXX.######, where MOD is the module (in this case "BET" for beetles), XX is the domain number, and the number contains 6-digits (see example in **Figure 67**).

BET.D10.000139

Figure 67. IndividualID label for a beetle specimen

K.1 INDIVIDUALID SPECIMEN LABELS

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

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

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

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

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

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

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

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