FIELD AND LAB PROTOCOL: MOSQUITO PHENOLOGY, ABUNDANCE, DIVERSITY AND INFECTIOUS DISEASE

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
-	03/30/2011	ECO-00159	Initial Draft Release
A_DRAFT	09/23/2011	ECO-00280	Update to new document number's/template throughout document.
B_DRAFT	07/30/2012	ECO-00442	Update Draft Release for Mosquito 2012.
C	01/10/2014	ECO-01139	Initial release. Will update to TOS template in next rev.

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1 DESCRIPTION

1.1 Purpose

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

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

1.2 Scope

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

- general safety practices
- site-specific safety practices
- general equipment maintenance

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

1.3 Acknowledgements

Dr. Cara Gibson, Kali Blevins, and Patrick Travers contributed significantly to earlier versions of this protocol.

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

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

AD [01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD [02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD [03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD [04]	NEON.DOC.001155	NEON Training Plan
AD [05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD [06]	NEON.DOC.000910	NEON Science Design for Mosquito Abundance, Diversity, and Phenology
AD [07]	NEON.DOC.000911	NEON Science Design for Vectors and Pathogens
AD [08]	NEON.DOC.001100	NEON Lab Protocols: Ground Beetle and Mosquito Specimen Processing
AD [09]	NEON.DOC.005003	NEON Level 0 Data Products Catalog
AD [10]	NEON.DOC.014002	FSU Science Requirements

2.2 Reference Documents

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

RD [01]	NEON.DOC.000008	NEON Acronym List
RD [02]	NEON.DOC.000243	NEON Glossary of Terms
RD [03]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD [04]	NEON.DOC.014051	Field Audit Plan
RD [05]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan

2.3 Acronyms

CDC	U.S. Centers for Disease Control and Prevention
BOLD	Barcode of Life Database
TEA	Triethylamine

2.4 Definitions

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

provide materials in support of skills acquisition in the topic areas including information on how to best train staff rather than detailing only the steps of the procedure.

3 BACKGROUND AND OBJECTIVES

3.1 Background

Mosquitoes are a diverse and widespread family of insects that have been extensively studied because of their ecological and epidemiological significance. As a dominant taxon in aquatic food webs, mosquitoes comprise a sizable proportion of the invertebrate biomass and act as a key food source for aquatic and terrestrial predators (e.g., fish, amphibians, spiders, birds). Mosquitoes also act as vectors for numerous parasites and pathogens of humans, livestock, and wildlife, and their biology and ecology have been extensively studied to characterize and mitigate impacts of associated diseases. Because of their sensitivity to environmental gradients and perturbations mosquitoes represent an ideal sentinel taxon for evaluating the ecological effects of global change phenomena. Their short generation time and high fecundity allow mosquitoes to respond quickly to environmental change, but because of the group's high diversity and varied ecological niches the nature and magnitude of these changes can differ markedly among species. Changes in global climate are predicted to affect the distribution, demography, and seasonal phenology of many mosquitoes, and associated effects on pathogen transmission cycles have also been posited. Because of their frequent association with humans and ability to thrive in human-modified environments, mosquito ecology is also likely to be significantly affected by land use changes. Based on these reasons mosquitoes were selected as a sentinel (focal) taxon to be monitored within the National Ecological Observatory Network.

3.2 NEON Science Requirements

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

3.3 NEON Data Products

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

4 PROTOCOL

Mosquito sampling involves the collection of mosquitoes, primarily using CDC CO_2 light traps. A CO_2 light trap consists of (listed from the top to the bottom of the assembled trap): a cylindrical insulated cooler (that holds dry ice), a plastic rain cover attached to a light/fan assembly (battery-powered), and a mesh collection cup. During deployment, dry ice in the insulated cooler releases CO_2 as it sublimates, and this gas attracts mosquitoes to the vicinity of the trap. Some mosquitoes may also be attracted to the trap by the light. The battery-powered fan sucks these mosquitoes into the mesh collection cup, where they remain until the trap is collected.

Following minimal in-house processing samples will be sent to one or more external facilities where mosquitoes will be identified to species to characterize patterns of mosquito abundance, diversity, and phenology at NEON sites. A subset of identified mosquitoes will be tested for infection by pathogens to quantify the presence/absence and prevalence of various arboviruses. Some mosquitoes will be set aside for DNA barcode analysis as well as for long-term archiving.

5 QUALITY ASSURANCE AND CONTROL

The procedures associated with this protocol will be audited according to the Field Audit Plan (RD[05]). Additional quality assurance will be performed on data collected via these procedures according to the Data and Data Product Quality Assurance and Control Plan (RD[06]).

Because of the wide range and variance of mosquito abundance and pathogen prevalence, algorithms that check mosquito data for irregularities may catch some errors but will not be a dependable way to fully quality control mosquito data from the field. Following the protocols exactly at each domain will be required to ensure data quality and this can only be known by conducting "hot checks" where someone who knows the protocols well goes out into the field with technicians and observes their data collection. For work done by external laboratories, QA/QC plans will be developed based on pre-existing laboratory protocols modified as needed to meet NEON requirements.

When unexpected field conditions require deviations from the field protocols outlined in this document, contingent decisions outlined in Table 1 and Table 2 below should be followed in the interest of maintaining data quality.

Table 1. Contingent decision for spatial sampling

Delay Duration	Action	Adverse Outcome?	Outcome for Data Products
<3 hours	Resume/continue with normal sampling at conclusion of delay. (Note the duration and cause of the delay)	None, but note that excessively delayed retrieval of mosquitoes from traps increases the likelihood of mosquito mortality, especially under hot/dry and wet conditions. Dead mosquitoes are more difficult to test for pathogens.	Quality of samples reduced, creating potential for complications with processing (identification, pathogen testing).
3 hours to 1 day	Scenario 1: If the delay occurs prior to trap deployment and prior to the start of the sampling bout then push the start date for the bout back one day. Scenario 2: If the delay occurs after the initial deployment of traps during a bout but prior to the collection/resetting of traps, then repeat any missed trapping on the subsequent	Consistent temporal interval of time series data interrupted.	Compromise statistical analysis of temporal trends in the data

Delay Duration	Action	Adverse Outcome?	Outcome for Data Products
	day. In both cases, a) do not adjust (push back) dates for subsequent sampling bouts, and b) note the duration and cause of the delay		
1-14 days	Scenario 1: If the delay occurs prior to trap deployment and prior to the start of the sampling bout then push the start date for the bout back for the duration of the delay. Scenario 2: If the delay occurs after the initial deployment of traps during a bout but prior to the collection/resetting of traps, then repeat the entire sampling bout at the conclusion of the delay. In both cases, a) do not adjust (push back) dates for subsequent sampling bouts, and b) note the duration and cause of the delay	Consistent temporal interval of time series data interrupted.	Compromise statistical analysis of temporal trends in the data

Table 2. Contingent decision for field season sampling

Delay Duration	Action	Adverse Outcome?	Outcome for Data Products
<3 hours	Resume/continue with normal sampling at conclusion of delay. (Note the duration and cause of the delay)	None, but note that excessively delayed retrieval of mosquitoes from traps increases the likelihood of mosquito mortality, especially under hot/dry and wet conditions. Dead mosquitoes are more difficult to test for pathogens.	Quality of samples reduced, creating potential for complications with processing (identification, pathogen testing).
3 hours to 1 day	Scenario 1: If the delay occurs prior to trap deployment and prior to the start of the sampling bout then push the start date for the bout back one day. Scenario 2: If the delay occurs after the initial deployment of traps during a bout but prior to the collection/resetting of traps, then repeat any missed trapping on the subsequent day. In both cases, a) do not adjust (push back) dates for subsequent sampling bouts, and b) note the duration and cause of the delay	Consistent temporal interval of time series data interrupted.	Compromise statistical analysis of temporal trends in the data
1-7 days (core), 1-14 days (relocatable)	Scenario 1: If the delay occurs prior to trap deployment and prior to the start of the sampling bout then push the start date for the bout back for the duration of the delay. Scenario 2: If the delay occurs after the initial deployment of traps during a bout but prior to the collection/resetting of traps, then repeat the	Consistent temporal interval of time series data interrupted (moderately).	Compromise statistical analysis of temporal trends in the data

Delay Duration	Action	Adverse Outcome?	Outcome for Data Products
	entire sampling bout at the conclusion of the delay. In both cases, a) do not adjust (push back) dates for subsequent sampling bouts, and b) note the duration and cause of the delay		
>7 days (core), >14 days (relocatable)	Cancel the impacted sampling bout and stop sampling until next scheduled sampling bout. Contact associated TOS staff scientists. Note duration and cause of the delay.	Consistent temporal interval of time series data interrupted (maximally)	Reduction in sample size as sampling bouts are missed

6 SAFETY

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

Follow guidelines provided in AD[02] to prevent mosquito bites. If used, insecticide must be applied at least 30 minutes prior to arriving in the field. If using insecticide in spray form do not apply in the vicinity of sampling equipment. After applying insecticide clean the palms of hands (e.g., with soap/water or alcohol swabs) before handling any sampling equipment.

7 PERSONNEL REQUIREMENTS

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

8 TRAINING REQUIREMENTS

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

9 FIELD STANDARD OPERATING PROCEDURE

9.1 Sampling Frequency and Timing

There are three distinct types of sampling associated with mosquito trapping.

1. Spatial sampling: this will occur during the first year of sampling at each site to provide data on mosquito abundance and diversity used to determine the permanent locations of mosquito sampling plots. In subsequent years mosquito sampling will consist of a combination of field season and off season sampling. The basic trapping techniques are exactly the same, but spatial sampling allocates more sampling effort to spatial versus temporal coverage. During spatial sampling at each site up to 30 plots will be sampled once per month for three consecutive spring/summer months. CO_2 light traps will be associated with Distributed Plots. During each sampling event mosquito sampling will involve ~40 continuous hours of sampling using one CDC CO_2 light trap at each plot. Plots will be randomly located in each of the major vegetation types ($\geq$10% of total cover), with the number of plots per vegetation type proportional to the percent cover of that type at the site. The specific timing of these activities depends on local patterns of seasonal phenology.

During a bout of spatial sampling traps will be initially deployed as close as possible to dusk (but no earlier than 4 hours before sunset) on the first day of the bout (Figure 1). Traps will be checked (full catch cups retrieved and replaced with new/empty catch cups) and reset (coolers refilled with dry ice) as close as possible to dawn (but no later than 4 hours after sunrise) on the second day of the bout. Traps will be checked and reset again as close as possible to dusk (but no earlier than 4 hours before sunset) on the second day of the bout. Traps will be checked and retrieved as close as possible to dawn (but no later than 4 hours after sunrise) on the third day of the bout.

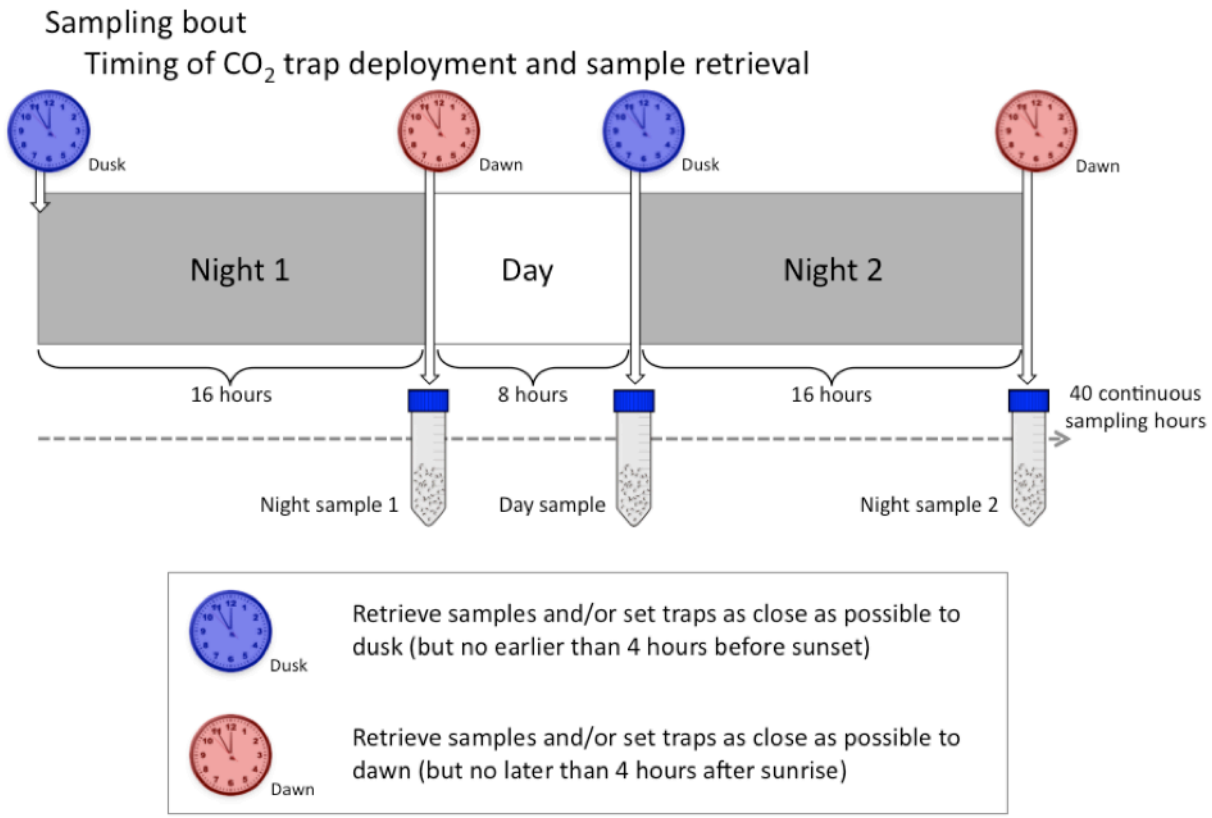

Figure 1. Mosquito trap servicing during a bout of spatial or field season sampling. Trapping occurs during a ~40 hour window, including two nights and the intervening day. This involves 4 trips to each sampling plot.

2. Field season sampling: Within a domain, bouts of field season sampling will occur every two weeks at the core site and every four weeks at relocatable sites (alternating between relocatable sites) such that sampling is conducted at one site every week (Figure 2). Sampling will be conducted until specified collection thresholds that designate the end of field season sampling and beginning of off-season sampling are crossed (Figure 3, Figure 4). A single bout of field season sampling will involve ~40 continuous hours of sampling using one CDC CO_2 light trap at each of 10 plots at a site (Figure 1, described below). CO_2 light traps will be associated with TOS Distributed Plots.

During a bout of field season sampling, traps will be deployed and initially set as close as possible to dusk (but no earlier than 4 hours before sunset) on the first day of the bout (Figure 1). Traps will be checked (full catch cups retrieved and replaced with new/empty catch cups) and reset (coolers refilled with dry ice) as close as possible to dawn (but no later than 4 hours after sunrise) on the second day of the bout. Traps will be checked and reset again as close as possible to dusk (but no earlier than 4 hours before sunset) on the second day of the bout. Traps will be checked and retrieved as close as possible to dawn (but no later than 4 hours after sunrise) on the third day of the bout.

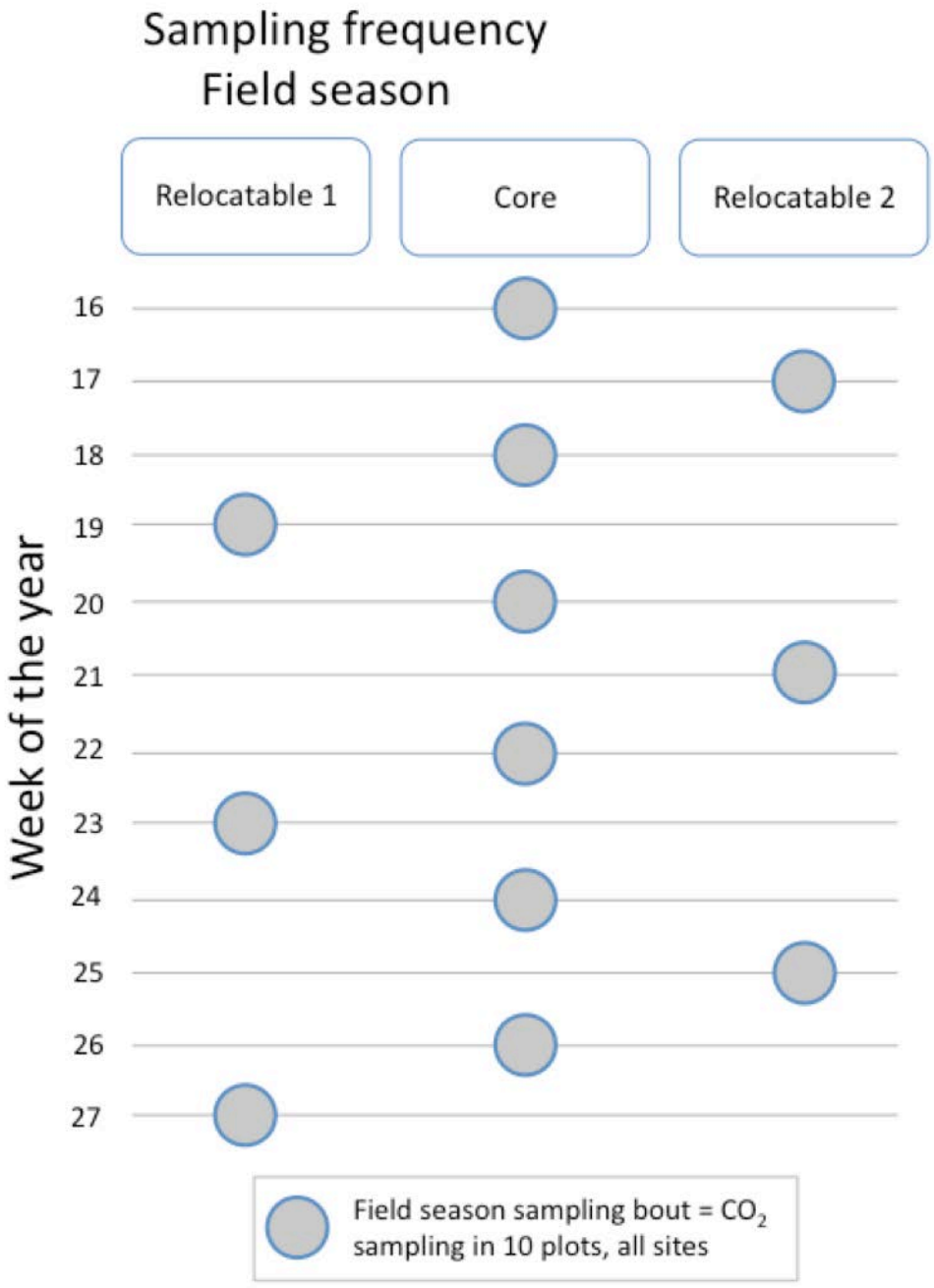

Figure 2. Sampling occurs at one site each week. Mosquitoes are sampled every other week at the core site and every four weeks at locatable sites. The number of weeks in the field season varies among domains.

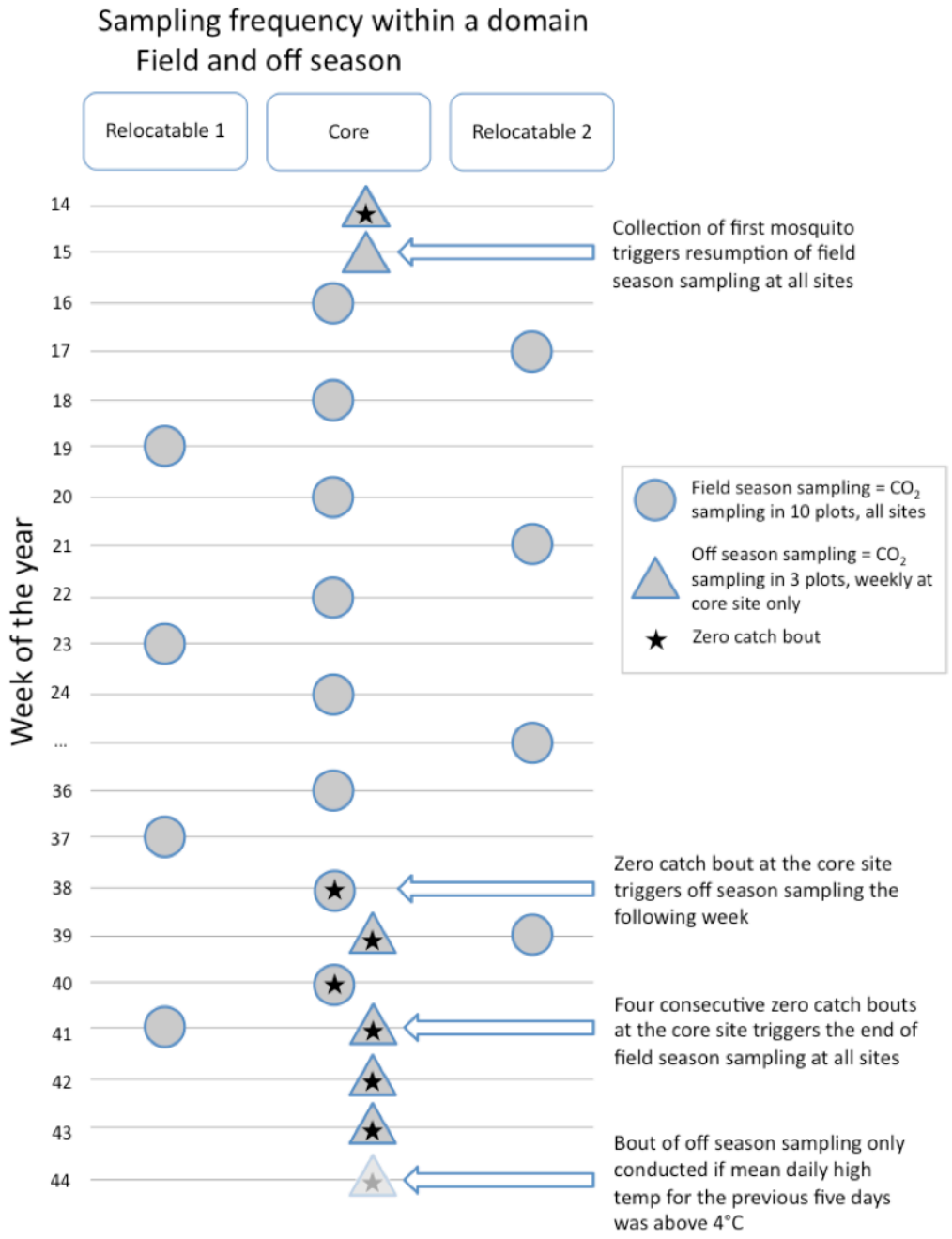

Figure 3. Schedule of field and off season sampling, including transitions between the two

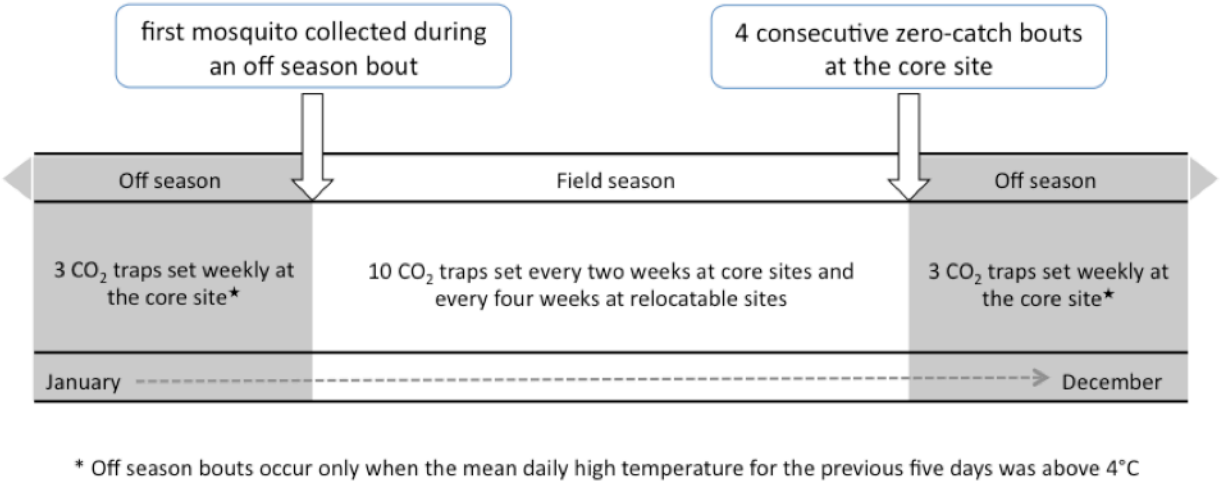

Figure 4. Mosquito sampling timeline for a representative domain.
Trapping occurs all year at the core site, with more traps during the growing season (i.e., warm months when mosquitoes are most abundant and active). During the field season, both the core and relocatable sites are sampled using ten traps per trapping bout. After 4 consecutive zero-catch bouts at the core site, off season trapping (3 traps set weekly at the core site) commences until mosquitoes are detected the following field season.

3. **Off season sampling:** Off season sampling is conducted exclusively at core sites for the purpose of determining the start of field season sampling each year. Information from this sampling can also be used to measure changes from year to year in mosquito phenology (the timing of life cycle events). A bout of off season sampling is conducted in the week following the first zero-catch bout of field season sampling at the core site. The following week a bout of field season sampling is conducted as scheduled. If all three of these are zero-catch bouts a second bout of off season sampling is conducted in the following week. If this results in the fourth consecutive zero-catch week then field season sampling stops and off season sampling continues (only at the core site). Off season sampling will be conducted weekly but a given bout will only occur if the mean daily high temperature for the previous 5 days was above 4°C. Off-season sampling will continue until the first mosquito is collected during an off season bout. This will mark the resumption of field season sampling at all sites in the associated domain (Figure 3, Figure 4).

During a bout of off season sampling traps will be deployed as close as possible to dusk (but no earlier than 4 hours before sunset) on the first day of the bout and retrieved as close as possible to dawn (but no later than 4 hours after sunrise) on the following day. In contrast to spatial and field season sampling, a bout of off season sampling involves only a single night of trapping.

9.1.1 Criteria for Determining Sampling Dates

In the first year of sampling the three monthly sampling bouts associated with spatial sampling occur once per month for three consecutive spring/summer months. The onset of sampling will likely be dictate by domain-specific hiring timelines but would ideally occur in June. The second and third bouts

of spatial sampling should occur as close as possible to one and two months later, respectively, in July and August.

9.2 Equipment and Materials

Table 3. Field Equipment List

Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
	Handheld GPS		No	No
MX103541	CO_2 light trap, battery-powered fan assembly	30	No	No
MX100695	CO_2 light trap, cylindrical insulated cooler	30	No	No
MX100694	CO_2 light trap, mesh collection cup	32	No	No
	Dry ice (pellet form recommended)	1.5kg per trap for 12-18 hours of sampling	No	Yes
	Gloves to handle dry ice		No	No
	Tape (masking recommended)		No	No
	Shepherd's hook	Up to 30	Yes	No
	Rope		No	No
	Scissors		No	No
	Aluminum foil		No	No
	Battery (for CO_2 light trap)		No	No
	Paper towels		No	No
	Resealable freezer bags (gallon size recommended)		No	No
	Razor-blade knife		No	No
	Safety pins		No	No
	Insulated cooler(s)		No	No
	Cardboard cards or cloth		No	No
	Printing paper (for datasheets and labels)		No	No
	Pencils		No	No
	Pens with archival, ethanol-safe ink		No	No

* Quantities are the minimum required to implement protocols

9.3 Preparation

1) Prior to a sampling bout:
   a) For each CO_2 light trap, remove the rain cover and make sure that in the red circuit assembly the first switch is in the closed position and the second and third switches are in the open position (Figure 5). On this setting the trap fan remains on at all times and the light comes on at dusk and shuts off at dawn.

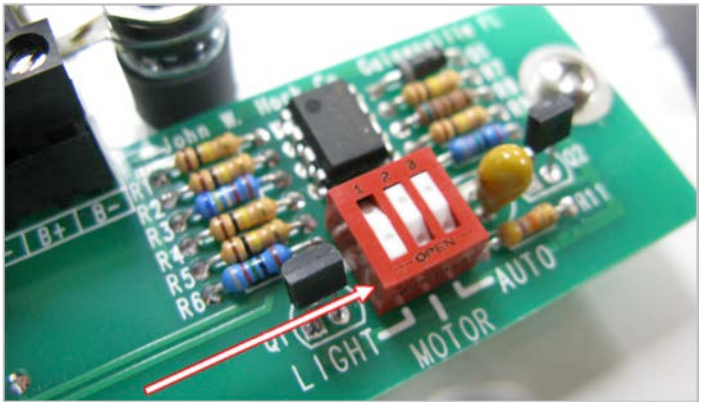

Figure 5. Picture showing CO2 light trap circuit assembly switches in the closed-open-open position

b) Identify the locations of distributed plots used for mosquito sampling access routes.

c) Test all trap components for proper functionality. This includes making sure that electronics are working (e.g., fan turns on when connected to a battery) and mesh of collection cup sleeves is not torn.

d) Make sure batteries are charged or charging (see appendix D for battery charging instructions).

e) Make sure that reusable ice packs are frozen or being frozen.

2) Print datasheets if PDA is not available (see Mosquito Sampling Datasheet, Appendix A).

3) Prepare locality labels:

a) Prepare template to print 0.28 in x 0.75 in label.

b) Prepare data for label.

Table 4 Required Information for Locality Label

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

c) Print labels.(see AD [08] for additional detail on locality labels).

GEORGIA Baker County. Jones Ecological Res Ctr. 38m
N31.1874 W84.4707 Pitfall trap
Aug2013. RNelson
NEON.JERC.000562.

Figure 6 Example of locality label

4) Just prior to heading to the field for sampling:
   a) Obtain enough dry ice to be able to fill the cylindrical insulated cooler of each trap (e.g., ~1.5kg of ice in pellet form). To ensure that enough dry ice will be available in spite of sublimation that will occur between ice pickup and trap deployment consider the duration of this period and the manner in which dry ice is stored and transported to the field. If coolers are filled individually in the lab and transported to the field with dry ice already in them, cover the vent hole on the bottom of each cooler with tape.
   b) If used insecticide must be applied at least 30 minutes prior to arriving in the field. If using insecticide in spray form do not apply in the vicinity of sampling equipment. After applying insecticide clean the palms of hands (e.g., with soap/water or alcohol swabs) before handling any sampling equipment.

9.4 Sample Collection in the Field

9.4.1 Setting Traps

1) Travel to the sampling plot using maps and/or a handheld GPS as necessary.
2) To set a trap hang it from a natural structure (e.g., a tree branch) or installed post (e.g., a Shepherd's hook) such that the height of hole in the bottom of the insulated cooler is five to six feet (1.52-1.83m).
   a) If possible deployment locations should be adjacent to and on the west side of elevated vegetation to allow shading from the morning sun and on the leeward side to afford protection from prevailing winds as these locations provide protection from the elements.
3) Hang the trap's insulated dry ice container from the elevated external structure and use the clip on the underside of the cooler to attach the rain guard/fan assembly. Secure the mesh collection cup to the lower end of the fan assembly using the elastic band sewn into the mesh.
4) Connect the fan to the power source by color-matching the wire leads and the battery terminals (red to '+' terminal, black to '-' terminal). The fan should immediately come on but because the light is activated by a light-sensitive switch it will not turn on until ambient light levels are sufficiently low.
5) Tie the battery cord once around the natural feature or Shepherd's hook from which the trap is suspended. Place the battery in a resealable freezer bag to keep it dry. If necessary wrap the battery cord with aluminum foil as necessary to provide protection from chewing animals (e.g., if cattle have access to sampling plots).
6) Remove any tape covering the hole in the underside of the insulated dry ice container.
7) Attach three locality labels to the catch cup, using safety pins to secure the labels to the nylon cuff of the mesh sleeve and not through the mesh itself.

8) Record appropriate information about the visit to the sampling plot on the field datasheet.

9.4.2 Retrieving samples from Traps

1) Travel to the sampling plot using maps and/or a handheld GPS as necessary.
2) With the fan still running carefully remove the collection cup from the fan assembly by sliding the mesh sleeve off of the fan assembly. The running fan ensures that the mosquitoes cannot escape from the collection cup as it is being removed from the fan housing. Gently tap flying mosquitoes down towards the bottom of the sleeve before separating the collection cup from the fan assembly.
3) Once separated from the fan assembly, cinch the top of the sleeve closed and tie off the drawstrings sewn into the mesh to seal the collection cup sleeve. Be careful not to crush any mosquitoes while removing the collection cup and tying the drawstrings. If possible gently stuff/tuck sleeve material into the hole cut into the top of the plastic cup but only to the extent that this does not crush mosquitoes.
4) Ensure a triplicate locality label is still attached to the collection cup.
5) Record appropriate sampling information on the field datasheet.
6) Use paper towels (recommended) to remove any water that has accumulated in the plastic portion of the collection cup prior to placing it an insulated cooler containing dry ice.
7) Place the catch cup into an insulated cooler containing dry ice. Be sure to place a cardboard card or cloth (recommended) between the catch cups and the ice so that they do not come into direct contact. Once frozen, samples must remain frozen until being transferred into a -80°C freezer at the domain lab.

9.5 Sample Preservation

1) Keep the samples frozen to ensure necessary sample preservation.
2) Once frozen by being placed into an insulated cooler containing dry ice samples must remain frozen at all times including during and after transfer into sampling storage vials.
3) Transfer samples into ultralow freezer as soon as possible upon return to the domain lab.

9.6 Data Handling

1) Enter data from field datasheets into the database provided by CI within seven days of returning to the lab after a bout of data collection.
2) Scan datasheets and save in PDF file format.
3) Save paper copy of datasheets.

9.7 Refreshing the Sampling Kit

1) Test traps to verify that they are still fully functional.
2) Refreeze ice packs.
3) Charge batteries (see recommendations in Appendix B).
4) Print new datasheets as needed.

9.8 Equipment Maintenance, Cleaning and Storage

1) If collection cups are wet or dirty following trapping, gently wash them by hand using fragrance-free laundry detergent and hang to dry. Make sure all collection cups are clean and free of insect parts.
2) If the catch bag is damaged or torn it should be replaced as captured mosquitoes are able to escape through holes in the mesh.
3) Clean any other equipment as necessary using dilute laundry detergent and a dish sponge.
4) Make sure all equipment is dry before placing it in storage.

10 LABORATORY STANDARD OPERATING PROCEDURE: SAMPLE PREPARATION

10.1 Sample Preparation Timing

10.2 Equipment and Materials

Table 5. Laboratory Equipment List

Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
	Battery charger		No	No
	Plastic bins for battery secondary containment		No	No
	Printing paper (for datasheets and labels)		No	No
	Pens with archival, ethanol-safe ink		No	No
	Centrifuge tubes (50 mL volume recommended)		No	No
	Centrifuge tubes (15 mL volume recommended)		No	No
	Tube racks for 50 ml centrifuge tubes (recommended)		No	No
	Tube racks for 15 ml centrifuge tubes (recommended)		No	No
	Forceps		No	No
	Tissues paper		No	No
	Fragrance-free laundry detergent		No	No
	Dish sponge		No	No

* Quantities are the minimum required to implement protocols

10.3 Preparation

1) Prior to processing samples clear and clean off bench space.

10.4 Sample Preparation in the Lab

1) Following return from the field samples can be processed directly out of the insulated cooler containing dry ice or transferred into a -20°C or -80°C freezer and processed from there.
2) Identify the first catch cup to be processed. Remove the triplicate locality labels attached to the catch cup.
3) Obtain enough sample vials to store mosquitoes from the catch cup.
4) Externally label each sample vial with a mosquito pooled sample unique number label (see RD[06]). If multiple vials are needed to accommodate samples collected in a single trap all of those vials should be marked with the identical label, which should be followed by a notation indicating of the number of vials (e.g., 1/3 indicating the first of three vials). We recommend labeling the body rather than the lid of the vial.

Figure 7. Mosquito pooled sample vial label

5) Record the sample vial external label information on the field datasheet.
6) Place up to three locality labels from the triplicate locality label removed from the catch cup into each empty sample vial. If more than one sample vial is needed to accommodate the mosquitoes in the catch cup distribute the locality labels among these vials. Print extra locality labels as needed so that every sample vial contains at least one locality label.
7) Place the empty sample vial(s) in a freezer for a period of time sufficient for the walls of the vial(s) themselves to become frozen to avoid transferring heat from warm vials to frozen mosquitoes.
8) Once the empty sample vial(s) is/are frozen (and each is externally labeled and contains a locality label) remove the vials from the freezer and the catch cup from the insulated cooler containing dry ice.
9) Transfer mosquitoes from the catch cup into the frozen sample vial(s). This must be done quickly to ensure that samples remain frozen at all times. Do not remove any bycatch. Depending on the sample vial used a funnel (e.g., a cone made by asymmetrically rolling up a sheet of paper) is recommended to expedite transfer. Consider positioning sample vials upright in a small cardboard box or beaker filled with dry ice.
10) Once all of the mosquitoes from the collection cup have been transferred into the sample vial(s) place part or all of a piece of tissue paper into the top of each vial. This will prevent samples from shifting and being damaged during subsequent handling and shipping.
11) Seal the sample vials and place them immediately into a -80°C freezer. Samples must remain frozen at all times.
12) Make sure to practice proper sample inventory techniques so that the storage location of a sample can be determined unambiguously at all times.

10.5 Sample Preservation

After each sample is processed, transfer the storage vial into a -80°C freezer.

10.6 Sample Shipping

1) Ship samples to external facilities for (e.g., for taxonomic identification and pathogen testing) on dry ice via an overnight delivery service.
2) Specific instructions for sample shipping will be provided.

10.7 Data Handling

1) Enter data from datasheets into the database provided by CI within seven days of returning to the lab after a bout of data collection.
2) Scan datasheets and save in PDF file format.
3) Save paper copy of datasheets.

10.8 Equipment Maintenance, Cleaning and Storage

1) Clean off the table where sorting activities were performed.
2) Clean the sorting dishes with ethanol and wipe dry.
3) Put away all supplies in their designated storage locations.
4) All materials should be put away in clearly marked receptacles or cabinets after each bout of laboratory work.
5) All flammables and chemicals must be returned to the appropriate cabinets.

10.9 Mounting Specimens and Preparing Samples for Genetic Analysis

Detailed instructions for mounting specimens and preparing samples for genetic analyses are provided in AD [08]. Pointing involves mounting specimens on a small paper triangle on a pin.

After mosquitoes have been identified to species by a taxonomist, a subset of mosquitoes will be submitted to a lab for DNA barcoding. The process of DNA barcoding involves removing a leg to submit as a tissue sample and requires a matching physical voucher specimen. The voucher specimen must be both pointed and photographed.Prepare and submit a subset of mosquitoes for DNA barcoding.

The process of DNA barcoding involves 3 steps: (1) pointing mosquitoes, (2) removing a leg to submit as a tissue sample, and (3) photographing mosquitoes (not necessarily in that order). These steps are essentially the same for both ground beetles and mosquitoes. Consider doing ground beetle and mosquito barcoding at the same time and refer to the detailed instructions in the Common Insect Lab Protocol(AD [08]). Specific instructions on pointing, tissue sample removal and photography of mosquitoes are described therein and will not be repeated here.

10.10 Pointing

Because a physical specimen is required for every individual submitted for barcoding, the same individual mosquitoes selected for DNA barcoding need to be pointed. Pointing involves mounting the mosquito on a small paper triangle on a pin. Detailed pointing instructions are provided in the Common Insect Lab Protocol (AD [08]) and will not be repeated here. All mosquitoes that need to be mounted on pins will be pointed.

10.11 Photographing

Similar to pointing, all mosquitoes that are submitted for DNA barcoding must be photographed. Detailed instructions for photographing pinned/pointed insects are provided in the Common Insect Lab Protocol (AD[08]) and will not be repeated here.

Appendix A MOSQUITO SAMPLING DATASHEET

NEON mosquito sampling, spatial sampling *page ____of____*

Locality:__________________________ Collector:________________________________

Plot ID	Protocol step	eventDate (YYYY/MM/DD)	Time (24 HR)	Fan on?	Catch cup on?	Dry ice left?	Label ID
	1st deployment						
	1st collection, 2nd deployment			Y / N	Y / N	Y / N	
	2nd collection, 3rd deployment			Y / N	Y / N	Y / N	
	3rd collection			Y / N	Y / N	Y / N	
Remarks							
	1st deployment						
	1st collection, 2nd deployment			Y / N	Y / N	Y / N	
	2nd collection, 3rd deployment			Y / N	Y / N	Y / N	
	3rd collection			Y / N	Y / N	Y / N	
Remarks							
	1st deployment						
	1st collection, 2nd deployment			Y / N	Y / N	Y / N	
	2nd collection, 3rd deployment			Y / N	Y / N	Y / N	
	3rd collection			Y / N	Y / N	Y / N	
Remarks							
	1st deployment						
	1st collection, 2nd deployment			Y / N	Y / N	Y / N	
	2nd collection, 3rd deployment			Y / N	Y / N	Y / N	
	3rd collection			Y / N	Y / N	Y / N	
Remarks							
	1st deployment						
	1st collection, 2nd deployment			Y / N	Y / N	Y / N	
	2nd collection, 3rd deployment			Y / N	Y / N	Y / N	
	3rd collection			Y / N	Y / N	Y / N	
Remarks							
	1st deployment						
	1st collection, 2nd deployment			Y / N	Y / N	Y / N	
	2nd collection, 3rd deployment			Y / N	Y / N	Y / N	
	3rd collection			Y / N	Y / N	Y / N	
Remarks							
	1st deployment						
	1st collection, 2nd deployment			Y / N	Y / N	Y / N	
	2nd collection, 3rd deployment			Y / N	Y / N	Y / N	
	3rd collection			Y / N	Y / N	Y / N	
Remarks							
	1st deployment						
	1st collection, 2nd deployment			Y / N	Y / N	Y / N	
	2nd collection, 3rd deployment			Y / N	Y / N	Y / N	
	3rd collection			Y / N	Y / N	Y / N	
Remarks							
	1st deployment						
	1st collection, 2nd deployment			Y / N	Y / N	Y / N	
	2nd collection, 3rd deployment			Y / N	Y / N	Y / N	
	3rd collection			Y / N	Y / N	Y / N	
Remarks							

Worksheet	Lookup Tables	Field	Description
mosquito_field	site_ids	domain	Number of the NEON domain
	site_ids	locality	full site name as specified in site_ids
	domain_personnel (TBD)	collector	full names of personnel that conducted the mosquito sampling
	plot_ids	plotID	plot ID (4-character site code _XXX)
	protocol_step	protocol_step	text indicating the protocol step associated with sample collection; "1st deployment", "1st collection, 2nd deployment", "2nd collection, 3rd deployment", "3rd collection"
		eventDate	date (YYYYMMDD) that the protocol step was executed
		eventTime	time (24 HR; HHMM) that the protocol step was executed
		fan_on	whether the trap fan was on when samples were collected, "Y" or "N"
		catch_cup_on	whether the trap catch cup was on when samples were collected, "Y" or "N"
		dry_ice_left	whether there was any dry ice in the trap cooler when samples were collected, "Y" or "N"
		labelID	The external vial label; year (two digits, e.g., 13), the site_ID (e.g., CPER), and a three digit numeric code (e.g., 13CPER001). For each site and each year the numeric code should begin at 001 and sequentially increase.
		remarks	notes written by field technicians about sampling
		num_vials_identical_label	total number of sample vials with the same label
		shipped_date	date (YYYYMMDD) that the sample vial was shipped to an analytical facility
	external_facilities (TBD)	location_shipped	name of the external facility to which samples were sent by NEON for processing
mosquito_taxonomy	external_facilities (TBD)	facility_name	the name of the facility where taxonomic ID was performed
		date_sample_rcvd	date (YYYYMMDD) the facility received the samples sent from a NEON domain lab
		labelID	the label on the sample vial applied at the NEON domain lab. This is a linking variable that can be used to fill in the domain, site, plot, and sampling date
		scientificName	name of a mosquito species identified in a sample (could be multiple species/data rows per label ID)
		num_ind_collected	the nubmer of individuals of a mosquito species identified in a vial with given label ID
			following ID the facility will pool samples of a given species across plots and dates within a site/bout combination and give these vials new lables. This is a linking variable that can be used to fill in the domain, site, and sampling dates (bout)

A description of each field in the datasheet shown above and in some cases an example of how to fill out the datasheet

Appendix B BATTERY MAINTENANCE

1) The batteries used to power the CDC CO_2 light traps are a 6V sealed gel electrolyte type. They pose little risk, but proper handling procedures should be followed. Charging should be performed in a designated area with batteries placed in plastic bins for secondary containment. Particular care should be exercised when charging depleted batteries as chargers can become hot and potentially cause burns. Chargers should not be touched until after the green light comes on. Warning signs should be placed around the batteries while charging.
2) To charge a battery, first connect the color-coded leads to the battery. Next, plug the charger into the AC outlet. If the battery is mistakenly connected backwards (negative to positive) or the charger leads are shorted together, a red light on the charger will be illuminated to indicate a possible mistake. When the charger is first plugged in it sends intermittent current pulses into the battery and monitors the battery's response to determine the charge state. A red light may come on briefly if the battery is excessively discharged, but it should go off within the first few minutes of charging.
3) Once the charger determines that the battery can safely take a charge, it goes into the bulk charging mode. During bulk charging, indicated by a yellow light on the charger, the full capacity of the charger is applied until the battery reaches 80% of its capacity.

4) Once the battery has reached 80% capacity charging enters absorption mode: the yellow light on the charger remains on but the charge output is reduced from 100% to 33% of capacity until the battery reaches full charge.
5) Once full charge has been reached a green light on the charger becomes illuminated to indicate that the battery is now in its ideal charge state. As long as the batteries remain connected, the charger will maintain them in this state. This means that batteries may be left connected to the charger overnight, over the weekend, or indefinitely, and when they are picked up, they are in the ideal state.