TOS PROTOCOL AND PROCEDURE: MOS – MOSQUITO SAMPLING

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See configuration management system for approval history.

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
-	04/27/2011	ECO-00159	Initial Draft Release
A_DRAFT	10/03/2011	ECO-00280	Update to new document numbers and template
B_DRAFT	07/30/2012	ECO-00442	Adjusted for known issues from 2011 prototype and revised for Domain 3 specific information
C_DRAFT	02/24/2014	ECO-01139	Draft release. Will be finalized in next rev.
D	03/27/2014	ECO-01672	Production release, template change, and other changes as detailed in Appendix C
E	03/27/2014	ECO-02353	Migration to new protocol template Sampling frequency and timing section updated to provide instructions on where to deploy off-season traps (FOPS-870) and how to process samples collected during off-season sampling (FOPS-1647) including how to distinguish mosquitoes from midges and crane flies Contingent table updates to provide instructions on when sampling should be cancelled/postponed due to high winds (FOPS-1260) Equipment list updated to include kimwipes to be used in packing mosquitoes in sample vials (FOPS-1457, FOPS-844, FOPS-815 SOP A: Text added clarifying permissible change to ring on top of fan assembly (FOPS-1313). Locality labels attached to catch cups have been replaced by sampleID labels SOP B: Text added to recommend that traps not be hung over or near water (FOPS-1099). Locality labels attached to catch cups have been replaced by sampleID labels. Instructions on how to deal with fans clogged by mosquitoes were added (FOPS-1215). Instructions on how to record instances in which no sample vials are generated for a catch cup were added (FOPS-1222…field and lab datasheet modified accordingly). Instructions added on a possible method to clean fan assemblies clogged with mosquito bodies. Associate equipment (tooth or bottle brushes) added to equipment list. SOP C: More detailed instructions for packing mosquitoes in sample vials with tissue prior to shipping added in response to FOPS-1457, FOPS-844, FOPS-815.

			Format for vial ID has been slightly modified (a period after D/N, 2-digit number for vial number(s)). Locality labels inserted into sample vials have been replaced by modified sample ID labels SOP E: deleted as content related to post-identification processing of mosquitoes at domain labs has been moved to the TOS Protocol and Procedure for Ground Beetle and Mosquito Specimen Processing. SOP F: Instructions on how to obtain the file used to create mosquito shipping manifests added per FOPS-1283. Shipping manifest and associated instructions were revised for consistency per FOPS-1316. Format of shipping manifest has been adjusted with the addition of fields and changes to the name and format of some existing fields
F	02/23/2015	ECO-02563	Update of mosquito TOS protocol based on 2014 field experience and budget analysis to improve workflow and reduce costs.
G	01/29/2016	ECO-02905	Clarified wording as to the sampling window (response to FOPS-2039) and trap placement during off-season (FOPS-2083). Removed bout number from sampleID and vial ID format. Updated shipping manifest figure to remove bout number reference. Remove reference to common insect lab protocol. Updated the timing of sampling section to include a modification for Alaska.
H	03/06/2017	ECO-04329	Added site specific appendices for all 47 terrestrial sites
J	02/16/2018	ECO-05255	Added barcode language; 2018 field season sampling of 24 hours (2 collections), instead of 40 hours (3 collections) for logistical/budget reasons; added clarification about Alaska sampling per NEON-7386
K	03/18/2019	ECO-05880	Updated figures and text to reflect 24 hours of sampling (2 collections per plot per bout); removing requirement for an external label on samples; substituting kimwipes in the packaging of mosquito vials; clarifying language for sample timing
L	03/05/2020	ECO-06259	• Updated to new template (NEON.DOC.050006vJ) • Added photos of specimens and diagrams of workflows • Added missed bout reporting; usage of the 'samplingImpractical' field • Missed bouts due to low temperatures during the field season count towards the three end-of-season mosquito absences needed to transition to 'off season' sampling

M	03/16/2022	ECO-06781	• Update to reflect change in terminology from relocatable to gradient sites
N	02/08/2024	ECO-07057	• Updated to new template (NEON.DOC.050006vL) • Updated NEON logo • Changed to use CO2 canisters instead of dry ice to bait traps across majority of sites • Added trap modifications for windy conditions • Added alternatives to dry ice for initial sample storage • Added appendix for troubleshooting mosquito trap fan issues • Clarified guidance for how and when to enter samplingImpractical records

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

1 OVERVIEW

1.1 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 aquatic invertebrate biomass and act as a key food source for both aquatic and terrestrial predators (e.g., fish, amphibians, spiders, birds and bats). Mosquitoes also act as vectors for numerous parasites and pathogens of humans, livestock and wildlife. Mosquito biology and ecology have also 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. Although a short generation time and high fecundity allow mosquitoes to respond quickly to environmental change generally, the high diversity and varied ecological niches of this group will result in marked differences in response between species. Changes in global climate are predicted to affect the distribution, demography and seasonal phenology of many mosquitoes; these changes are thought to have associated effects on pathogen transmission cycles. 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 (NEON).

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.

1.1.1 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

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

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

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

AD[01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[05]	NEON.DOC.004104	NEON Science Data Quality 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

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Data Products Catalog
RD[04]	NEON.DOC.001271	AOS/TOS Protocol and Procedure: DMP – Data Management
RD[05]	NEON.DOC. 001581	Datasheets for TOS Protocol and Procedure: MOS – Mosquito Sampling
RD[06]	NEON.DOC.003282	NEON Protocol and Procedure: SIM – Site Management and Disturbance Data Collection
RD[07]	NEON.DOC.005247	AOS/TOS Standard Operating Procedure: NEON Aquatic and Terrestrial Site Navigation
RD[08]	NEON.DOC.001025	TOS Protocol and Procedure: PLT – Plot Establishment
RD[09]	Available via download of data from NEON portal	NEON Raw Data Ingest Workbook for TOS Mosquito Abundance, Diversity, and Phenology
RD[10]	NEON.DOC.005224	NEON Protocol and Procedure: SCS – Shipping Ecological Samples and Equipment
RD[11]	NEON.DOC.005346	OS Standard Operating Procedure: FRZ – Preparation and Use of Dry Ice Alternative Freezing Materials

2.3 Acronyms

Acronym	Definition
CDC	U.S. Centers for Disease Control and Prevention
SOP	Standard Operating Procedure

2.4 Definitions

Fulcrum: Software tool used to create NEON electronic data entry applications.

ServiceNow: Software tool used for problem/incident tracking and resolution.

3 METHOD

The Standard Operating Procedures (SOPs) presented in this protocol describe tasks that, when taken together, allow estimation of mosquito abundance, diversity and pathogen status. These SOPs are:

- SOP A: Preparing for Sampling
- SOP B: Field Sampling
- SOP C: Post-Field Sampling Tasks
- SOP D: Data Entry and Verification
- SOP E: Sample Shipping

Mosquito sampling involves preparing to sample, collection in the field, minor laboratory processing, data handling, and shipping to external facilities. Field collection of live mosquitoes is conducted using Centers for Disease Control and Prevention (CDC) CO2 light traps. A CO2 light trap consists of (listed from the top to the bottom of the assembled trap): a cylindrical insulated cooler to hold dry ice, a plastic rain cover attached to a light/fan assembly (battery-powered), and a mesh collection cup. The light from the light/fan assembly will be disabled to reduce by-catch. During deployment, dry ice in the insulated cooler releases CO2 as it sublimates, and this gas attracts mosquitoes to the vicinity of the trap. Beginning in 2024 the majority of NEON sites shifted to use canisters to release CO2 gas instead of dry ice for a more consistent gas flow rate and reduced long-term supply costs. The trapType field indicates which bait method was used. The battery-powered fan sucks these mosquitoes into the mesh collection cup, where they remain alive 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.

Mosquito sampling plots are randomly located in each of the major vegetation types (i.e., any vegetation type >5% of total cover at the site), with the number of plots per vegetation type proportional to the percent cover of that vegetation type at the site. Plots are located within 30m of a road to facilitate expedient sampling (**Figure 1**).

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 this protocol across all NEON domains, for the life of the project. 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.

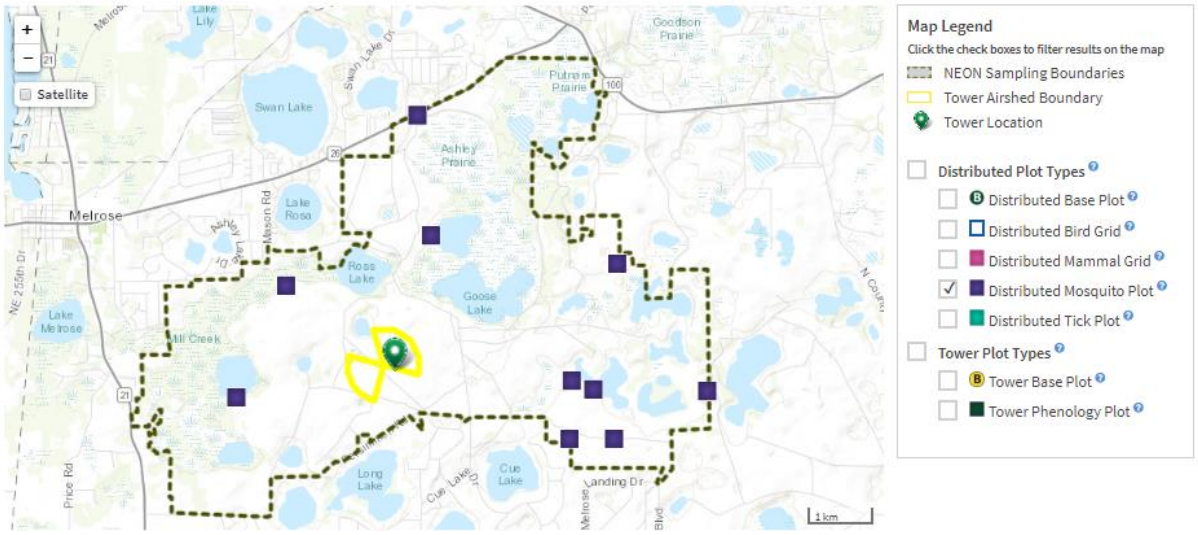


Figure 1. Mosquito points distributed across the Florida core site location using a randomized design stratified by vegetation type; plots are located near roads for logistical ease of access.

Quality assurance is performed on data collected via these procedures according to the NEON Science Data Quality Plan (AD[05]).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Sampling must occur whenever mosquitoes might be active (typically when temperatures are above 10°C) and must capture species occurrences at the very start and end of each season to accurately capture mosquito phenology. Thus, there are two distinct types of sampling associated with mosquito trapping: **off-season** and **field season** sampling. Estimated dates of off-season and field season sampling are provided in 1 for scheduling purposes.

In general, off-season sampling is conducted at core sites for the purpose of (1) determining the start of field season sampling each year and (2) providing valuable absence data to inform our understanding of mosquito phenology. In contrast, field season sampling generates samples and data that feed into all mosquito data products. Off-season sampling is conducted weekly but a given bout will only occur if the mean daily high temperature for the previous 5 days was above 10°C. Within a domain, bouts of field season sampling will occur every **two weeks at the core site** and **every four weeks at each gradient site**, with sampling alternating between gradient sites, where applicable (**Figure 2**).

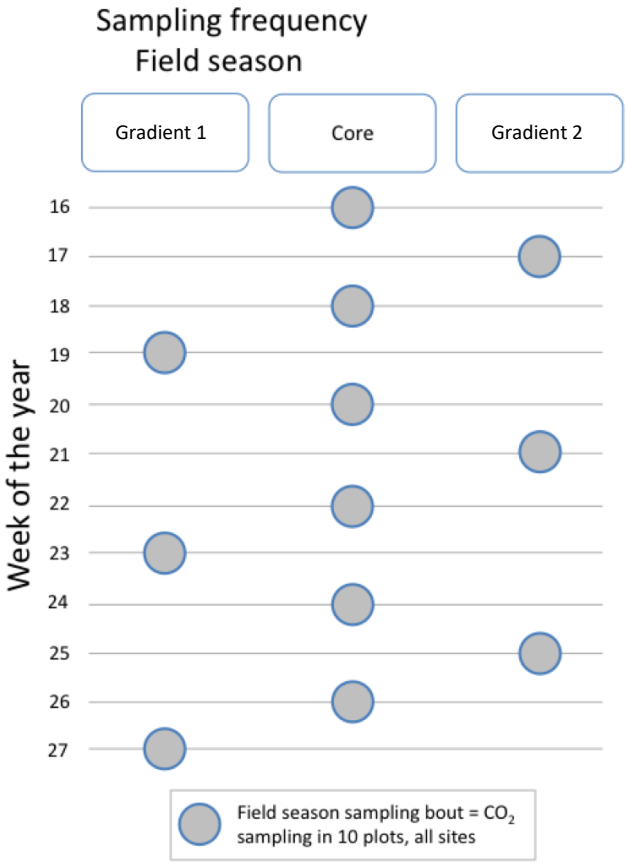


Figure 2. Frequency of sampling bouts at a domain; weekly sampling alternating between core and gradient sites.

1. **Off-season sampling**

Off-season sampling is conducted at core sites for the purpose of determining the start of field season sampling each year (but see exceptions in Appendix F). Once mosquitoes are detected from off-season sampling at the core site, field season sampling will begin for all sites within the domain.

During a bout of off-season sampling, one trap is deployed at each of three points at the core sites. For logistic ease, deploy these 3 traps at the three field season mosquito sampling plots that are readily accessible and spread across the core site (i.e., if possible, do not choose three sampling plots immediately adjacent to one another). Once chosen, these same plots will be consistently sampled for every bout of off-season sampling at the core site unless instructed otherwise by Science. During 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 field season sampling, a bout of off-season sampling is shorter in duration, does not collect diurnal mosquitoes, and occurs at a smaller number of locations.

2. **Field season sampling**

Field season sampling bouts will involve ~24 continuous hours of sampling using one CDC CO_2 light trap at each plot (**Figure 3**, described below). If delays in sampling occur due to contingent events (see **Table 2**), the duration of a bout will not exceed a three-day window. The specific timing of these activities depends on local patterns of seasonal mosquito abundance.

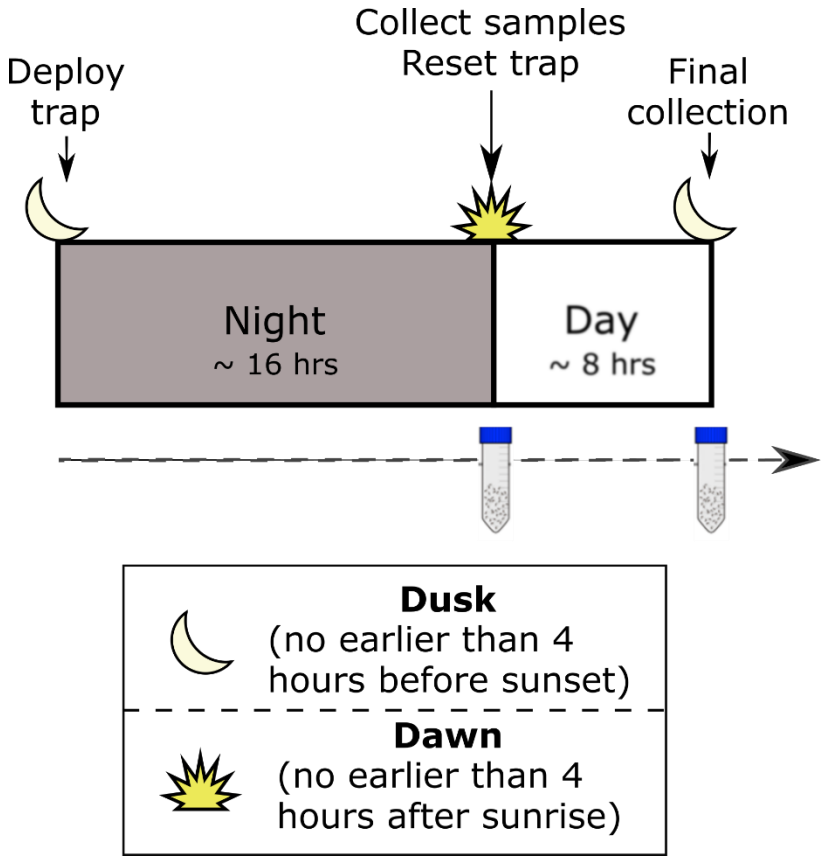


Figure 3. Timing of a mosquito sampling bout that generates two samples (designated by blue-capped vials), one night and one day of trapping. (Daytime and night-time durations shown are for illustrative purposes only.)

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 3**). Traps will be checked and reset (catch cups with target taxa retrieved and replaced with new/empty catch cups and dry ice refilled if applicable) as close as possible to dawn (but no later than 4 hours after sunrise) on the second day of the bout. Traps will be retrieved later that day following the daytime trap collection period. All samples generated from field season sampling will be processed and sent for taxonomic identification; however, taxonomic information resulting from sample collections that occur outside the recommended period (described above) may be compromised.

Note: In Alaska and any other sites where summer day length exceeds 17 hours, the window for deployment and evening trap checking will occur between 4:00 PM and 8:00 PM local time (observing daylight savings where applicable) in lieu of 4 hours prior to dusk. Initial trap checking and dawn recovery times will occur between 6:00AM and 10:00 AM local time. In this case, 6:00 PM (for dusk) and 8:00 AM (for dawn) are the target trap deployment and recovery time points.

Table 1. Sampling frequency for mosquito sampling procedures across site types and season.

SOP	Site Type	Plots Sampled	Bout Duration	Bouts Per Year	Bout Interval	Yearly Interval	Remarks
SOP B	Core site (off season)	3	~ 16 hours	variable	weekly	annually	Off season sampling occurs weekly until mosquitoes are detected at the core site
	Gradient (off season)	NA	NA	NA	NA	NA	Gradient sites not sampled during 'off season' period
	Core site (field season)	10	24 hours	up to 26 per year	every 14 days	annually	
	Gradient (field season)	10	24 hours	up to 12 per year	monthly	annually	

4.2 Criteria for Determining Onset and Cessation of Sampling

Sampling occurs year-round at core site locations, alternating between more frequent but less intensive off-season sampling and more comprehensive field season sampling. Once mosquitoes are observed at the core site, NEON staff conduct field season sampling until mosquitoes are absent at the core site for three consecutive bouts (**Figure 4**, **Figure 5**). Until mosquitoes are confirmed as absent, field season bouts at the gradient sites should continue as scheduled. Note that in some cases, the timing of sampling at a gradient site will be based on conditions at a core site in a different domain. Please see Appendix F for details about these exceptions.

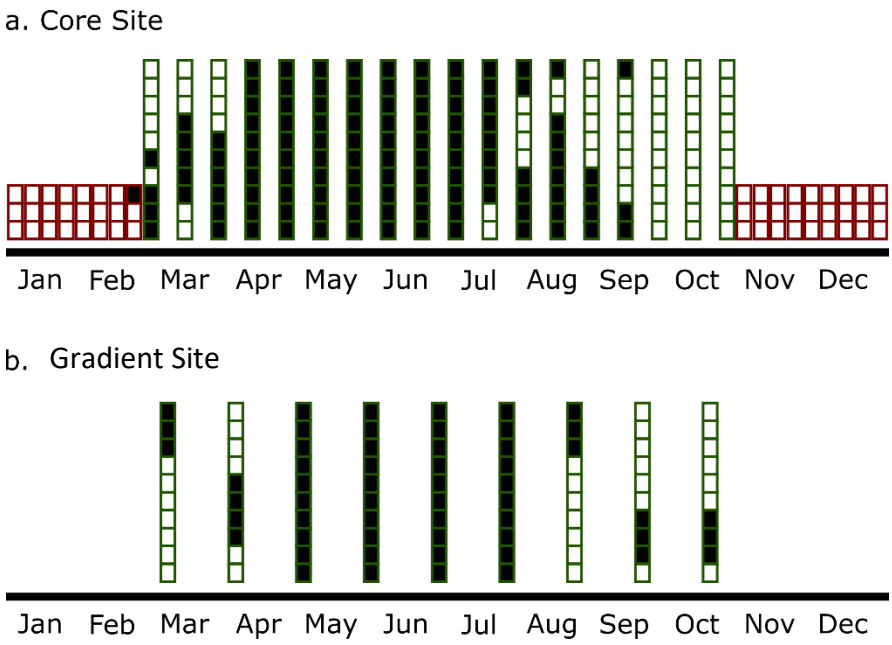


Figure 4. An example sampling schedule for a site highlighting the transitions between field season (green boxes) and off-season (red boxes) mosquito sampling at (a) core and (b) gradient site locations. In this diagram, each box represents one plot sampled at the site during the bout. Filled boxes represent mosquito captures at one plot. Mosquito capture at the core site location during off-season sampling, initiates field season sampling at the core and all gradient sites. Absence of mosquitoes for three consecutive bouts at the core site ends field season sampling at all site locations.

Sampling only occurs if the mean daily high temperature at the site (or nearest location with reliable temperature data) for the previous 5 days is above the domain-specific temperature threshold (in Alaska, this threshold is 4°C; all other locations the threshold is 10°C). Sampling bouts are cancelled when temperatures are below the threshold, but resume when temperatures rise above the 10°C (or 4°C in Alaska) threshold. Off-season sampling continues until the first mosquito is collected during an off-season bout. Initial "identification" of insects collected during off-season sampling will be done informally by domain staff. This is because the time required to have samples sent to and identified by external facilities would introduce an unacceptably long delay between the collection of mosquitoes during off-season sampling and the initiation of field season sampling. For insects collected during a bout of off-season sampling, species-level taxonomic identification of mosquitoes is not necessary. Instead, samples need only be identified as *likely* to be mosquitoes (family Culicidae) based on general morphology (see **Figure 6**, **Figure 7**).

The collection of one or more insects likely to be mosquitoes during a bout of off-season sampling will mark the resumption of field season sampling at all sites in the associated domain (**Figure 4**, **Figure 5**). For each bout of off-season sampling during which insects *likely* to be mosquitoes are collected, save samples in a labeled vial that can be sent to a taxonomic identification facility later in the season (i.e., together with field season samples) if resources allow at the end of the season as determined by Collections and Laboratory Analysis staff. Alternatively, you may detect mosquitoes by means other than

off-season sampling (e.g., observing them while in the field at either a core or gradient site). In this case, you may begin field season sampling without an off-season bout capturing mosquitoes. When field season sampling is initiated, it is acceptable to start with either the core site or a gradient site per the master schedule. If resources are not sufficient to sample at all sites within the domain when field season sampling is initiated (e.g., spring arrived very early and seasonal technicians are not yet available) or concluding, sampling will be prioritized at the core site.

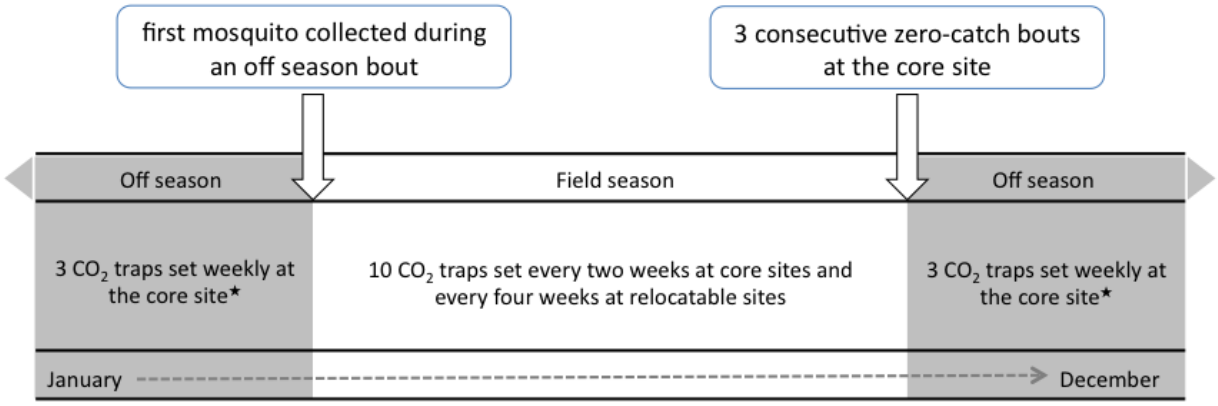


Figure 5. Annual summary of mosquito sampling intensity including transitions from off-season to field season and back to off-season sampling. Bouts occur only when the mean daily high temperature for the previous five days is above the temperature threshold (4°C in Alaska, 10°C elsewhere).

When low temperatures result in the cancellation of a *field season* sampling bout at the core site (see section on **Missed or Incomplete Sampling**), that bout is treated as though it was a zero-catch bout for the purposes of initiating off-season sampling. If a field season sampling bout is cancelled for any other reason (e.g., hazardous conditions, site access, staffing issues), that is not considered toward the three zero-catch bouts needed to initiate off-season sampling.

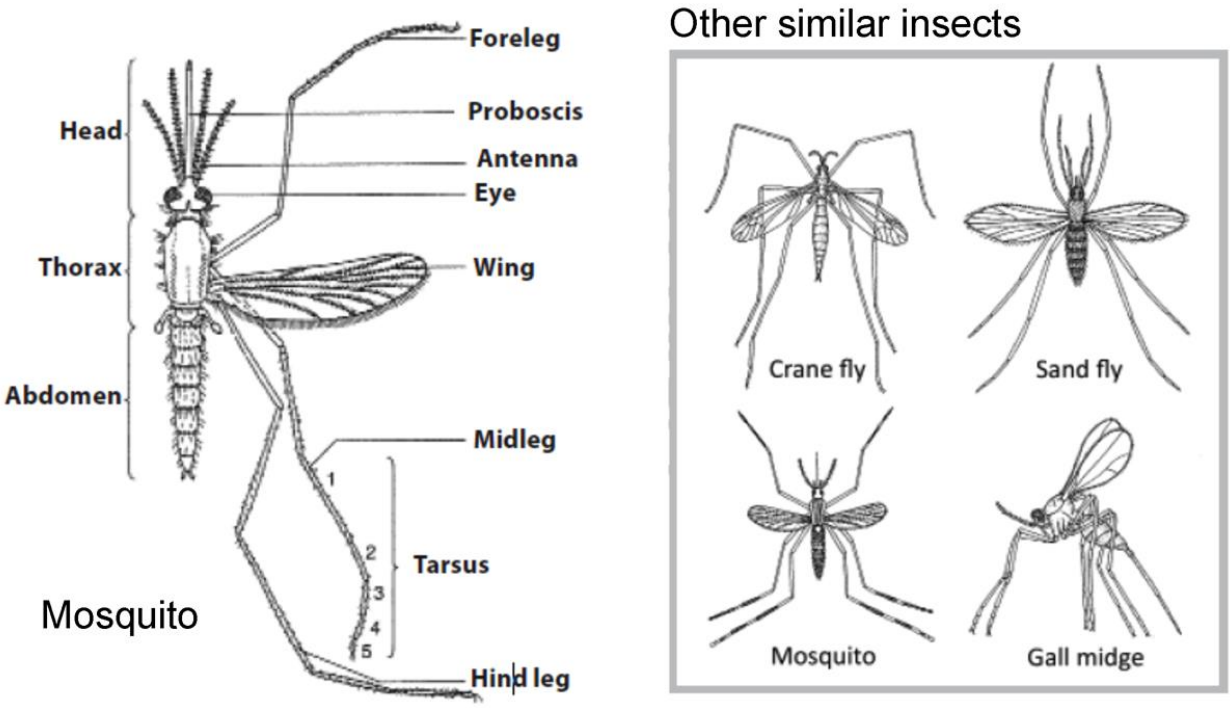


Figure 6. Basic morphology of a mosquito and comparison with other similar-looking insects.

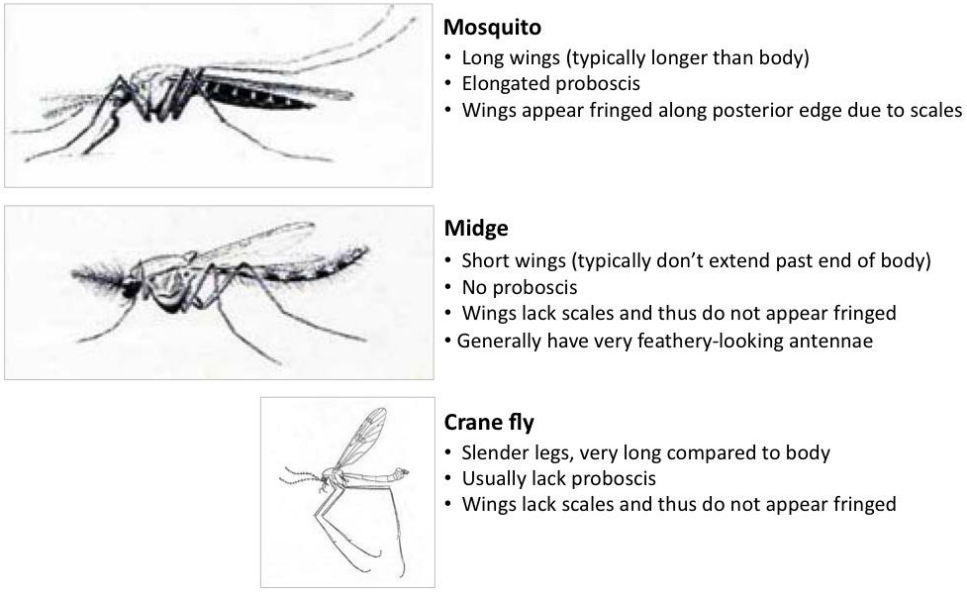


Figure 7. Morphological features that distinguish mosquitoes from midges and crane flies.

4.3 Timing for Laboratory Processing and Analysis

Process samples as soon as possible after returning from the field, ideally within one week of sample collection.

4.4 Sampling Timing Contingencies

When field conditions require deviations from the protocol, follow the contingent decisions outlined in **Table 2** below to maintain data quality. When indicated by **Table 2**, the cause of cancellations must be recorded in the sampling impractical field of the 'MOS: Trap Setting and Collection [PROD]' Fulcrum application or in a data sheet and issue a ServiceNow ticket for cancellations other than those covered in the protocol (e.g., too windy or cold to sample).

Table 2. Contingent decisions for mosquito sampling.

Delay/Situation	Action	Outcome for Data Products
High winds >= 25mph	It is required that traps are not deployed if wind gusts reach >=25 mph. Mosquitoes are not active in windy conditions and equipment has potential to be damaged. Reschedule bout if possible to a time when wind speed is less than 25 mph. If rescheduling is impossible, populate the 'sampling impractical' field with 'extreme weather' for all impacted collections (up to 20 records per bout)	Sampling equipment could be damaged and the quality of samples is low.
Bout is delayed < 3 hours	Resume/continue with normal sampling at conclusion of delay.	Quality of samples reduced, creating potential for complications with processing (identification, pathogen testing). Note also 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 identify and test for pathogens.

Delay/Situation	Action	Outcome for Data Products
Bout is delayed 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.[1] In both cases, do not adjust (push back) dates for subsequent sampling bouts.	Brief interruption in consistent interval of time series data compromises statistical analysis of temporal trends in the data.
Bout is delayed 1-7 days (core) OR 1-14 days (gradient)	**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, do not adjust (push back) dates for subsequent sampling bouts.	Moderate interruption in consistent interval of time series data compromises statistical analysis of temporal trends in the data.
Bout is delayed > 7 days (core) OR > 14 days (gradient)	Cancel the impacted sampling bout and stop sampling until next scheduled sampling bout. Create a sampling impractical record for each missed sample (up to 20 records per bout). Note the cause of the delay in each record where sampling was cancelled. If applicable, also create a record in the Site Management and Disturbance application.	Maximal interruption in consistent interval of time series data compromises statistical analysis of temporal trends in the data. Reduction in sample size as sampling bouts are missed.

[1] For example: A thunderstorm prevents collection/reset of traps for the morning bout by more than 3 hours (result is a deviant collection timing). The bout should be rescheduled as soon as possible (within 1-7 days core site, or 1-14 days gradient site). If the bout can be rescheduled, the samples from the deviant bout should be discarded and the data should be deleted. If rescheduling is not possible, the data and samples should be retained for processing as usual, but the data product will indicate the extended duration of collection. Repeating collection to capture the correct intervals is pending on staff availability.

4.5 Missed or Incomplete Sampling

Sampling according to the schedule is not always possible, and multiple factors may impede work in the field at one or more plots or sampling locations in a given bout. For example:

- Logistics – e.g., insufficient staff or equipment
- Environment – e.g., deep snow, flooding, inclement weather
- Management activities – e.g., controlled burns, pesticide application

Instances such as those listed above must be documented for scheduling, tracking long-term plot suitability, and informing end users of NEON data availability. Some types of missed sampling are due to events that should be recorded in the Site Management App; refer to the Site Management and Event Reporting Protocol for more detail (RD[06]).

Missed or Incomplete Sampling Terms

Terms that inform Missed or Incomplete Sampling include:

- **Protocol Sampling Dates**: Bout-specific sampling dates (1).
- **Scheduled Sampling Dates**: Bout-specific sampling dates scheduled by Field Science and approved by Science. These dates coincide with or are a subset of the Protocol Sampling Dates.
- **Missed Sampling**: Incidence of *scheduled sampling* that did not occur. Missed Sampling is recorded at the same resolution as data that are ordinarily recorded.
- **Sampling Impractical**: The field name associated with a controlled list of values that is included in the data product to explain a Missed Sampling event – i.e., why sampling did not occur.
- **Rescheduled**: Missed Sampling is rescheduled for another time within the *protocol sampling dates*, resulting in no change to the total number of sampling events per year.

The documentation that must accompany missed sampling depends on the timing, subsequent action, and the audience appropriate for numerous scenarios (**Figure 8**).

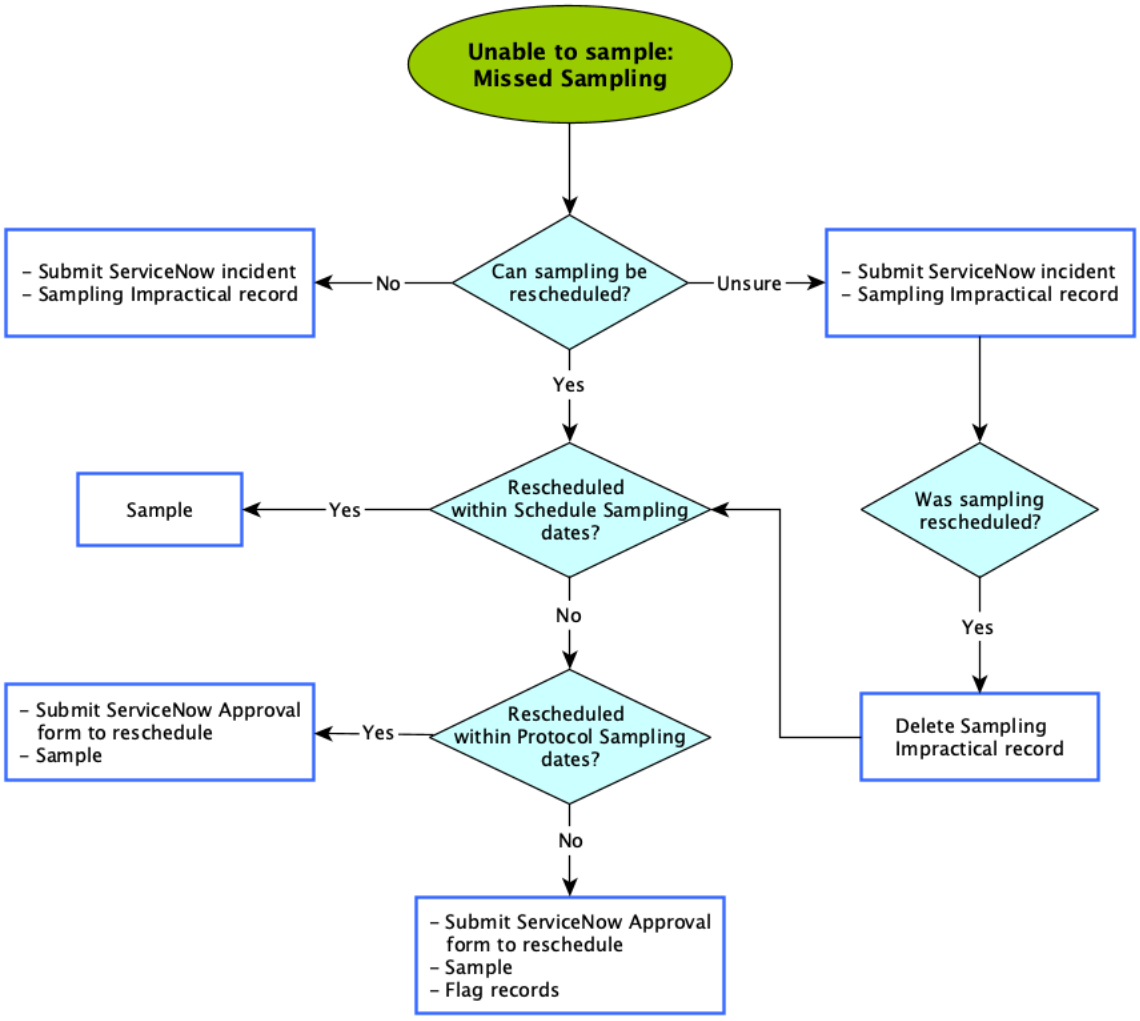


Figure 8. The documentation to account for a Missed Sampling event depends on the situation for each plot of each bout that is not sampled. Light blue diamonds represent contingencies, blue line boxes describe the required actions. Required delay and cancellation actions are outlined for each protocol in the 'Scheduled Field Activities – Delays and Cancellations' spreadsheet available in a Field Science SharePoint library. Missed Sampling events may also require the creation of a Site Management record.

To Report Missed or Incomplete Sampling:

1. Missed or Incomplete Sampling that cannot be rescheduled within the Schedule sampling dates must be communicated to Science by a ServiceNow Incident.
    a. For Missed Sampling that is Rescheduled, there are some cases that require approval by Science and Operations (**Figure 8**).
    b. Consult **Table 3** below to determine required actions if scheduled activities are delayed or canceled. Guidance for this and other NEON protocols is summarized for ease of use

in a table posted to a Field Science SharePoint library. However, this protocol is the ultimate source of information should any discrepancy exist.

2. Create a Fulcrum record for each Missed Sampling event using the samplingImpractical field. That is, data are recorded at the plot level, a record must be made for each plot & time point missed.

    a. Record each scheduled plot visit of the bout where traps were not deployed in Fulcrum. If no plots were sampled during a scheduled field season bout, 20 samplingImpractical records would be created in the 'MOS: Trap Setting and Collection [PROD]' application. If no plots were sampled during a scheduled off-season bout, 3 samplingImpractical records would be created in the 'MOS: Trap Setting and Collection [PROD]' application.

    b. The 'setTime' and 'collectTime' should be the same for samplingImpractical records so that the total number of trap hours is 0. For missed daytime collections the 'setTime' and 'collectTime' should be 8AM and for missed nighttime collections the 'setTime' and 'collectTime' should be 6PM.

    c. Some sites experience temperatures that are too low for sampling during the 'off season' when weekly bouts to check for mosquito activity would otherwise be occurring. Beginning in the 2024 field season the guidance for creation of samplingImpractical records at core sites under these circumstances is that the season during which temperatures are too low to support mosquito sampling should be "book-ended" by at least one set of off-season samplingImpractical records marking "temperature low" at the start of the cold season and at the end of the cold season. The second set of low temperature records can be created retrospectively once temperatures have warmed above protocol-specified temperatures. Creation of samplingImpractical records at gradient sites should continue to occur as long as the core site that determines sampling is still in active field season sampling.

    d. At some sites temperatures may fluctuate above and below the sampling threshold for a period of several weeks. During this time, if the temperature goes above the protocol threshold, the data for that bout should be recorded and then the subsequent bout would require an additional samplingImpractical record for the missed sampling. After that no additional samplingImpractical records are needed until just before the next resumption of sampling.

    e. Example Scenario: A site enters off-season sampling on November 20 after 2 zero-capture bouts and a low-temperature cancellation. The next off-season bout of sampling is completed on November 27 because temperatures warmed to just above the threshold. After that the temperatures are below protocol threshold until April 15. In this scenario, there should be a set of off-season samplingImpractical – 'low temperature' records on December 4 and April 8 to "book-end" the period of time where temperatures were consistently too cold to sample; however no

additional samplingImpractical records are needed for the other cancelations between 12/04 and 4/08.

3. For each samplingImpractical record, the **samplingImpractical** field must be populated in the mobile collection device to describe the reason why sampling could not occur (**Table 3**, **Table 4**)

Table 3. Guidance for responding to delays and cancellations encountered during implementation of the Mosquito Sampling protocol. Update table rows to reflect protocol-specific scenarios.

Activity Name	Days Delayed from Schedule	Delay Action	Cancellation Action
Mosquito sample shipping	> 5 days	Notify ^List-CLA	Notify ^List-CLA
TOS Mosquito Sampling	See **Table 2**	See **Table 2**	Submit incident ticket if reason for cancellation is not already covered by the protocol (e.g., low temperature / high wind)

Table 4. Protocol-specific Sampling Impractical reasons entered in the Fulcrum application. In the event that more than one is applicable, choose the dominant reason sampling was missed.

Sampling Impractical reason	Description
Location snow covered	Location snow covered; traps should be deployed such that the bottom of the trap fan hood is 1.2 -1.8 meters (4-6 feet) above the ground, use this reason when snow is high enough that the trap (if it were to be deployed) would be closer than 1.2 meters to the ground
Location flooded	Standing or flowing water too deep to complete sampling
Temperature low	Ambient temperature lower than requirements specified in protocol
Logistical	Site or plot access compromised, staffing issues, errors (e.g., equipment not available in the field)
Management	Management activities such as controlled burn, pesticide applications, etc.
Extreme weather	Events (e.g., thunderstorms, hurricanes) that compromise safety and access
Other	Sampling location inaccessible due to other ecological reason (describe reason in the remarks)

4.6 Estimated Time

The time required to implement a protocol will vary depending factors such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe provided below (**Table 5**) 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. Please note that if sampling at particular locations requires significantly more time than expected, Science may propose to move these sampling locations.

An experienced two-person team will require approximately 15-25 minutes to complete deployment or retrieval of samples from at a single plot. This entails travel time between the plot and the road and time required for either a) hanging a trap for an initial deployment, b) transferring a catch cup containing samples into a cooler and replacing the catch cup (and potentially battery/fan) for a redeployment, or c) transferring a catch cup containing samples into a cooler and taking down trap equipment at the conclusion of sampling bout.

Table 5. Estimated staff and labor hours required for implementation of mosquito sampling.

SOP	Estimated time	Suggested staff	Total person hours
SOP A: Preparing for sampling	0.5 - 1 h per bout	2	1-2 h/bout
SOP B.1: Setting traps	0.25 – 0.5 hrs/plot	2	5-10 hrs/bout
SOP B.2: Retrieving samples from traps	0.25 – 0.5 hrs/plot	2	10-20 hrs/bout
SOP C.2: Laboratory Processing – transferring mosquitoes	0.5-3 hrs/bout	2	0.5-3 hrs/bout
SOP D: Data Entry and Verification	1 h per bout	1	1 h per bout
SOP E: Sample shipment	0.5 h per bout	1	0.5 h per bout

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, Field Ecologist 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. The use of insect repellent is recommended; however, repellent must be applied at least **30 minutes prior** to arriving in the field if used. **NEVER** apply insect repellent in the vicinity of sampling equipment. After applying insect repellent, clean the palms of hands (e.g., with soap/water or alcohol swabs) before handling any sampling equipment. Note: using ethanol on skin immediately prior to using DEET is not recommended as it increases absorption of the chemical into the skin.

Field personnel are collecting biting insects, but there is no increased risk of infection by zoonotic pathogens during implementation of this protocol than in general fieldwork.

Dry ice should be handled with extreme care. Refer to EHS Safety Policy and Program Manual (AD[01]), Section HM-01, Cryogenic Safety. There are additional safety considerations when using CO_2 canisters that are described in the training modules that technicians handling CO_2 canisters are required to complete (see Training Requirements section).

6 PERSONNEL

6.1 Training Requirements

All technicians must complete protocol-specific training as required in the Field Operations Job Instruction Training Plan (AD[04]). This includes watching the mosquito processing video, which documents proper handling of mosquito samples in the laboratory. Additional protocol-specific required skills and safety training are described in this section. Technicians working with CO_2 canisters must complete two additional training courses that can be found in Success Factors:

(1) Compressed Gas Cylinder Safety Awareness Training, and

(2) Transportation of Materials of Trade Training.

6.2 Specialized Skills

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

7 STANDARD OPERATING PROCEDURES

SOP Overview

NEON staff execute the following SOPs to complete mosquito sampling:

- **SOP A**: Tasks to complete in the domain support facility prior to sampling
- **SOP B**: Procedure for mosquito field sampling; including initial deployment and trap recovery
    - Occurs biweekly (core sites, **Figure 9**) or monthly (gradient sites, **Figure 10**)
- **SOP C**: Post-Field Sampling tasks (includes missed/incomplete bout reporting, lab processing)
- **SOP D**: Data Entry & Verification
    - Occurs 14 days post-sampling bout
- **SOP E**: Sample shipment from domain support facility to external lab for taxonomic analysis

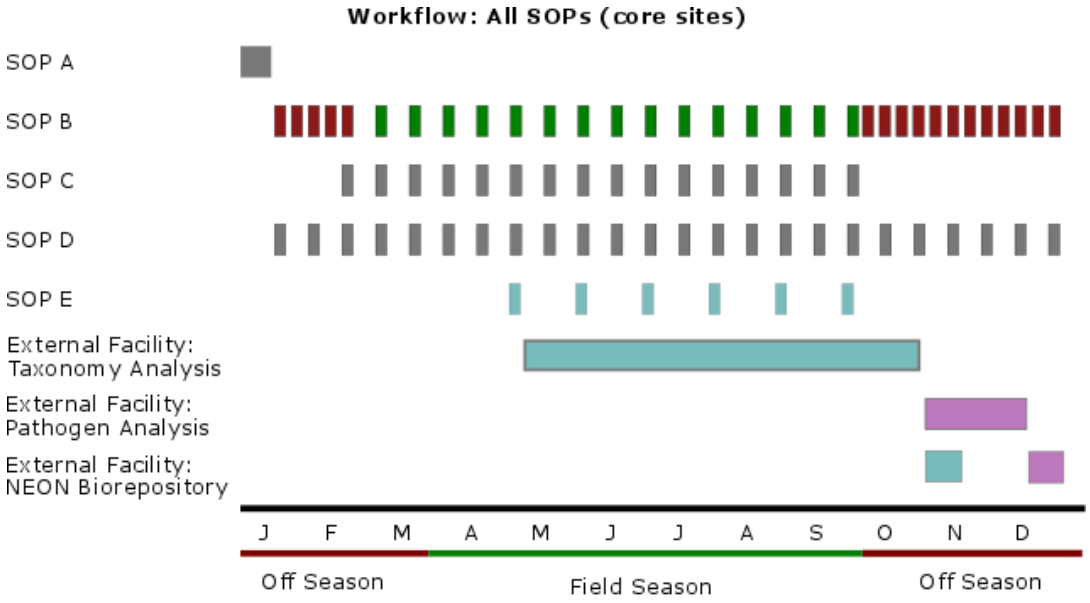


Figure 9. High-level diagram showing timing of activities at core site locations and connection between SOPs and external lab activities. Red and green boxes show timing of activities that occur in the off-season and field season, respectively. Grey boxes show lab-based activities that occur at the domain support facility. Blue boxes indicate activities associated with taxonomic identification, purple boxes indicate activities associated with pathogen analysis.

NEON staff collect mosquitoes and send samples to contracted external facilities throughout the field season; these taxonomists identify a subset of collected mosquitoes (up to 200 per sample) to species-level, where possible. The taxonomist points 10-20 mosquito vouchers of each species from each domain; a portion of the vouchers are DNA barcoded. The remaining mosquitoes identified by the taxonomist are grouped into pools of individuals of the same sex and species collected from the same

sampling bout. Some pools are sent for pathogen testing (according to the criteria laid out in NEON Science Design for Vectors and Pathogens; AD[07]); the remaining pools and voucher specimens are archived at the NEON Biorepository. Following mosquito pathogen testing and DNA barcoding, genetic extracts left over from each analysis are shipped from these analytical facilities to the NEON Biorepository.

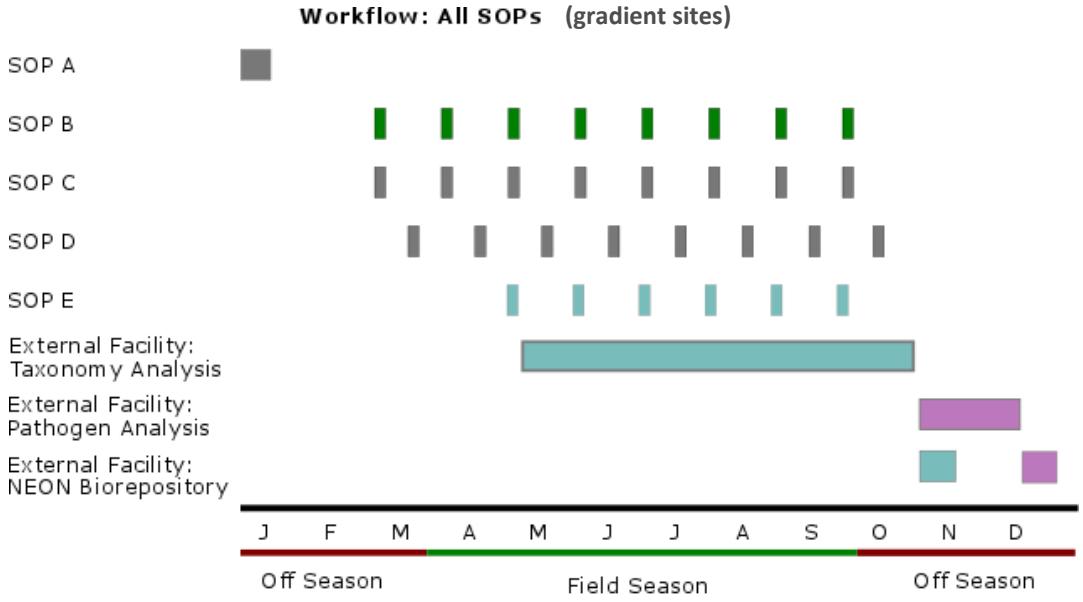


Figure 10. High-level diagram showing timing of activities at gradient site locations and connection between SOPs and external lab activities. Green boxes show timing of activities that occur in the field season (no off season activities occur at gradient sites). Grey boxes show lab-based activities that occur at the domain support facility. Blue boxes indicate activities associated with taxonomic identification, purple boxes indicate activities associated with pathogen analysis.

The mosquito sampling workflow for a particular bout (**Figure 11**) begins with an evaluation of whether conditions are suitable for sampling (see Section 4.5 and 0 for details on missed/incomplete sampling). If a bout is cancelled or a subset of traps are not deployed, a site management record may be required (see NEON Protocol and Procedure: Site Management and Disturbance Data Collection; RD[06]). If conditions are suitable for sampling, NEON staff will execute SOP A, which describes trap deployment and recovery for a typical sample. If mosquitoes are present, the sample will be transferred from the mesh catch cup into the final container (**Figure 12**). Sample trap deployment and collection is identical for off season and field season sampling; only differing in the number of records and samples generated (see Section 4.1 for details). Each off-season bout generates 3 records from the core site only; all data entry and verification is complete within 14 days of sample collection (SOP D).Each field season bout generates 20 records per site per bout; all data entry and verification is complete within 14 days of sample collection (SOP D).

Figure 11. An expanded workflow diagram showing major activities within field and lab SOPs. Actions are squares and decisions are diamonds. This workflow requires creation of a data record that is subsequently modified; record status of the data record is indicated in each oval. Deciding if sampling is impractical is the first step before initiating sampling. Some 'sampling impractical' reasons may also require a record in the "Site Management and Event Reporting [PROD]" application; see NEON Protocol and Procedure: Site Management and Disturbance Data Collection for additional details.

Sampling quick reference

Each collected mesh catch cup yields one mosquito sample. Each mosquito sample is transferred in the lab from the mesh catch cup into one or more vials and associated with a single barcode. The size of the vial(s) used depends on whether a small, medium, or large number of mosquitoes is present in the catch cup. If the sample will fit in a single vial, the barcode is placed directly on the vial. If more than one vial is needed to hold the sample, the barcode is placed onto a secondary cryosafe bag instead of a single vial.

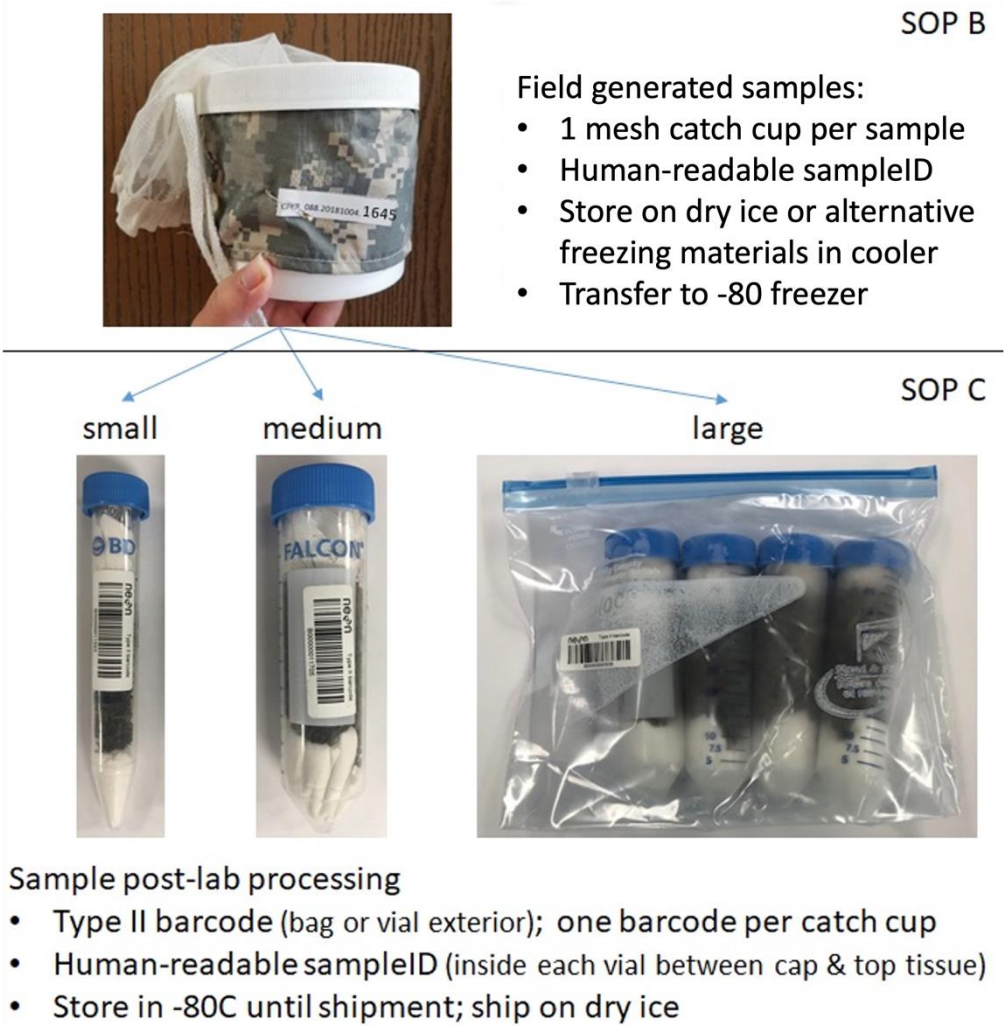


Figure 12. Sample identifiers, barcodes and storage needs for each mosquito sample; up to 3 (off-season) or 20 (field season) samples are possible during a collection bout.

SOP A Preparing for Sampling

A.1 Preparing for Data Capture

Mobile applications are the preferred mechanism for data entry. Mobile devices should be fully charged at the beginning of each field day, whenever possible.

However, given the potential for mobile devices to fail under field conditions, it is imperative that waterproof paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times.

A.2 Labels and Identifiers

This protocol uses a mix of human intelligible labels and barcodes in the workflow. Barcodes are useful for minimizing transcription errors and tracking samples from the domain support facility to external locations. **All samples being shipped from the DSF must have a barcode.**

A sampleID label (**Figure 13**, **Figure 14**) printed on waterproof paper is generated for every trap deployment; up to 20 will be needed per bout. These labels incorporate the plotID location, the collection date and the time of collection. Because the collection time is unknown in advance, these labels should be printed without the last four digits. If preferred, the date can also be hand-written instead of pre-printed. The collection time will be written on the label when the catch cup is retrieved from the field.

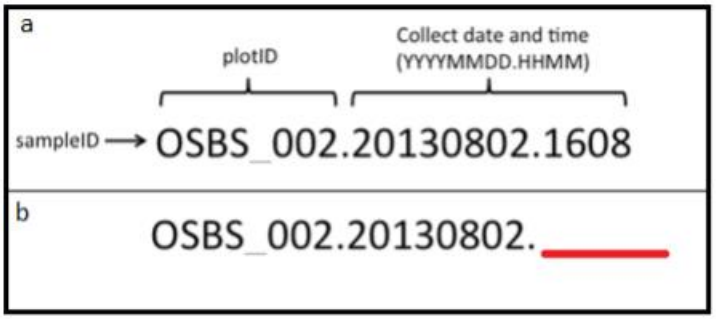


Figure 13. (a) Annotated sampleID example. (b) SampleIDs deployed in the field will have all but the last four-digits pre-filled.

SampleID labels are pinned to the camo sleeve of the catch cup during trap deployment (**Figure 14**). This maintains sample provenance in transit from the field to the domain support facility through final sample processing and shipment. The sampleID label is placed between the upper plug of tissue and the vial cap of the final sample during processing.

Important Note: If this step of pinning the label to the camo sleeve of the catch cup is forgotten then the samples can no longer be differentiated after collection resulting in the loss of all samples and data from this portion of the bout.

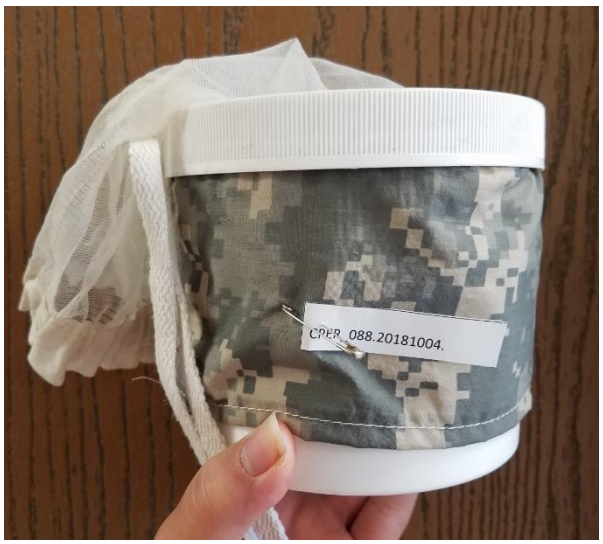


Figure 14. Human-intelligible sample identifier used in the field.

During sample processing in the domain support facility, the sample of mosquitoes is transferred from the mesh catch cup (**Figure 14**) into a final sample container with an affixed Type II barcode (**Figure 15**). Each sample will be affiliated with a single barcode. Small mosquito samples (less than a few hundred individuals collected) are placed from the catch cup into a single vial with single barcode. Very full catch cups have too many mosquitoes to fit into a single vial; this type of sample is split into multiple vials placed into a single cryosafe bag. The sample bag of vials receives a single barcode (**Figure 12**).

Figure 15. An example of a Type II barcode. These large-size, cryo-safe barcodes have a prefix of 'B' followed by 11 numbers.

About Barcode Uses and Placement

This protocol generates up to 20 samples per bout (2 samples per plot) from the field that are sent for further taxonomic identification and pathogen testing. For the mosquito protocol, barcodes are absolutely required and **we only use Type II**, (see **Table 6**). The rule of thumb is that the primary field sample will ALWAYS need a barcode due to its importance in generating future samples. Likewise, the final disposition of all vialed samples must have a barcode affixed to assist in the shipping and receipt of samples destined for the Biorepository or an external laboratory.

Adhesive Type II barcodes labels should be applied at least 30 minutes prior to sample storage in -80C freezer so barcodes can dry at room temperature and stick to cryosafe vials/bags, (this may be done at the start of the season).

- If a sample can fit into a single vial, barcode labels should be oriented such that it is possible to scan them; the scanner will not work on a curved surface. This means aligning the barcode lengthwise along a vial, *not* horizontally wrapping around a vial.
- If multiple vials are required to contain a sample from one trap, place the barcode on the cryosafe plastic bag that will contain all vials associated with that sample.
  Example: A sample collection fills ten 50-mL falcon tubes. The single barcode is applied to the cryosafe bag that contains all ten vials, not each vial containing 1/10 the sample.

Barcode labels must be associated with a unique sample and each barcode must be mapped to one sample in the database. Neither the data entry mechanism nor our database can handle 10 barcodes mapping to the same sample. Barcodes are unique, but are not initially associated with a particular sample, so adhering barcode labels to containers in advance is encouraged.

It is very important that any vials returned by the taxonomy laboratory for re-use in mosquito collection be thoroughly cleaned according to the steps above and have all old barcodes removed prior to re-use. Vials that have been used and returned by the taxonomy laboratory **must be clearly labeled as such** so that they do not get used to store newly collected samples. Re-use of old vials without removing the barcode causes duplicate barcode errors that prevent data from loading.

Table 6. Sample types and barcodes used.

Sample Type	Description	Example Identifier	Fulcrum App	Container Type	Barcode Used	Barcode Required ?	Barcode Qty
Field samples	Mosquitoes from the catch cup	CPER_001.20180904.0813 *(plotID.collectDate. collectTime)* [used for internal label only]	MOS: Trap Setting and Collection [PROD]	1. Cryosafe vial OR 2. Cryosafe bag containing cryosafe vials	Type II	**Always Required**	1 per sample; up to 20 per bout per site

A.3 Preparing for Field Sampling

1. Plan and save sampling routes for field teams using standard site navigation procedures (RD[07]). Hand-written directions with approximate mileage can also help in the case of electronics failure. Route planning enhances sampling efficiency and helps avoid accidental foot traffic within NEON plots.

2. 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 16**). On this setting, the trap fan remains on at all times.

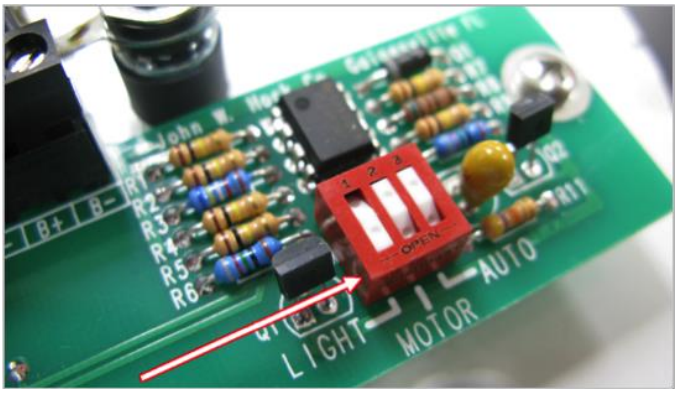


Figure 16. Switches inside circuit assembly of a CDC mosquito trap.

3. Remove the light bulb from each trap before the first use. Cover the hole with tape (masking tape recommended) store the light bulb in the lab. Traps are deployed without lights.
4. Optionally replace small key ring on the top of the rain cover and clip with a slightly larger key ring not to exceed 2.5cm (1 inch) in diameter and small carabineer to aid in removing/hanging traps in field.
5. Ensure that all traps have a drop cable loop support installed on the wire that leads into the fan to prevent it from being pulled out in windy conditions (**Figure 17**).
    a. For already fully assembled fans, remove the rain shield from the fan by unscrewing all three plastic topped screws.
    b. Select one of the metal screws securing the white aluminum plate to the clear plastic fan housing, making sure to **avoid the screw with heat shrink wrapping** securing the motor leads.
    c. Remove the chosen screw, and the long and short plastic sleeves that house it. Slip the key ring around the longer plastic sleeve, and then replace both plastic sleeves and thread the screw back through them, tightening to secure it in place.

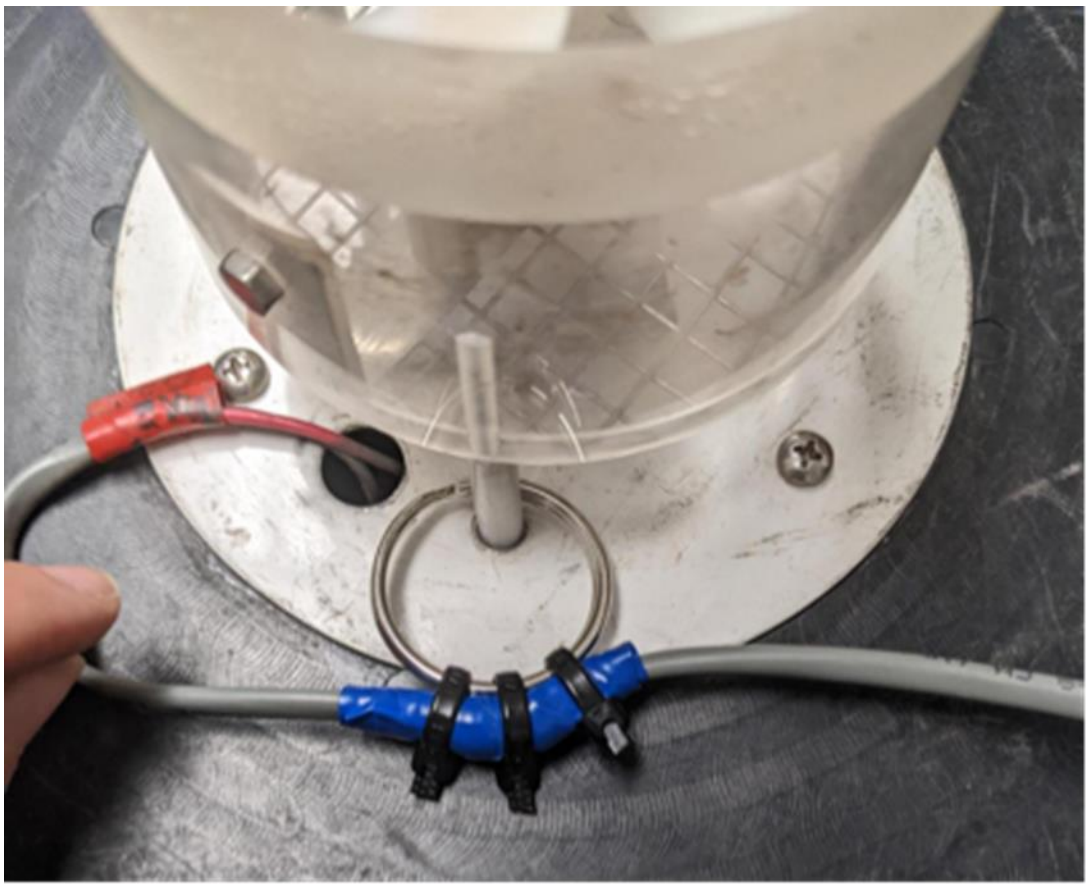


Figure 17. Example photo of a drop cable loop support to protect the wire leading into the fan from damage in windy conditions.

d. Take the battery leads and hold them against the attached key ring, adjusting to create 3-4 cm of slack in the leads between the key ring and the point where the leads pass through the white aluminum plate, making sure they will not be crimped when secured. Mark this point on the battery leads.

e. At the selected point, wrap the battery leads in electrical tape to create a padded sleeve of tape about 4 cm long to protect the leads from the cable ties.

f. Use one of the cable ties to secure the battery leads to the key ring at the midpoint of the taped section. Pull gently on the battery leads from a variety of angles to ensure that the leads will not be pinched and that little-to-no force is exerted on the point where the leads pass through the white aluminum plate. If crimping or pulling is observed, clip the cable tie and reposition the battery leads and/or tape pad to eliminate it and repeat the process to verify range of motion. If the leads have a sufficient range of motion with no problems, secure an additional cable tie on either side of the first, spacing them out to distribute the force between them.

g. Cut the excess length from the cable ties and reinstall the rain shield.

6. Ensure that all fans have a fuse installed on a wire leading to the battery connection (**Figure 18**).
    a. Place fuse inside fuse holder attach one spade connector to each side.
    b. Inspect fan wires for damage. Repair or replace damaged wires before continuing.
    c. Slide heatshrink tubing over positive (red) wire.
    d. Plug spade connector on fuse holder into positive (red) wire.
    e. Use electrical tape to secure the connector. Stretch the tape tightly. The purpose of the tape is to ensure the connector does not pull apart under tension.
    f. Slide heatshrink tubing over connector and shrink using a heat gun. The purpose of the heatshrink tubing is to make it difficult for a new user to use the trap without the fuse holder.

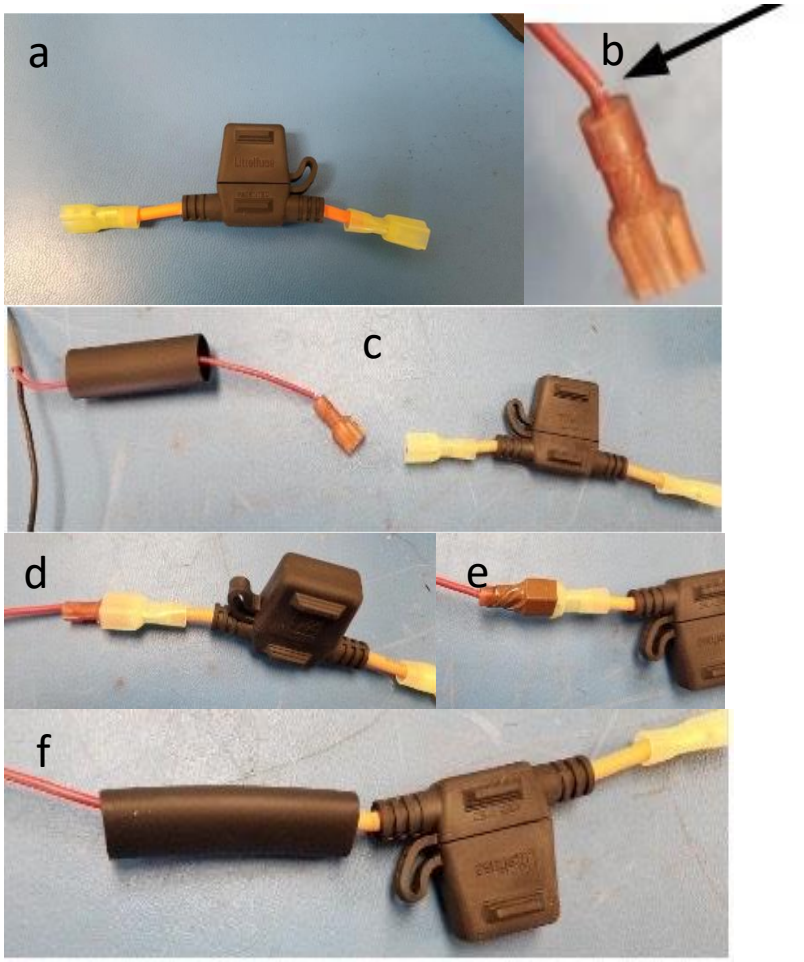


Figure 18. Diagram of steps involved in attaching fuse to battery wire. Place fuse inside holder and attach spade clips (a), inspect wire for damage (b), slide heatshrink tubing over red wire (c), plug fuse into wire (d), use electrical tape on connectors (e), and use heatshrink (f).

NOTE: If your domain experiences windy conditions at any sites create trap leashes to deploy during windy sampling (**Figure 19**).

g. Measure the length of a straight line from the fan housing where it hangs at rest, to the top of the curved support leg of the Achla 2-piece shepherd's hook. For domains that use an alternative set up such as U-posts or T-posts, measure to a point on the post approximately halfway between the point level with the fan housing and the ground. Record this measurement and add ~80 cm to get the needed length of cable.

h. Cut a length of vinyl coated cable using wire cutters. At each end of the cable, thread on a crimp sleeve, and create a loop just large enough to fit your hand through. Use a hammer to crimp the sleeve, fixing the loop in place. Attach a carabiner to one of the loops.

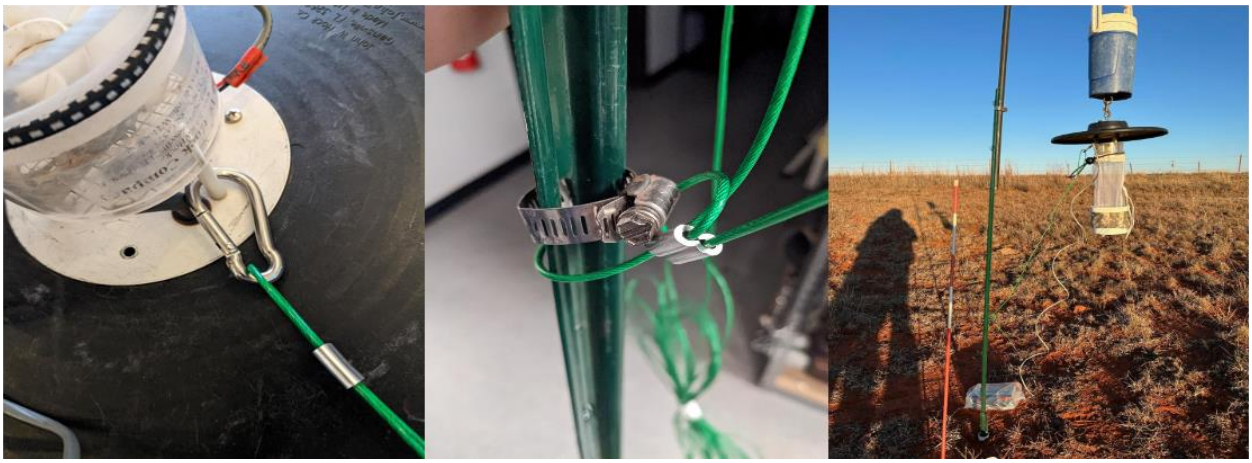


Figure 19. Example of trap leash showing two connection points and deployment in the field.

7. 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 spins in the proper direction). The fan may spin in the reverse direction if the battery leads are not connected properly (e.g., positive wire to negative battery terminal). Ensure that the mesh of collection cup sleeves is not torn and that the lid is tightened over the heavier-duty cloth part of the sleeve (not the finer mesh), as this may result in damage to the sleeve. When damage to the sleeve occurs, it is permissible to mend any holes in the sleeve, if possible. Use a sewing machine or needle and thread to make repairs.

8. The majority of sites will be using CO_2 canisters to bait traps. Unless pre-filled 5lb canisters are being purchased directly from a vendor, most sites will need to fill each 5lb CO_2 canister from a 50 lb siphon tank a minimum of a few days prior to sampling. Note that the less expensive 'industrial grade' CO_2 is sufficient for this protocol. Details and steps for preparing the canisters and regulators for this method of trapping are found in Appendix C.

9. If dry ice will be used to bait the traps, ensure that the interior of the coolers are marked at the half-way point using a permanent marker. This will allow for quick evaluation in the field as to the level of dry ice.
10. If alternative freezing materials will be used for storage after initial sample collection follow all procedures described in RD[11] for pre-chilling the ice packs/aquarium rocks and cooler for up to 48 hours. Charge trap batteries (see SOP A for battery charging instructions) and mobile data entry devices (data entry devices should be synced to the cloud prior to use).
11. If they will be used, make sure that reusable ice packs are frozen.
12. Identify the locations of plots used for mosquito sampling (use GPS and/or maps).
13. Print waterproof field datasheets (RD[05]) and two sets of waterproof sampleID labels for each trap (total of 10 labels per set). The format of the sampleID is plotID.collectionDate.collectionTime (example **Figure 13**). Note that because the time of trap retrieval will not be known when these labels are printed, enough space should be left on the label to allow the time to be written in by hand when the trap is retrieved. If preferred, the date can also be hand-written instead of pre-printed.
14. If vials are going to be re-used to store specimens, the following protocol should be followed to clean the vials prior to re-use. **All previous barcodes must be removed prior to re-use.** The vials and lids should be placed into a sink filled with warm soapy water. A test tube or bottle brush should be used to give each vial a quick scrub. Vials and lids should be rinsed in clean tap water followed by an individual rinse with a squirt bottle of DI water. Vials can be dried on a tray in a drying oven on low temperature overnight if needed. Vials should be stored separately by protocol and site (if possible) for re-use.
15. Prepare final sample containers by affixing one adhesive barcode label to each vial and/or Ziploc bag used to contain each sample (Type II cryosafe label, see **Figure 15**; **DO NOT** use other types of barcodes on the vial).

A.4 The Morning of Field Sampling:

1. Obtain enough dry ice to be able to freeze any samples during transport from the field to the lab (typically 2.5 – 4.5 kg is sufficient, but depends on drive time). If dry ice will be used as bait in the cylindrical insulated cooler of each trap an additional ~1.5 kg of dry ice in pellet form will be needed. If it is not possible to obtain dry ice follow the guidance in RD[11] to keep samples cold after collection.
    a. Acquire sufficient dry ice to account for sublimation between dry ice delivery/pickup and trap deployment.
    b. If trap 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 to avoid

release of CO_2 in enclosed spaces. Please keep in mind that **you need to remove tape at trap deployment**.

2. If using CO_2 canisters for bait secure the canisters into the truck with the approved transportation rack system that has been vetted by the safety team (**Figure 20**).

Figure 20. Transportation rack for CO_2 canisters

3. The use of insect repellent is recommended but only if you can apply it 30 minutes prior to trap deployment/reset; see Section 5 (Safety) for details on application.
4. Cover battery terminals for fans prior to transport into the field. If battery terminals are not adequately covered, contact with metal can result in arcing and/or smoking during transport.
5. Use the checklist (Appendix B) to ensure that all required materials are in the field truck prior to sampling.

SOP B Field Sampling

Table 7. Data collection resources for mosquito sampling protocol.

Resource Type	Name & Location
Fulcrum application	MOS: Trap Setting and Collection [PROD]
Fulcrum Manual	Fulcrum Manual for MOS: Trap Setting and Collection

B.1 Setting Traps

1. Navigate to the sampling plot using maps and/or a handheld GPS. Verify the identity of the plot location with plotID listed on the permanent plot marker (created during plot establishment).
    a. If plots cannot be accessed or sampling is otherwise impractical follow guidelines in the section Missed or Incomplete Sampling.
2. Hang a single trap at each mosquito trapping plot from a natural structure (e.g., a tree branch) or Shepherd's hook such that the height of the hole in the bottom of the insulated cooler is 1.2 - 1.8 meters (4-6 feet) above the ground.
    a. Hang the trap within 10m of the mosquito sampling point assigned during plot establishment (RD[06]).
    b. On more exposed sites, if possible, deployment locations should be adjacent to and on the west side of any available tall-statured vegetation to allow shading from the morning sun and on the leeward side to afford protection from prevailing winds.

       Alternative 1: At sites with sandy soils, drive a T-post support into the ground and secure the Shepherd's hook to the T-post to prevent the trap from falling over.

       Alternative 2: A Shepherd's hook may instead be placed into a 5-gallon bucket containing concrete that is anchored to the ground. The concrete in the bucket will act as a weighted anchor to prevent the Shepherd's hook from falling over in soils too unstable to use the T-post method or soils too compacted to insert the Shepherd's hook alone.

       See Appendix F for a list of sites using either the T-post method of securing Shepherd's hooks (Alternative 1) or the concrete method (Alternative 2).
    c. Avoid hanging traps over or within 5m of standing or flowing water. (Note: if it is not possible to place the trap within 10m of the monumented mosquito sampling point and 5m away from a water source, issue a problem ticket to NEON Science).
    d. At each sampled plot, hang the trap in the same location throughout all bouts of a season. If resampling the same plot over multiple years, continue to use the same location (if possible). In many cases the number of suitable locations for hanging traps will be limited, but make note of the location for use in subsequent seasons.

3. If it is windy enough that the trap is swinging around its attachment point use a trap leash (**Figure 19**).
    a. For Achla 2-piece hooks, or similar designs, slip the loop without the carabiner over one of the hook supports before pushing the hook into the ground, then clip the carabiner to one of the plastic sleeved screws connecting the fan housing to the white aluminum disk.
    b. For hook set ups using T-posts or U-posts, reach through the loop without the carabiner and grab the length of the cable, pulling it through to create a second loop. Slip this loop over the post and slide it to a point halfway between the fan and the ground, tugging gently to tighten the loop, preventing it from sliding up or down. Clip the carabiner to one of the plastic sleeved screws connecting the fan housing to the aluminum disk.
    c. With any hook set up, for the leash to work as intended it should be connected to a point on the hook that is lower than the fan, and the leash prevented from sliding up the hook or post when pulled, to reduce the risk of the fan being blown up and over the hook in strong winds. Modify this design as needed to achieve this goal.
4. Deploy the CO_2 'bait' method that will be used to attract mosquitoes to the area.
    a. If a 5 lb CO_2 canister will be the method of deploying CO_2 bait:
        i. Secure the canister with Velcro/bungees to the Shepherd's hook/post. If you are on a slope place the field canister on uphill side.
        ii. Attach the regulator to the field canister (**Note: Chemical Hygiene and Safety Plan prohibits carrying/transporting gas canisters with a regulator attached**). Insert regulator washer, attach to canister outlet, and tighten attachment nut, but do not over tighten. Ensure delivery pressure control knob is closed by turning knob counterclockwise until there is no resistance (**Figure 21**). Open 5lb field canister valve by screwing counterclockwise until completely open, then back off ¼ turn to ensure connections are not leaking.

Figure 21. Example regulator with parts labeled.

iii. Set delivery pressure gauge to 15-20 PSI using delivery pressure control knob. The PSI setting could vary by site and date and can be adjusted after checking the flow rate in the next step. Listen carefully for a leak where the regulator is connected to the tank. Spray soapy water on the connector to test for suspected leaks. If bubbles form at the site of the soapy water applications that indicates a leak. If the flow rate seems low for a given regulator setting it may also be indicative of a leak.

iv. Use a flow meter to check that the flow rate is between 0.5 – 0.7 Lpm or equivalent. Adjust as needed. Checking flow rates ensures that technicians are aware of any problems with the setup such as a plugged orifice restrictor or a problem with CO_2 delivery.

v. Hang trap from Shepherd's hook or T-post and attach CO_2 supply tube so that the end rests on top of the fan hat using either a binder clip on the cord from which the trap hangs, or snug re-usable zip ties on the Shepherd's hook. Take care not to exceed a 75-degree bend in the canister supply hose to allow for the free flow of gas. You may also want to attach the supply tube to the Shepherd's hook with Velcro strips.

vi. In the MOS:Trap Setting and Collection app be sure to change the trapType to **CO2Canister**. This is very important for documentation.

b. If dry ice will be the method of deploying CO_2 bait:

i. Hang the trap's insulated dry ice container from the elevated external structure and use the clip on the underside of the cooler (or optional carabineer) to attach the rain guard/fan assembly.

ii. Ensure that the clip on the cooler does not cover the hole where the CO_2 escapes.

iii. If coolers were prefilled at the DSF, please remove the tape covering the hole in the underside of the insulated cooler containing dry ice. **Easy to forget but critical step! In the MOS: Trap Setting and Collection app be sure that the trapType is set to **dry ice**. This is very important for documentation.

5. Secure the mesh collection cup to the lower end of the fan assembly using the elastic band sewn into the mesh. Rubber bands are a good addition to the elastic band (should the elastic wear or environmental conditions be windy).

6. Apply any weather-dependent trap modifications to help the samples stay as dry as possible.

    a. OPTIONAL: If conditions are extremely rainy, use a plastic sleeve to cover the mesh catch cup and prevent samples from becoming waterlogged in the mesh and icy when frozen. Plastic sleeve is made by cutting the bottom from a 1 Mil Gusseted Poly Bags. Slide the plastic sleeve over the cup and mesh sleeve as pictured (**Figure 22**). Use a rubberband to attach the bag to the fan assembly over the top of the sleeve. It is important to ensure that the bag does not extend above the plastic shroud of the fan assembly, which could potentially block mosquitos from entering the trap.

Figure 22. Optional plastic sleeve to cover mesh catch cup; only for use in rainy weather.

b. Note: Do **NOT** use this modification in misty or non-rainy conditions. If used in medium to low moisture situations, this modification can result in the fan pulling moisture in from the air. This *increases* moisture within the catch cup by trapping mist between the plastic sleeve and catch cup.

7. Connect the fan to the 6V battery by color-matching the wire leads and the battery terminals (red to '+' terminal, black to '-' terminal). The fan should immediately come on.

8. If possible, tie any loose battery cord around the natural feature or Shepherd's hook from which the trap is suspended. Place the battery in a re-sealable plastic bag to keep it dry. Best practice is to seal the bag around the wire and fold the top of the bag underneath the battery (this will prevent water from entering the battery assembly).

    a. At sites with cattle present, wrap the battery cord with aluminum foil to provide protection from chewing livestock and wildlife. Note that this measure may not have any effect on deer or elk.

9. If you have not already done so, attach a sampleID label to the catch cup of the trap. Use a safety pin to attach the label to the nylon 'cuff' of the catch cup sleeve. **Reminder: failure to complete this step will result in the loss of samples that cannot be differentiated.**
10. Record appropriate information about the visit to the sampling plot on the mobile data entry device (**Table 7**) or field datasheet (RD[05]) if the device is non-functional. All records must have plot locations, set dates and set times recorded.

B.2 Retrieving Samples from Traps

1. Navigate to the sampling plot using maps and/or a handheld GPS. Verify the identity of the plot location with plotID listed on the permanent plot marker (created during plot establishment). If plots cannot be accessed or sampling is otherwise impractical follow guidelines in the section Missed or Incomplete Sampling.
2. If the optional plastic sleeve was used to guard against heavy rain, simply slide the plastic up to access the top of the mesh sleeve. If the plastic sleeve is still in good condition, it can be reused when resetting the trap.
3. With the fan still running, gently tap flying mosquitoes down towards the bottom of the sleeve and into the cup, blowing air to move them down can also be helpful to avoid damaging samples. Tie the laces on the mesh sleeve of the catch cup to seal the opening.
    a. Take care not to crush any mosquitoes while tapping them down and tying the laces. Keeping the fan running during this process ensures that the mosquitoes cannot escape from the collection cup during this process. This step may be best completed as a two-person operation.
4. Remove the collection cup by sliding the mesh sleeve off the fan assembly, while keeping the fan running.
5. If possible, gently stuff/tuck sleeve material into the hole in the top of the catch cup but only to the extent that this does not crush mosquitoes.
6. **Ensure that a sampleID label is still attached to the collection cup**. If the sampleID label is no longer attached to the collection cup, attach a second (duplicate) label. Use an ethanol-safe, fine tipped pen or a pencil (if the label is wet) to write in the trap collection time on the sampleID label.
7. Redeploy trap as necessary. **Remember to attach a new sampleID label to the new (empty) collection cup attached to the re-deployed trap**.
    a. If dry ice is being used as bait, note that ice may have formed on or around the vent hole of the trap's insulated cooler. During deployment, condensation drips down the sides of the cooler and may freeze around the vent hole depending on ambient conditions. This is normal and should only be considered a problem if the insulated cooler is still more than half-full of dry ice at the conclusion of a deployment. The

halfway point was demarcated in permanent marker on the cooler interior during preparation, which allows for quick evaluation of the remaining dry ice.

Should this icing over of the vent hole occur and > 50% of the dry ice remains, the CO2Status field should be marked as flow obstructed, and the blockage should be noted in the remarks.

b. If CO_2 canisters are being used, check that the flow rate is between 0.5-0.7Lpm and adjust the flow with the delivery pressure control knob until desired rate is achieved, then rehang the supply hose. If the CO_2 flow rate is near zero mark the sampleCondition as sample incomplete and mark the CO2Status field as "Absent".

c. If CO_2 canisters are being used and traps are not being reset, you will need to bring the canister back to the lab. To remove the canister, first close the canister, bleed CO_2 off until pressure gauge indicates 0 PSI, disconnect from regulator, and remove all equipment from field. Handle regulators with care and coil hoses to prevent snagging on vegetation. When returning from the field weigh and store empty tanks in the freezer.

d. If traps are scheduled to be reset but will not be reset be sure to create Sampling Impractical records and choose the appropriate rationale from the samplingImpractical dropdown field (**Table 4**).

8. In instances where mosquito abundance is exceedingly high, trap fans may become clogged and have their function impaired by dead mosquitoes. In these instances, it is recommended that the trap fan assembly be swapped out for a new (clean) assembly when the trap is serviced (samples retrieved). The old (clogged) fan assembly can then be returned to the lab and dead mosquitoes cleaned from the fan blades, housing, motor, and intake screen.

9. Transport catch cup containing sample back to field vehicle.

10. It is very important that catch cups are as dry as possible before they are placed on dry ice or alternate freezing materials (RD[11]). Wet samples can become frozen into unidentifiable masses that are challenging to separate. Prior to placing the catch cup into an insulated cooler for transport back to the lab, use paper towels (recommended) to remove any water that has accumulated in the catch cup. Do this by swabbing the mesh-covered hole in the bottom of the catch cup. **Do not untie the laces.**

11. Place catch cup into insulated cooler for transport back to the domain lab. To prevent mosquitoes sticking to the metal mesh, make sure to place the catch cup 'upside down' in the cooler such that the metal is facing up. The cooler should ideally contain dry ice but may contain alternative freezing materials (RD[11]) if logistics (e.g., duration of field visit, local availability of dry ice) preclude the use of dry ice.

12. Place cardboard or a cloth (an old tick sampling cloth works very well) between the catch cups and the ice so that they do not come into direct contact. Moisture on the outside of the catch cup or mesh bottom can freeze to the ice and cause cups to stick, potentially damaging

equipment or samples. Once frozen, samples must remain frozen at all times. Covering the dry ice is optional when temperatures are so high that the samples may not freeze sufficiently, or if using alternate freezing materials.

13. Record appropriate sampling information on the mobile data entry device or field datasheet (RD[05]) if the mobile device is non-functional. This includes the:
    a. plotID (4 letter site code and 3 digit plot number; e.g., HARV_001),
    b. collectDateTime (date and time that the protocol step was completed),
    c. fanStatus (On or Off)
    d. cupStatus (OK, missing, or disturbed)
    e. CO2Status (Present, Absent, Flow Obstructed, or Other – see remarks). Absent should be used when the dry ice or CO_2 has run out, while flow obstructed is used for situations where the dry ice vent hole or delivery tube is clogged.
    f. "targetTaxaPresent" (may be revised during sample processing in SOP C)
        i. This field can be populated in Fulcrum with values of Yes, No, or Maybe and can be filled out in the field or in the lab when the catch cup is processed.
        ii. Enter "N" if the catch cup is completely empty or contains only a very small number of by-catch specimens (e.g., a couple of moths) that can be quickly identified and removed. In this case (targetTaxaPresent=N), no sample vial is generated.
        iii. Enter "Y" if the catch cup definitely contains mosquitoes. In this case (targetTaxaPresent=Y) one or more sample vials will be generated.
        iv. Enter "M" (maybe) if you think that there might be mosquitoes in the sample. In this case (targetTaxaPresent=M) the catch cup will contain insects when examined in the field at the time of collection but when examined in the lab, the cup will contain either a) some insects that look like (or might be) mosquitoes, or b) so many insects, including potentially many by-catch individuals, that the sample cannot be sorted (by-catch removed) quickly and instead is simply transferred into one or more vials for sorting and ID at an external facility. In this case (targetTaxaPresent=M), one or more sample vials will be generated.
    g. sampleCondition (may be revised during sample processing in SOP C)
        i. No known compromise – sample intact/good condition.
        ii. Cold chain broken – sample thawed at any point in the treatment of this sample.

iii. Sample incomplete – some mosquitoes escaped & the sample sent to the identification lab is incomplete; could also be used if the sample was stored in 10 falcon tubes, but one tube is misplaced/lost.

iv. Handling error – sample was damaged (e.g. catch cup dropped).

v. Other (describe in remarks) – use this option if multiple types of compromise occur or a compromise occurs not in this list. One example is when the timing of trap setting or collection falls outside the allowable window.

h. sampleFate (may be revised during sample processing in SOP C)

i. active – a sample with mosquitoes (or insects that might be mosquitoes) was collected.

ii. lost – a sample with mosquitoes (or insects that might be mosquitoes) was collected, but all insects escaped and thus no sample exists (entire sample lost).

14. If using a Shepherd's hook, leave the hook at the plot for the duration of the sampling season, if the site use permit allows.

B.3 Sample Preservation

1. Upon returning to the lab, immediately transfer catch cups into an ultra-low freezer (-80°C). Keep catch cups from different collection events separate (e.g., 1st collection cups should be in a different labeled bag than 2nd collection cups to aid in differentiating them during the transfer to vials in the lab). Processing samples by transferring catch cup contents into vials soon after returning from the field can minimize data entry errors.

2. **Once frozen, samples must remain frozen at all times.**

B.4 Refreshing the Sampling Kit

1. Test traps to verify that they are still fully functional.

2. Refreeze reusable ice packs.

3. Obtain fresh consumable items and stock sampling kit with replacements of all necessary supplies (i.e., 20 new collection cups, spare fan assemblies, etc).

4. Print new datasheets (RD[05]) and sampleID labels as needed; note that it is encouraged to do this in advance at the start of the season.

5. Sync mobile device/tablet at end of field day at Domain Support Facility, and place mobile device on charger when not in use.

B.5 Equipment Maintenance, Cleaning, and Storage

1. If mesh sleeves/collection cups are wet or dirty following trapping, gently wash them by hand using fragrance-free laundry detergent and hang/stack to dry. Make sure all trap components are clean and free of insect parts.
2. If the mesh sleeve is damaged or torn it should be replaced as captured mosquitoes may be able to escape through holes in the mesh.
3. Check the mosquito fan wires for damage (e.g., chewed wires, cords caught in vehicle doors or storage bin lid). Minor damage is repairable by covering hole(s) with electrical tape. Unrepaired damage to wires can result in inoperative fans in the field.
4. Clean fans as necessary. Please note that if mosquito traps will be used at multiple sites it is imperative to visually inspect fans and catch cups for stray mosquitoes and clean as necessary. Best practice is to keep traps separate between sites when possible to avoid accidental transfer of mosquito specimens from incompletely cleaned traps between sites.
    a. Optional step: Remove the fan assembly from the rain cover.
    b. Fill a small tray with warm soapy water to a height that, when one or more fan assemblies are placed in the tray, the water level reaches just below the fan motor.
    c. Soak fan assemblies for 10-20 minutes and then remove from the water tray and clean with bottle brush.
        i. Alternatively, a toothbrush with a small amount of dish soap can be used to clean the fan assembly in lieu of a soaking tray. This method may be more appropriate in cases with especially persistent mosquito debris.
    d. Once clean, set the fan assembly(ies) on a paper towel to dry before reattaching the rain cover.
5. Clean any other equipment as necessary using fragrance-free laundry detergent.
6. Make sure all equipment is dry before placing it in storage.
7. Charge mosquito trap batteries. 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 and transportation procedures should be followed.
    a. Use plastic covers or tape to cover terminals when not in use.
    b. Charge batteries 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. The green light indicates charging is complete and batteries on chargers should not be touched until after the green light comes on.
    c. Warning signs should be placed around the batteries while charging.

d. Connect the color-coded leads to the battery.

e. 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.

f. 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.

g. 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.

h. 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.

Important note: When *transporting* batteries into the field, each battery must be placed in its own plastic bag as batteries can arc and/or smoke if uncovered terminals meet. Uncovered batteries are a safety risk and can also damage the battery itself.

SOP C Post-Field Sampling Tasks

C.1 Document Incomplete Sampling Within a Site

Mosquito sampling is scheduled to occur at all prescribed sampling locations according to the frequency and timing described in Section 4 and 1 (off season: 1 collections per plot per bout; up to 3 samples generated per site per bout; field season: 2 collections per plot per bout; up to 20 samples generated per site per bout). Ideally, sampling will occur at those 10 sampling locations for the lifetime of the Observatory (core sites) or the duration of the site's affiliation with the NEON project (gradient sites). However, sampling may be shifted from one location to another when sampling is compromised. In general, a sampling location is compromised when sampling becomes so limited that data quality is significantly reduced.

There are two main pathways by which sampling can be compromised. First, sampling locations can become inappropriately suited to answer meaningful biological questions – e.g., a terrestrial sampling plot is compromised after road-building activities, or a terrestrial sampling plot becomes permanently aquatic. Second, sampling locations may be located in areas that are logistically impossible to sample on a schedule that is biologically meaningful.

For the mosquito sampling program, a given plot must be sampled at least 50% of the bouts expected for the site (see 1**, Table 10** for the number of expected bouts) over a two-year period. Plots that cannot be sampled on this schedule should be considered compromised.

If sampling at a given plot is not possible during a given bout for reasons other than those already covered by the protocol (e.g., low temperatures or windy conditions) a problem ticket should be submitted by Field Science staff.

To document locations not sampled during the current bout:

1. Review Fulcrum records to determine which locations were scheduled for sampling but were not sampled.
2. Create an incident with the following naming convention to document the missed sampling: 'TOS Sampling Incomplete: MOS – [Root Cause Description]'
    a. Example: 'TOS Sampling Incomplete: MOS – Could not access plot due to permanently closed road'
3. If sampling at a plot is considered compromised due to one of the reasons listed above, alert Science staff with a ticket.

C.2 Laboratory Processing – Transferring Mosquitoes from Catch Cups to Sample Vials

1. Ensure that mosquitoes have reached cold enough temperatures to kill (and not just immobilize) them prior to sorting samples into vials. In cases where alternate freezing materials were used (RD[11]) or dry ice levels in the cooler are low, please store samples in the ultralow (-80) freezer

for at least 30 minutes prior to transferring to vials. If mosquitoes are not cold enough they can appear dead, but then thaw during processing and fly away resulting in incomplete samples.

2. Clear and clean off bench space prior to processing samples.

3. Obtain enough sample vials to hold samples from each catch cup. Use vials and/or bags that have been pre-labeled with barcodes at least 20-30 minutes before processing samples (SOP A). Be sure that you are using clean vials with new barcodes. If the taxonomy laboratory returns dirty vials these should be clearly labeled within the laboratory to prevent their accidental use prior to appropriate cleaning and removal of old barcodes.

    a. In the case of large volume samples, you may need multiple vials for each catch cup.

    b. Approximate the number and size of vials based on typical catch cup volumes at your site (10 mL vials for sites with few mosquitoes per sample, 50 mL vials for sites with moderate numbers of mosquitoes per sample). Preparing extra vials is recommended in the event that cup contents are higher than expected.

Figure 23. Example of 2 mosquito samples; medium (upper) and small (lower) capture events, respectively. Note that the human-readable sampleID is placed between the cap and upper tissue, away from the mosquitoes.

    c. Note: Each barcode-labeled container must be filled with a unique sample. If multiple vials are required to contain a sample from one trap, the barcode must be placed on the cryosafe bag that will contain all vials associated with that sample (**Figure 24**). Barcodes are unique, but are not initially associated with a particular sample, so you are encouraged to prepare these in advance (see SOP A).

Example: If a catch cup from collection fills ten 50-mL falcon tubes, the single barcode is applied to the cryosafe bag not each vial containing 1/10 the sample; the database cannot handle 10 barcodes mapping to the same catch cup.

Barcodes must be adhered to each vial or cryosafe bag for 20-30 mins *before* introducing the container to dry ice or -80C. Place the barcode on the white surface of the cryosafe bag (**Figure 24**).

Figure 24. Example of a large volume sample that was placed into multiple vials; only one Type II barcode is used on the exterior of the cryosafe bag.

4. Fold a square of toilet paper (1-ply is sufficient) into quarters to create a smaller square.
5. Open each vial, use your thumb to make a pocket in the center of the toilet paper square, and place the folded toilet paper into the bottom of the vial for packing. For smaller vials, use a smaller amount of paper and the end of a brush to make a pocket.
    a. The paper padding will help absorb moisture, prevent mosquitoes from sticking to the bottom of the vial, and cushion the sample during transit.
    b. At this time you can also prepare the tissue "plugs" that will be used at the top of your sample/below lid. This plug holds the mosquitoes in place so they cannot bounce around, but does not squash them.
6. Prior to sample transfer, pull up the record in the mobile data entry application of the one catch cup you are about to process and scan the barcode that corresponds to that one sample. Note: Data must be entered into Fulcrum prior to placing mosquitoes from the catch cup into one or more vials.
7. The sampleID label generated in the field at time of collection needs to be placed into each vial once it is full of mosquitoes (see step 10).

a. For the samples that fit into a single vial, the sampleID label attached to the catch cup is used as the internal sampleID label. When used, make sure that the trap collection date and time areincluded on the label. Verify that the sampleID label matches the auto-populated sampleID in the electronic file.

b. If multiple vials are required to hold the sample, print additional sampleID labels. These will be inserted into each vial with mosquitoes. Printing extra labels is recommended.

8. Gather and/or prepare any equipment necessary for transferring mosquitoes from catch cups into sample vials.

9. Set up a chilling station to keep sample vials cold following removal from freezer/dry ice and during transfer.

a. A simple version of such a station involves a Tupperware container filled with dry ice or ice, with a cardboard “lid” that has holes cut into it for insertion of chilled sample vials (**Figure 25**).

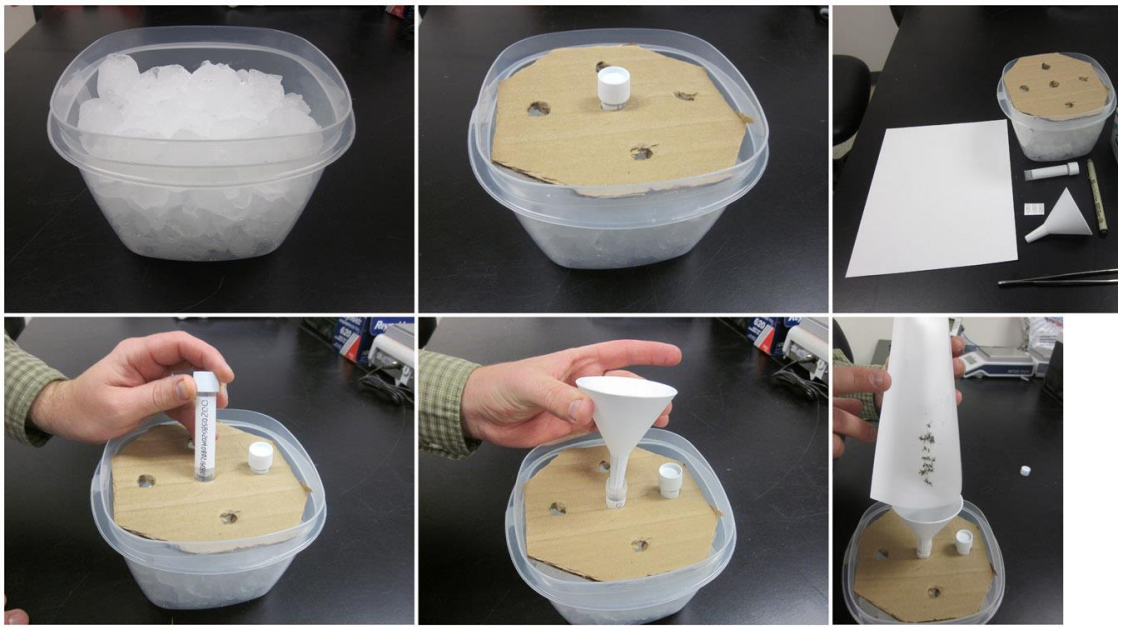


Figure 25. Example of a chilling station and associated laboratory setup for transferring mosquitoes from catch cups into sample vials.

10. Optional: If processing large numbers of samples, it is helpful to have an intermediate cooler filled with dry ice or alternative freezing materials. If used, multiple traps can be placed into this intermediate cooler from the -80°C freezer. This prevents having to repeatedly reopen the freezer. Keep in mind, all catch cups should be placed with the wire mesh facing up as mosquitoes can be damaged if they are in contact with the frozen wire mesh.

11. Remove one catch cup and the corresponding labeled empty sample vial(s) from the freezer (or intermediate cooler as described above). Place empty vials into chilling station. Note that samples should be placed in the ultralow freezer for at least 30 minutes prior to processing mosquitoes if alternative freezing materials were used at initial sample collection. This is because the temperatures are sometimes not cold enough to kill the mosquitoes and they can warm up and fly away prior to processing.
12. Gently transfer mosquitoes from the catch cup into the empty sample vial(s).
    a. Insert a funnel into a frozen sample vial.
    b. Unscrew the lid of the catch cup and remove mesh. Be sure that no mosquitoes are trapped in the mesh or stuck to the sleeve.
    c. Remove obvious/large by-catch (e.g., moths, beetles) that are clearly not mosquitoes and that could damage mosquito specimens when sample vials are sent to external facilities.
        i. While by-catch removal can improve sample quality, prioritize keeping mosquito samples frozen (i.e., do not spend too much time removing by-catch).
        ii. If by-catch is frozen to mosquitoes (and is of similar size to the mosquitoes), do not attempt to disentangle.
        iii. Do not spend more than 5-10 seconds per sample removing by-catch.
    d. If you need to revise "targetTaxaPresent" based on the contents of the trap, pull up the record on the mobile data entry tablet.
        i. This field can be populated with values of Yes, No, or Maybe (choose one).
        ii. Enter "N" if the catch cup is completely empty or contains only a very small number of by-catch specimens (e.g., a couple of moths) that can be quickly (5-10 seconds) identified and sorted out when the catch cup contents are being processed. In this case (targetTaxaPresent=N), no sample vial is generated. Do not send empty vials to the taxonomist.
        iii. Enter "Y" if the catch cup definitely contains mosquitoes. In this case (targetTaxaPresent=Y) one or more sample vials will be generated.
        iv. Enter "M" (maybe) if you think that there might be mosquitoes in the sample. In this case (targetTaxaPresent=M) the catch cup will contain either a) some insects that look like (or might be) mosquitoes, or b) so many insects, including potentially many by-catch individuals, that the sample cannot be sorted (by-catch removed) quickly and instead is simply transferred into one or more vials for sorting and ID at an external facility. In this case (targetTaxaPresent=M), one or more sample vials will be generated.

e. Guide mosquitoes from the catch cup gently into the vial using a funnel (in **Figure 25** a piece of paper is used for this purpose). Alternatively, if mosquitoes are getting stuck in the funnel, the catch cup can be emptied by placing its contents on a white sheet of paper. The mosquitos can then be gently swept into the vial using a soft brush.

Note: Mosquitoes may rarely become encased in ice within the catch cup when excessive moisture at the time of collection is not removed promptly. Ice within the vial can damage specimens and result in a 'mosquito meatball', where specimens are mangled and unidentifiable by the taxonomist. Depending on how much excess ice is in the sample, one of two options are available:

OPTION A: **Only a small proportion of individuals are encased in ice**

Place as many mosquitoes as possible into the vial. The remainder of the mosquitoes in the sample may be discarded; during data entry, select 'sample incomplete' in the sampleCondition field. This should be done if just a small amount of the sample is encased in ice (i.e., <1% of the sample).

OPTION B: **Many (>1% of sample) of the mosquitoes within the sample are encased in ice**

Allow the affected sample to thaw until mosquitoes are able to be placed into a vial. During data entry, select 'cold chain broken' in the sampleCondition field. Samples may be allowed to thaw and dry out for as long as 10 minutes. This should only be done for samples where a large number of mosquitoes are encased in ice (e.g., 200) or a large proportion of individuals from the trap (i.e., >1% of the sample)

f. Use soft touch or feather-weight forceps to transfer any mosquitoes that may remain in the catch cup or associated mesh into the sample vial (i.e., are stuck to the mesh). Mosquitoes may be gently picked up by the wings, but should not be grabbed by the leg (legs tend to fall off) nor the body (easily pinched and then hard to identify). Take care to thoroughly check the catch cup, sleeve, and mesh around all edges for mosquitoes to ensure complete transfer of the sample so that mosquito specimens do not get inadvertently transferred between bouts/plots/sites upon re-use of the trap.

    i. **Do not overfill** the sample vial. Overfilled samples with too many mosquitoes will result in individuals being crushed and body parts being disassociated from the mosquito. Without the legs and wings intact, taxonomists cannot identify mosquitoes to species. See **Figure 26** for appropriate and inappropriate levels of mosquitoes.

Figure 26. Proper amount of tissue cushioning the top and bottom of two mosquito samples. Note that it is important to place the sampleID label between the cap and upper tissue.

g. Add an upper layer of toilet paper on top of the mosquito samples. Gently push this upper plug against the mosquitoes firmly enough to prevent sample movement but gently enough to avoid crushing samples. This will prevent samples from shifting and being damaged during subsequent handling and shipping.

Add additional tissue above the upper plug if the mosquito sample is small and there is empty space in the upper part of the vial. Alternatively, if the mosquito sample is too large to fit into a single vial, use additional vials as necessary until all mosquitoes from a catch cup have been transferred into sample vials.

13. Place a folded sampleID label into each vial above the upper plug of tissue. If only one vial is needed to contain the sample, the sampleID label from the field may be used (if it's in good condition). Otherwise, the sampleID should be printed and included in each vial associated with the sample.

    a. If using small vials, fold the label and/or slip it along the edge of the vial above the top layer of toilet paper to avoid crushing mosquitoes.

14. Seal each vial and immediately place it into an ultralow freezer at -80°C.

    a. When storing samples, take steps to keep samples of similar origin together. This organization will reduce the probability of samples thawing when they are inventoried and sorted at external processing facilities.

    b. Rubber band or bag multiple vials from a single site/bout/trap combination.

c. Store all vials from a sampling site/bout combination together in a re-sealable cryosafe bag or vial rack.

d. Vials from different sampling bouts within a site, and from different sites, should not be mixed.

15. For each sample vial, record the number of vials used to contain this unique sample directly into the fulcrum application (SOP D).

16. Wipe off any metal or plastic implements used during sample processing (e.g., forceps, plastic funnel) with a paper towel moistened with ethanol before processing the next sample.

a. It is acceptable to use the same paper towel to clean up after multiple samples.

Figure 27. Three mosquito vials. The center vial has an appropriate amount of wadding above and below the sample of mosquitoes. The sample on the left and right have too little material to effectively cushion the sample. Note that the sampleID label must be placed between the upper tissue plug and the lid of the vial.

C.3 Sample preservation

After each sample is processed, transfer the storage vial into an ultralow freezer (-80°C) until shipment.

C.4 Equipment Maintenance, Cleaning and Storage

1. Clean off the surface of the lab bench where processing activities were performed.
2. Clean any non-disposable equipment used during processing (e.g., funnel) with ethanol.
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.

SOP D Data Entry and Verification

Mobile applications are the preferred mechanism for data entry. Data should be entered into the protocol-specific application as they are being collected, whenever possible, to minimize data transcription and improve data quality. Mobile devices should be synced at the end of each field day, where possible; alternatively, devices should be synced immediately upon return to the Domain Support Facility.

However, given the potential for mobile devices to fail under field conditions, it is imperative that waterproof paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times. As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

If paper datasheets are used, the procedure is as follows:

1. Enter data from field datasheets and the number of vials generated from sample processing into the Mosquito mobile application or web user interface (WebUI), according to instructions in the AOS/TOS Protocol and Procedure: DMP – Data Management (RD[04]).
2. Scan datasheets and save in PDF file format.
3. Save paper copy of datasheets.

Before entering data, all personnel must read RD[04] for complete instructions regarding manual data transcription. Prior to entering data via WebUI or mobile application, each technician shall enter a plot (or subplot) of data from one bout into the protocol-specific WebUI or mobile data application housed on the Training portal, as described in RD[04].

Be sure to enter data for all plots within a bout that were visited even if traps were not set as scheduled, due to unforeseen circumstances (i.e. traps left out for 24 hours should still be entered with the actual time set and collected).

Quality Assurance

Data Quality Assurance (QA) is an important part of data collection and ensures that all data regarding observations and samples are accurate and complete. This protocol requires that certain QA checks be conducted in the field (i.e., before a field team leaves a plot or site), while others can be conducted at a later date in the office (typically within a week of collection). Field QA procedures are designed to prevent the occurrence of invalid data values that cannot be corrected at a later time, and to ensure that data and/or sample sets are complete before a sampling window closes. Incomplete data and/or sample sets cannot be supplemented by subsequent sampling efforts if the sampling window has closed. Invalid meta-data (e.g., collection dates, plotIDs) are difficult to correct when field crews are no longer

at a sampling location. Office QA procedures are meant to ensure that sampling activities are **consistent** across bouts, that sampling has been carried out to **completion**, and that activities are occurring in a **timely** manner. The Office QA will also assess duplicative data to maintain data **validity** and **integrity**.

All QA measures needed for this protocol are described in the AOS/TOS Protocol and Procedure: DMP – Data Management (RD[04]). There is an additional QC Checklist available from the sampling support library that details all checks that should be performed in the field as well as after returning to the laboratory.

SOP E Sample Shipment

1. Follow sample shipping timelines in Section 4 to maintain appropriate sample hold times and storage conditions.

    a. Discrepancies between this protocol document and the Shipping Protocol should be communicated to Science.

2. Follow instructions in the NEON Protocol and Procedure: Shipping Ecological Samples and Equipment in order to ship samples to external laboratories or the biorepository (RD[10]).

8 REFERENCES

Menne, Matthew J., Imke Durre, Bryant Korzeniewski, et al. 2012. Global Historical Climatology Network - Daily (GHCN-Daily), Version 3. [subset: 2006-01-01 to 2015-12-31]. NOAA National Climatic Data Center. https://doi.org/10.7289/V5D21VHZ

APPENDIX A QUICK REFERENCES

Quick Reference: Getting Ready for Sampling

STEP 1 – Gather all needed supplies (and extras). Be sure to start a few days ahead of time if using alternate freezing materials or CO_2 canisters to allow enough time to chill supplies and fill tanks.

STEP 2 – Test functionality of mosquito trap components.

STEP 3 – Upload sample locations to GPS unit and obtain maps.

STEP 4 – Charge & sync mobile data entry device. Print waterproof datasheets (used only if there is a failure in the mobile data recorder).

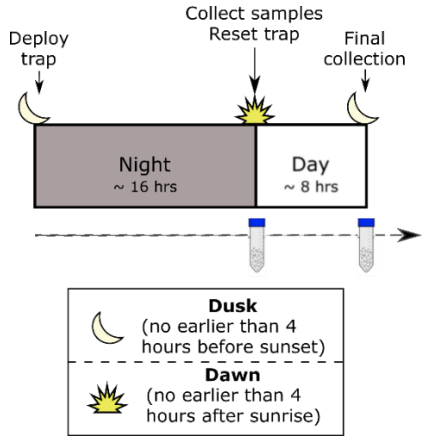
STEP 5 – Generate and print sampleID labels for each trap (plotID and collectDate pre-printed with space for collect time). You will need 20 labels per bout but you need to print extras.

On the field day:

STEP 6 – If using dry ice for bait or sample storage: obtain enough dry ice to set/reset traps. Bring additional dry ice to keep samples frozen during transport back to the lab.

STEP 7 – In coolers used to transport samples from field to lab, cover dry ice with cardboard or cloth. Covering the dry ice is optional when temperatures are so high that the samples may not freeze sufficiently, or if using alternate freezing materials.

Mosquito trap servicing during a bout occurs during a ~24-hour window, including one night and one day. This involves three trips to each sampling plot.



Keep samples frozen. The genetic material (that will be analyzed for pathogens) degrades when a sample thaws.

Quick Reference: Setting the Trap and Collecting Mosquitoes

For each bout, on the first visit:

STEP 1 – Pin sampleID label onto the mesh sleeve for the plot being visited. Verify that the sampleID (with plotID and collectDate) are correct.

STEP 2 – Set up CO_2 canister or fill cooler with dry ice pellets depending on bait method being used.

STEP 3 – Assemble trap components. Attach fan assembly to rain cover using screws if not already attached. Attach catch cup (with mesh sleeve) to fan. Connect fan to battery and seal battery in reusable 4-mil bag on the ground. If using dry ice and you pre filled coolers at DSF **remove tape from cooler vent hole**.

STEP 4 – Suspend trap approximately 1.2-1.8m above the ground. Hang on leeward side of tree, shrub or alternate Shepherd's hook/post. When possible shield from heavy wind, morning sunlight or rain.

STEP 5 – Record all metadata—especially plotID, set date, and set time—and any irregularities on mobile application (or paper datasheet if mobile application is unavailable).

Subsequent visits:

STEP 7 – After elapsed time, return to trap with replacement catch cup, dry ice or alternate freezing materials (RD[11]) and spare parts.

STEP 8 – Keep fan running. Tie off mesh sleeve and remove catch cup from fan assembly.

STEP 9 – Gently tuck mesh sleeve into catch cup.

STEP 10 – Record time of collection on the sampleID label. Place catch cup 'upside down' into cooler containing dry ice or alternative freezing materials.

STEP 11– Record all metadata and any irregularities on mobile application (or paper data sheet if mobile application is unavailable).

STEP 12 – Reset trap, if required. On final visit, bring trap components back to the lab.

STEP 13 – Carefully transport catch cup in cooler back to vehicle.

APPENDIX B REMINDERS

Getting Ready for Sampling

At least one to two days before field effort: Make sure to…

- ☐ Fill CO2 canisters a few days before the bout and pre-chill any freezer packs or aquarium rocks
- ☐ Test equipment at least one day before a sampling bout
- ☐ Print Mosquito Sampling Datasheet
- ☐ Bring a synced and charged tablet that has the Mosquito application loaded
- ☐ Upload sample coordinates to GPS and obtain maps
- ☐ Bring all supplies and extras

Labels: Be sure to…

- ☐ Print labels (Rite in the Rain) with correct plots and collection dates
- ☐ Cut labels into strips or bring scissors into field
- ☐ Bring safety pins for attaching sample labels
- ☐ Bring extra blank labels

Equipment: Be sure to…

- ☐ Inspect catch cup and mesh sleeve for tears
- ☐ Check circuit switches on traps (1st switch closed, 2nd and 3rd switches opened) at the beginning of the season.
- ☐ Test fan by connecting it to battery
- ☐ Inspect fan wires for damage
- ☐ Charge batteries
- ☐ Charge and sync mobile data entry device
- ☐ Print datasheets (Rite in the Rain)
- ☐ Check that sufficient dry ice is available when it is being used in the field

Collecting Quality Mosquito Specimens

Before heading into the Field: Make sure you have…

- ☐ All supplies and extras
- ☐ Printed paper datasheets, mobile data recorder, and sampleID labels (20 per bout plus extras)
- ☐ Tape the hole in the canister cooler (if it is being used and was filled with dry ice in lab)
- ☐ If used, applied insect repellant away from sampling equipment at least 30 mins before heading into the field. Wash hands before handing sampling equipment

Sample collection: Be sure to…

- ☐ Plan your day so that you service traps within the required window of time.
- ☐ Double check that the location written on the label matches the plotID on the permanent plot marker.
- ☐ Check mesh sleeve for tears or holes.
- ☐ CHECK THAT SAMPLE LABELS WITH DATE ARE ATTACHED TO MESH SLEEVE.
- ☐ Battery is connected to fan housing and fan is running in the correct direction.
- ☐ Place catch cups with target taxa in cooler with dry ice or alternate freezing materials RD[11].
- ☐ Check that the CO_2 flow rate is between 0.5-0.7 Lpm or that the cooler has been refilled with dry ice as needed.
- ☐ Record all required data and irregularities on mobile application (or paper datasheet if mobile application is unavailable).
- ☐ Keep cooler secure in the truck bed (not the cabin) of the vehicle so that it does not tip during transport.
- ☐ Transfer catch cups to a -80C freezer in the domain lab as soon as possible to maintain sample integrity.

Note on transporting dry ice: Coolers should be in the truck bed not the cabin (due to dry ice sublimation, and risks of CO_2 being released inside the enclosed truck)

Processing Specimens in the Lab

Specimen quality: Be sure to…

- ☐ Store specimens in the ultra-cold freezer (-80°C)
- ☐ Work with specimens from one catch cup at a time
- ☐ Look carefully for mosquitoes caught in folds of mesh sleeve
- ☐ Work quickly, sorting mosquitoes from obvious bycatch so that mosquitoes remain frozen and cold chain is not broken
- ☐ Provide sufficient padding for the mosquitoes on the bottom, tap the vial after filling to reduce space being specimens, and add a tissue plug to the top
- ☐ Put sampleID label (plotID.collectDate.collectTime) in every sample vial between the cap and top tissue plug

APPENDIX C DOCUMENTATION FOR THE USE OF CO_2 CANISTERS IN THE FIELD

C.1 Health and Safety

CO_2 canister handling can expose staff to hazards such as high-pressure release of compressed gas. The use of personal protective equipment includes gloves and eye protection when handling canisters as well as hearing protection when bleeding tanks. To mitigate the risk caused by expanding gases when the canisters are potentially exposed to high temperatures and direct sunlight, the following health and safety measures should be implemented:

- Limit direct sun exposure and cover canisters when the potential of direct sun is present.
- Routine temperature readings on the canisters using a laser unit to check external canister temperature and adjust positioning as needed. For temperature readings above 95°F, operator should take mitigation measures such as moving canisters to shade or covering them with an insulated pouch such as a backflow winter pipe cover to reduce direct exposure to sunlight.
- Brief staff on the overpressure valve, how it works when excessive pressure builds in the canister and to avoid the release valve nozzle (direct away from staff when handling).
- Secure tanks when filled and ensure release valve nozzles are directed away from staff.
- Canisters with liquids should not be filled above 80% full to allow for expansion.
- It is recommended that canisters should have an attached carry handle for ease of transit and to protect the valves in case they are dropped.
- Do not carry or transport gas canisters with a regulator attached.
- Transport canisters in a safety-approved rack system that is secured to the truck bed (**Figure 20**).

C.2 Initial Regulator Set-up:

1. Cut a ~10 ft length of 1/8” ID vinyl tubing into two segments.
2. Connect one end of a length of tubing to the outlet fitting of the regulator and the other end to the orifice restrictor.
    a. The original outlet fitting on the regulator will need to be removed and replaced with 1/8” barb x ¼” male NPT (**Figure 29**).
    b. Remove existing hose barb from the regulator using ¾” wrench and 9/16” socket (**Figure 29**). Note that removing the outlet fitting requires a lot of force and securing the regulator in a vise may be required.
    c. Install Teflon tape on 1/8” barb x ¼” male NPT fitting threads and insert barb fitting into regulator and tighten.

d. If the tubing is difficult to maneuver over the barbs of the orifice restrictor, use a heat gun/lighter to warm the end of the tubing. This will allow the tubing to easily slide over the barbs. As the tubing cools, it will create a tight seal on the barbs.

e. **Note the arrow on the orifice restrictor to indicate proper gas flow direction** (**Figure 28**).

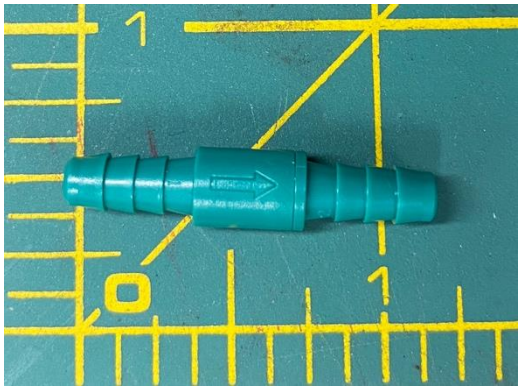


Figure 28. Barb showing arrow indicating direction of air flow.

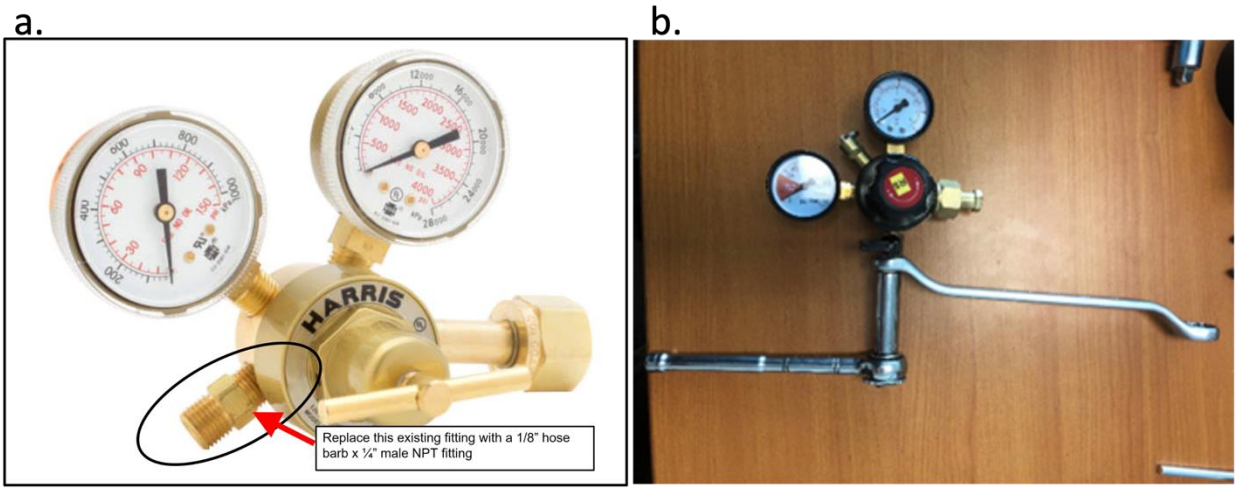


Figure 29. Harris 301-100-320 CO2 regulator showing the outlet fitting that should be replaced with a 1/8" hose barb x 1/4" male NPT fitting (a). Placement of wrench and socket to facilitate removal of barb (b).

3. Connect the 2nd length of tubing to the other end of the orifice restrictor. It may be necessary to super glue or tape the connectors if connections are loose or leaks are found during testing. Small zip ties may help secure these better under field conditions. Please note that these orifice restrictors can become clogged and lower the flow so they should be checked/replaced if the flow is reduced.

4. Attach a CO_2washer to the regulator using a twist tie or loose-leaf ring. This will ensure a washer is with the regulator and readily available at deployment. Be sure to also pack spare washers in case the one attached gets lost.

5. Wrap up tubing and secure in place with a Velcro-type strap attached to the regulator.
6. Number or name the regulator so that leaks discovered in the field are easily documented and communicated.

C.3 Filling the 5 lb CO_2 field canisters at the domain support facility:

Important safety warning – Canisters with liquids should not be filled above 80% full to allow for expansion.

Important Note – 5 lb CO_2 field canisters are filled with CO_2 from a 50 lb siphon canister. This process should be completed a minimum of 1-2 days before deployment. This will ensure enough time to fill all canisters, which may require multiple cycles of freeze-fill before they reach capacity. Note that the cheaper 'industrial grade' CO_2 is sufficient for the purposes of this protocol.

Required PPE – Note that the tanks in the -20 freezer will be extremely cold. Gloves should be worn to protect hands from the cold during transport. Eye and ear protection should also be used since the release of the gas when opening the bleed valve can be very forceful and loud.

1. If you have multiple siphon canisters, label each for ease of tracking the amount of CO_2 withdrawn from each canister.
2. Establish a target maximum weight for each 5 lb canister and add this as a label for future reference. Do this before connecting the transfer unit to the 5 lb field canister. The target fill weight is the empty canister tare weight (from stamp on canister) + 4 lb (1.8 kg) + weight of carrying handle if applicable. This step is only needed once in the lifetime of a 5 lb field canister.
3. Prior to filling the 5 lb field canisters, they should be stored in a -20C freezer for a minimum of 45 minutes before attempting to fill them. This process will allow the canister to be filled more effectively.
4. Connect the CO_2transfer unit to the siphon canister (**Figure 30**).
    a. Remove the safety cap from the 50 lb siphon canister. Note the hose end will attach to the field canister.
    b. Ensure CO_2 washers are placed between the canister valves and the transfer unit fittings before tightening in place with a 10" adjustable wrench.
    c. **After ensuring the transfer and bleed valves are closed**, open the siphon canister to the full open position.

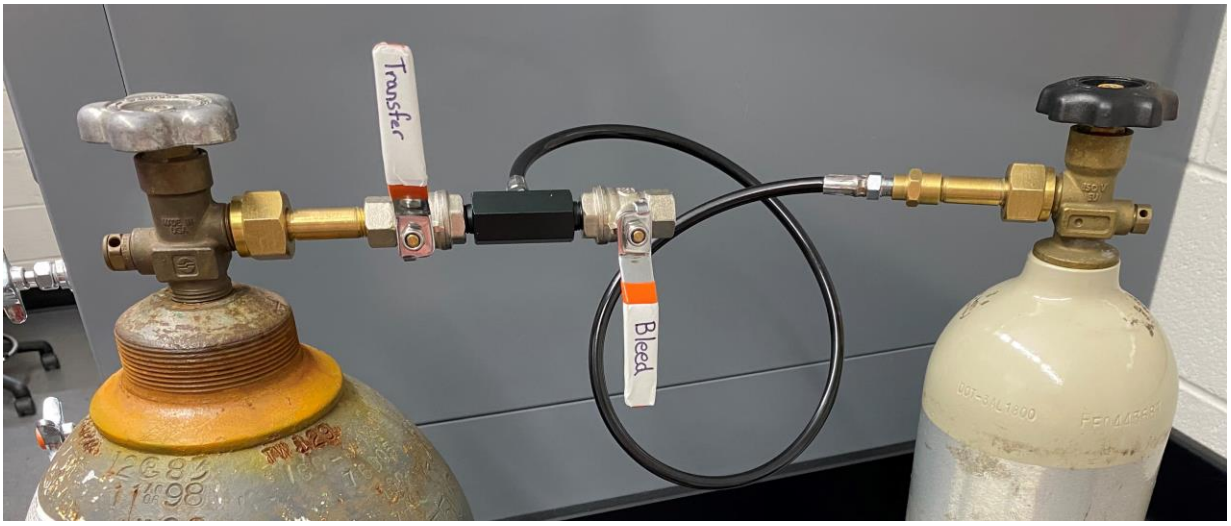


Figure 30. Transfer unit between a supply 50 lb CO_2 siphon canister (left) and a 5 lb CO_2 canister (right) used for field sampling.

5. Fill 5 lb field canister to target maximum weight without going over and check progress with a 0-25 lb scale or similar.
    a. With a CO_2 washer in place, connect the other end of the transfer unit hose to the 5 lb field canister, and place the field canister onto a zeroed scale.
    b. Open 5 lb field canister valve (counterclockwise) to full open position and back off (clockwise) ¼ turn.
    c. Open transfer valve to full open position to fill the 5 lb field canister.
    d. Close transfer valve and field canister valve **before** exceeding the target weight of CO_2. **Do not overfill canisters!**
        i. Siphon canister may be getting low if you cannot meet the fill requirement. Note that one siphon canister can typically fill 7-8 field canisters. You need to have at least one extra siphon canister than you have field sites since it will take more than one siphon canister for a single bout.
        ii. Place the field canister back into the freezer and attempt to fill again after field canister is chilled to -15C +/- 5C.
    e. To disconnect the transfer hose from the field canister, first open the bleed valve to relieve pressure in the transfer unit. Be sure to close the bleed valve afterwards. (WARNING: relieving the pressure will be loud and forceful. Be mindful of your surroundings and the direction of the valve opening).

f. It may be useful to internally record the final weight of the field canister to ensure that the proper amount of CO_2 was used during the bout and help with tracking CO_2 use from the siphon canister.

6. Place field canister into transport/storage rack. Repeat Steps 4-5 for all field canisters.
7. After the final field canister has been disconnected, close the siphon canister valve and open the bleed valve and transfer valve to release pressure in the transfer unit.
8. Disconnect the transfer unit and replace the canister cap.
    a. Open 5 lb field canister valve (counterclockwise) to full open position and back off (clockwise) ¼ turn.

C.4 Instructions for canister transport to the field for D.O.T. compliance:

Because compressed gases are considered hazardous materials, the Department of Transportation (D.O.T.) in the United States, regulates their transportation. Transportation of compressed gases triggers many regulatory requirements, some of which are covered here:

- Compressed gas canisters must be properly labeled with a UN 1013 label before they can be transported. The labels should never be removed or defaced.
- All canisters, after being loaded for transit, when in transit, and when waiting for unloading after transit should be secured by adequate means to protect the canisters. The means of securing should be sufficient to hold the canisters in place, yet not cause damage to the canisters. A safety-approved rack system has been developed for this purpose (**Figure 20**).
- Canisters must meet D.O.T.'s requirements for labeling, marking, and placarding, including the use of a UN 1013 label.
- The valve cap must be on the canister to protect the valve stem.
- The canister must be secured in an upright position in the back of the truck to prevent canister damage, especially the valve stem, during transport. Inspect the canister for existing damage prior to attempting transport.
- The canister must be located in the back of the truck to provide adequate ventilation in the event of a leak. Direct sunlight or excessive temperatures can result in a release of the canister contents.

C.5 Instructions for field deployment:

1. Load the supplies into the truck using the transportation rack (**Figure 20**), and drive/walk to the plot. Note that a bag may be helpful for carrying all the separate pieces of tubing and regulator.

Figure 31. Example regulator with parts labeled.

2. At the plot, place the field canister on at the base of Shepherd's hook or T post in the field and secure with Velcro/bungees. If you are on a slope place the field canister on uphill side.
3. Attach the regulator to the field canister (**Note**: Chemical Hygiene and Safety Plan prohibits carrying/transporting gas canisters with a regulator attached).
    a. Insert regulator washer, attach to canister outlet, and tighten attachment nut. Note this only needs to be snugged down, do not over tighten.
    b. Ensure delivery pressure control knob is closed - turn knob counterclockwise until there is no resistance (**Figure 31**).
    c. Open 5lb field canister valve by screwing counterclockwise until completely open, then back off ¼ turn to ensure connections are not leaking.
4. Set delivery pressure gauge to 15-20 PSI using delivery pressure control knob. The PSI setting could vary by site and date and can be adjusted after checking the flow rate in the next step.
    a. Listen carefully for a leak where the regulator is connected to the tank. You may also bring soapy water to spray on the connector to test for suspected leaks. If bubbles form at the site of the soapy water applications that indicates a leak. If the flow rate seems low for a given regulator setting it may also be indicative of a leak.
5. Check flow rate with flow meter and adjust as needed.
    a. The reading should be 0.5 – 0.7 Lpm or equivalent.
    b. The flow rate should be checked at canister set up as well as when traps are re-set.
    c. Checking flow rates ensures that technicians are aware of any problems with the setup such as a plugged orifice restrictor or a problem with CO_2 delivery.

6. Hang trap from Shepherd's hook or T-post and attach CO_2 supply tube so that the end rests on top of the fan hat using either a binder clip on the cord from which the trap hangs, or snug re-usable zip ties on the Shepherd's hook. Take care not to exceed a 75 degree bend in the canister supply hose to allow for the free flow of gas. You may also want to attach the supply tube to the shepherds hook with Velcro strips.
7. In the MOS: Trap Setting and Collection app be sure to change the trapType to **CO_2 canister**. This is very important for documentation.
8. Morning Collection:
    a. Perform mosquito sample collection per protocol.
    b. Check flow rate.
        i. If flow rate is above or below desired output (0.5-0.7 Lpm), adjust the flow with delivery pressure control knob until desired rate is achieved.
        ii. Re-hang supply hose.
    c. If the CO_2 flow rate is near zero mark the CO2Status field as "Absent".
9. Afternoon Collection:
    a. Perform mosquito sample collection per protocol.
    b. Check flow rate.
    c. If the CO_2 flow rate is near zero mark the CO2Status field as "Absent".
10. Close canister, bleed CO_2 off until pressure gauge indicates 0 PSI, disconnect from regulator, and remove all equipment from field. Handle regulators with care and coil hoses to prevent snagging on vegetation.
11. When returning from the field, record final tank weights on data sheet, store empty tanks in the freezer, and complete mosquito sampling protocol as usual.
    a. If ultra-low freezer packs or aquarium rocks were used at sample collection be sure to place all catch cups with samples into an ultra-low freezer to kill the mosquitoes prior to processing.

APPENDIX E ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

The dates in the table below are estimated from the 5-day running average of observed daily maximum temperatures (TMAX) from NOAA NCDC between 2006-2015 (Menne *et al.* 2012). Dates presented here are only a guide, and are derived according to the logic presented in Section 4.2. Because individual years may vary widely from the average dates provided below, 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.

Mosquitoes are typically active after mean temperatures rise above 10 degrees Centigrade, so historical averages of the start & end of the season are provided. Mosquitoes in Alaska are cold-adapted and a 10 degree Centigrade threshold has proven to be too warm and thus a date range associated with 4 degree Centigrade is provided.

Domain staff should schedule field season sampling to occur between the estimated field season start and end dates for each site as indicated in the table. Limited off-season sampling bouts at **core sites** should be scheduled ahead of the field season (prior to the estimated field season start date) and following the field season (after the estimated field season end date).

However, it is essential that domain staff monitor real-time conditions to determine when to transition between off-season and field season sampling or when to discontinue scheduled bouts, as described in Section 4 of this protocol.

Table 8. Estimated dates of sampling season based on historical temperature thresholds (data from NOAA NCDC 2006-2015). Actual initiation of field season sampling will be based on the presence of mosquitoes discovered during off-season sampling at the core site.

Domain Number	Site ID	Average 5-day temp above 10°C	
		Approx. Start Date (Field season)	Approx. End Date (Field season)
1	HARV	16-Mar	29-Nov
2	SCBI	8-Jan	24-Dec
3	OSBS	1-Jan	30-Dec
4	GUAN	6-Jan	26-Dec
5	UNDE	6-Apr	17-Oct
6	KONZ	20-Jan	18-Dec
7	ORNL	7-Jan	26-Dec
8	TALL	3-Jan	29-Dec
9	WOOD	3-Apr	13-Nov
10	CPER	26-Jan	12-Dec
11	CLBJ	3-Jan	28-Dec
12	YELL	1-Apr	6-Nov
	Site ID	Average 5-day temp above 10°C	

Domain Number		Approx. Start Date (Field season)	Approx. End Date (Field season)
13	NIWO	15-May	19-Oct
14	SRER	1-Jan	29-Dec
15	ONAQ	19-Mar	26-Nov
16	WREF	18-Mar	14-Nov
17	SJER	1-Jan	29-Dec
20	PUUM	14-Mar	3-Oct

Table 9. Estimated dates of sampling season based on historical temperature thresholds in Alaska (data from NOAA NCDC 2006-2015). Actual initiation of field season sampling will be based on the presence of mosquitoes discovered during off-season sampling.

Domain Number	Site ID	Average 5-day temp above 4 °C	
		Approx. Start Date (Field season)	Approx. End Date (Field season)
18	TOOL	1-Jun	17-Sep
19	BONA	13-Mar	5-Oct

Table 10. Number of expected bouts at core and gradient sites for each domain. Mosquito sampling is intended to capture the full range of mosquito activity at each site; the expected number of bouts presented here is not a minimum or maximum on the number of bouts to be performed. It is only to be used for evaluating whether a particular plot is compromised by providing less than the expected number of sampling events.

Domain Number	Core site	Gradient site
1	17	8
2	26	12
3	26	12
4	26	12
5	13	6
6	24	12
7	26	12
8	26	12
9	14	7
10	24	11
11	26	12
12	14	NA
13	10	MOAB: 8
14	26	12

Domain Number	Core site	Gradient site
15	16	NA
16	16	8
17	26	12
18	7	3
19	13	6
20	12	NA

APPENDIX F SITE-SPECIFIC INFORMATION

F.1 D04 – LAJA – Lajas Experimental Station

At Lajas Experimental Station (gradient site) a modification will be implemented due to difficult soil substrates. However, unless detailed in this paragraph, sampling will occur at Lajas in a way that is identical to sampling conducted at any other site. At each deployment location, the Shepherd's hook is attached to a steel U post with anchor plate. The tightly welded plate also helps to minimize sideways or rotational movement of the post preventing the Shepherd's hook from falling over the compacted soil (soil that otherwise prevents insertion of the Shepherd's hook alone).

A.1 D13 – MOAB – Moab, UT

The Moab (gradient site) is officially affiliated with the Niwot Ridge Mountain Research Station (core site) within D13, but the staff who conduct sampling at Moab are based at the D15 support facility in Utah. In the absence of this site-specific modification, monthly sampling at Moab would not be initiated until mosquitoes were detected at Niwot, which is problematic for both logistical and scientific reasons. From a biological perspective, Moab is closer in elevation (at 1767 m) to the D15 Onaqui-Ault core site (1685 m) than it is to the D13 Niwot core site (3513 m) and mosquitoes are present much earlier in the year at Moab than its D13 core site. Delaying sampling at Moab until the detection of mosquitoes at Niwot would result in missing phenological data at the Moab site. Thus, monthly sampling at Moab will be based on the initiation of field season sampling of mosquitoes by the ONAQ site location rather than the Niwot site. Apart from this change, sampling will occur at Moab in a way that is identical to sampling conducted at any other gradient site.

F.2 D14 – SRER/JORN – Santa Rita Experimental Range & Jornada LTER

At Santa Rita Experimental Range (core site) and Jornada LTER (gradient site), a modification will be implemented due to sandy/unstable soils at the site. However, unless detailed in this paragraph, sampling will occur in Domain 14 in a way that is identical to sampling conducted at any other site. At these sites, a T-post support is driven into the ground at the trap deployment location. The Shepherd's hook is secured to the T-post to prevent the trap from falling over.

F.3 D17 – TEAK – Lower Teakettle

For D17, the core site is at a significantly lower elevation than its associated gradient sites. As such, the switch from off-season sampling to field season sampling at SJER will not automatically trigger field season sampling at the TEAK gradient sites until later in the season (when the landscape is snow-free). Instead, TEAK will initiate monthly sampling based on the scheduled dates for NIWO (on the basis of similar high elevation). Heavy snow makes year-round phenology sampling at TEAK impractical, with multiple plots inaccessible from November to April. This site-specific adjustment does not apply to SOAP, which will follow the standard gradient collection schedule (e.g., monthly sampling after mosquitoes are detected at the core site), with the expectation that 3-4 winter bouts may be cancelled due to low temperatures or snow cover.

F.4 D20 – PUUM – Pu'u Maka'ala Natural Area Reserve

At Pu'u Maka'ala Natural Area Reserve (core site), a modification will be implemented due to permitting restrictions at the site. However, unless detailed in this paragraph, sampling will occur in Domain 20 in a way that is identical to sampling conducted at any other site. At this site, sampling occurs monthly instead of biweekly.

APPENDIX G TROUBLE-SHOOTING TIPS FOR MOSQUITO TRAP FANS:

G.1 Fan Troubleshooting:

1. Plug the fan into more than 1 battery to confirm the fan is the issue and not just the battery.
2. Check the battery connection terminals for proper connection and confirm there is no damage. If a terminal is missing or damaged proceed to “Replacing Battery Connection Terminals”.
3. Using an ohm meter, check the connection between the battery connection terminals and where the wires connect to the motor (**Figure 32**). Do this for both the positive and negative wires. **Note:** It is difficult to know which is positive/negative on the wires to the motor, so be sure to check both connection points if your first attempt reads zero.
4. If the ohm meter reads zero proceed to “Shortages”.
5. If ohm meter reads a number more than zero on both wires, then do a finger test and spin the fan to confirm it’s not hitting the walls of the assembly.
6. If the fan is hitting the walls, carefully press on the metal mesh to bend the whole motor and fan mount back into place.
7. If the fan spins freely and is just not getting power, then the motor is most likely the issue, proceed to “Replacing Motor”.

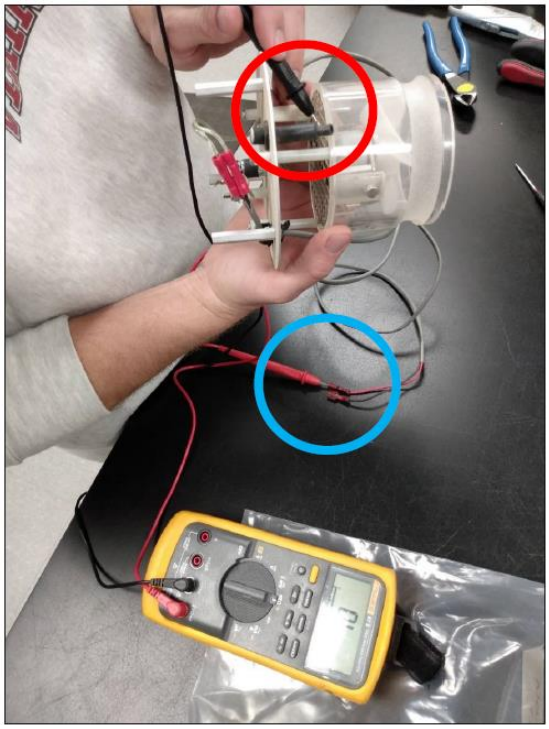


Figure 32. Blue circle: one end of the ohm meter plugs into the battery terminal. Red circle: other end of the ohm meter hits the wire where it connects to the motor.

G.2 Replacing battery connection terminals:

1. If the wire ends are bent or broken, use a wire cutter to cut the wire and strip the plastic to expose new straight wire.
2. Place the fresh end into a battery terminal size 22-18 and crimp the end where the wires went in using needle nose pliers.
3. Tug the connection to make sure the crimping was adequate.
4. Use a lighter or heat gun to shrink the connection (**Figure 33**).
5. Confirm a good connection by plugging the battery terminals into a battery.

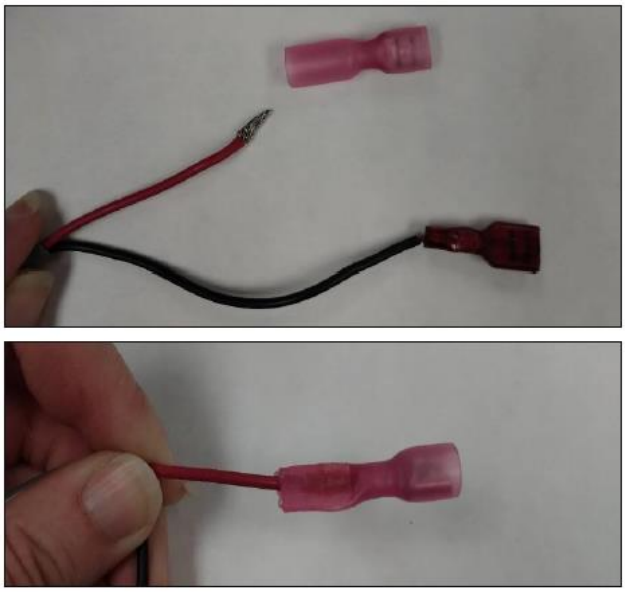


Figure 33. Battery terminal that needs a replacement (top). Completed heat shrunk battery terminal (bottom).

G.3 Shortages:

1. Check for a short between the circuit board and the battery connection terminal.
    a. Place one end of the ohm meter on the battery connection terminal and one where the wire connects to the circuit board (**Figure 34a**). Do this for both the black and red wire.
    b. If ohm meter reads “zero” you found the short. Usually happens at the 90° grommet, where the wire goes through the metal plate (**Figure 34b**). If the ohm meter reads > 0, proceed to step 2.
    c. Push the black grommet through the metal plate and remove wire from grommet.
    d. Cut wire below where the bend from the grommet is.
    e. Strip the wires and re-test with the ohm meter to ensure the short has been removed.

f. Replace the grommet with the wire inside, but this time put the gromet in the opposite direction (push grommet in from top side of the fan). The original orientation of the grommet causes the wire to bend at 90°, twice. Flipping it allows for only one 90° bend.

2. Using the ohm meter, test the circuit board for a short by placing one end of the meter where the red/black wire connects to the circuit board (**Figure 34**) and the other where the wire connects to the motor. If a short exists here, it is likely in the circuit board.

    a. Proceed to "Circuit Board Removal" section. **Note:** Even if the short does not occur here, it is a good practice to remove the circuit board anyways to eliminate a future potential source of failure. It is not needed for our uses.

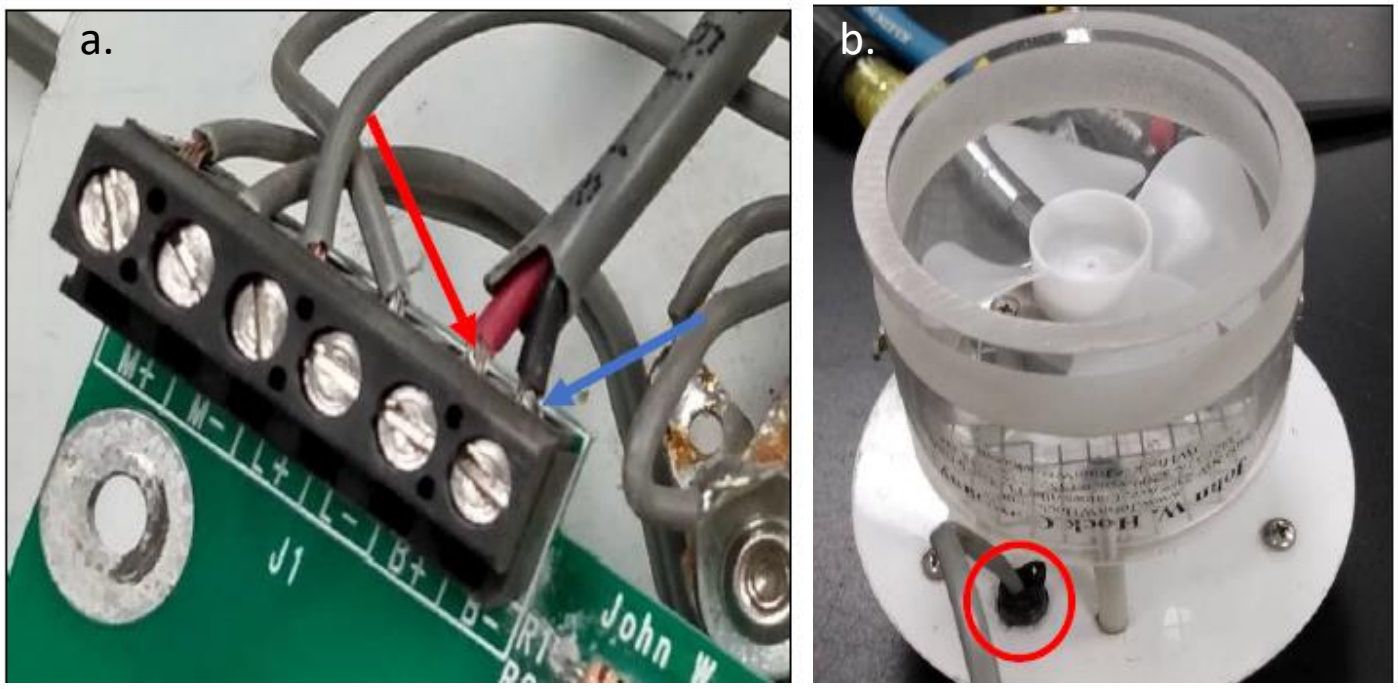


Figure 34. (a) Red arrow indicates where to place one end of the ohm meter when testing the positive wire. Blue arrow indicates where to place one end of the ohm meter when testing the negative wire; (b) the other end of the ohm meter is placed in the battery connection terminal. Red circle shows the 90-degree grommet, another common place for shortages.

G.4 Circuit Board Removal

1. Using a small flat head screwdriver loosen the screws holding the red and black wire connectors and the 2 opposite end wire connectors (**Figure 35a**).
2. Cut the wires connected to the light housing, using wire cutters, to remove the circuit board completely (**Figure 35b**).
3. Discard circuit board. The circuit board is not necessary for our uses and is a potential source of failure in the future.

4. Using butt splices, connect the red wire to the copper wire and the black wire to the other wire. **HINT:** Test the connection by plugging in the battery terminals into a battery before shrinking or crimping the wires to make sure the wires aren't crossed (positive to negative). You should feel air being blown out the bottom of the fan. If you don't the fan is likely spinning in reverse.
5. If using heat shrink butt splices, use a lighter or heat gun to melt the plastic and form a seal on all connections (**Figure 35c**). If using crimp butt splices, use needle nose pliers to crimp the connections.
6. Test the connection by plugging in the battery terminals into a battery.
7. Reassemble the hat and fan components.

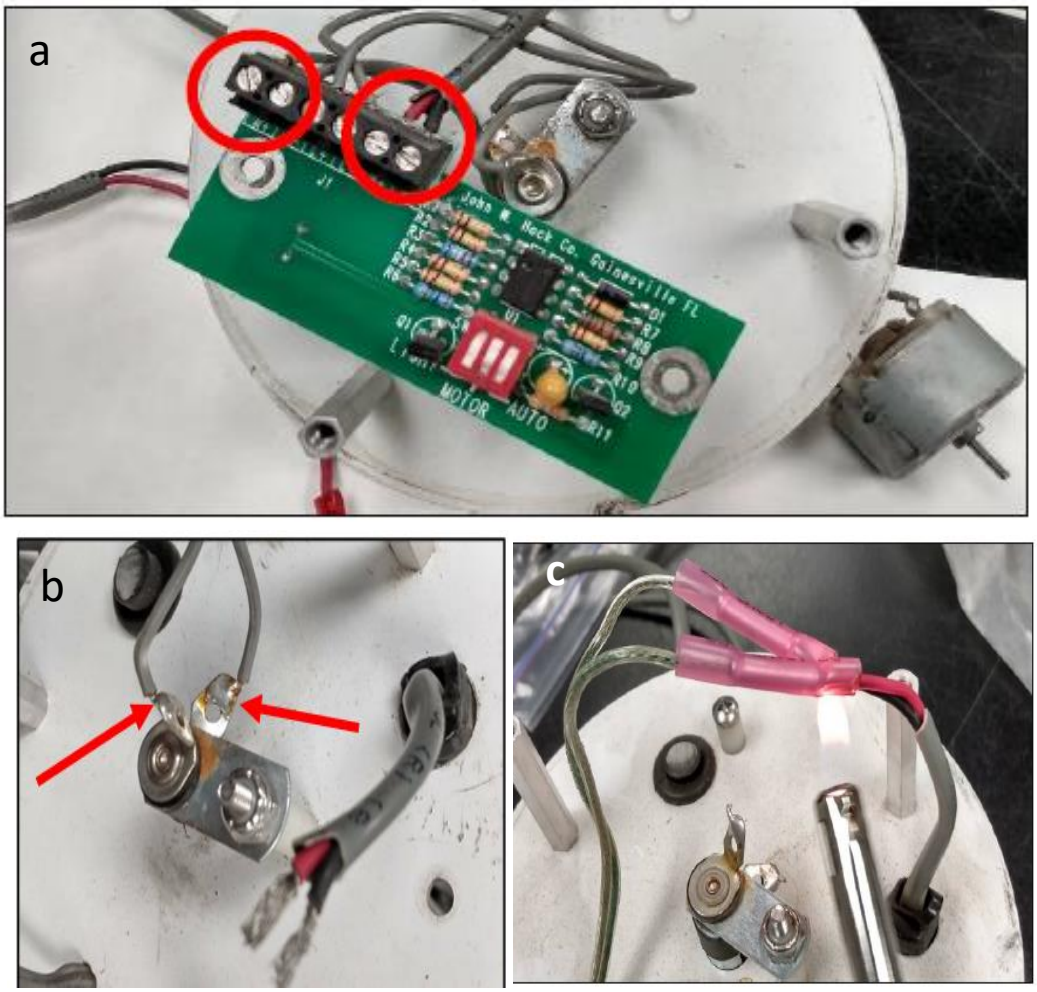


Figure 35. (a) Screws to loosen to access wire ends; (b) arrows showing wires to cut connected to light housing; (c) using lighter to heat shrink butt splices.

G.5 Replacing Motor (Figure 36):

1. Remove rain cover by unscrewing the 3 black screws.
2. Using the box cutter carefully cut the white tape that holds the wire to the support posts.
3. Remove the fan blades with pliers by pulling straight out.
4. Using a small screwdriver, unscrew the 2 screws holding the motor in place.
5. Using a screwdriver, remove the other 2 screws holding the face plate.
6. Cut off the wires from the existing motor, as close as possible to the terminals.
7. Strip the wires about half a centimeter using a wire stripper.
8. Grab a new motor and thread the wires through the small hole in the terminal (**Figure 36**). **HINT:** The positive terminal has a red dot next to it on the new motor and the positive wire on the fan is copper, it should appear red-ish in color. It's helpful to confirm the red wire by tracing it from the top of the fan to the terminal connection.
9. Solder the wires to the terminal using a soldering gun.
10. Check the motor is working by plugging the battery terminals into a battery.
11. Begin to assemble the motor back together, replacing the face plate, and screwing the motor back into the mount.
12. Firmly press the fan back into place (Reference Photo 4 in Photo Library shows the proper orientation for the fan).
13. Test the assembly by plugging in the battery terminals into a battery.
14. If the fan doesn't spin, confirm its not hitting the walls or unplugged incorrectly to the battery terminals.
15. If the fan is hitting the walls, carefully press on the metal mesh to bend the whole motor and fan mount back into place.
16. Test fan again to confirm it's working.
17. Reassemble the rain cover and fan components.
18. Tape the wire back to the support post using electrical tape.

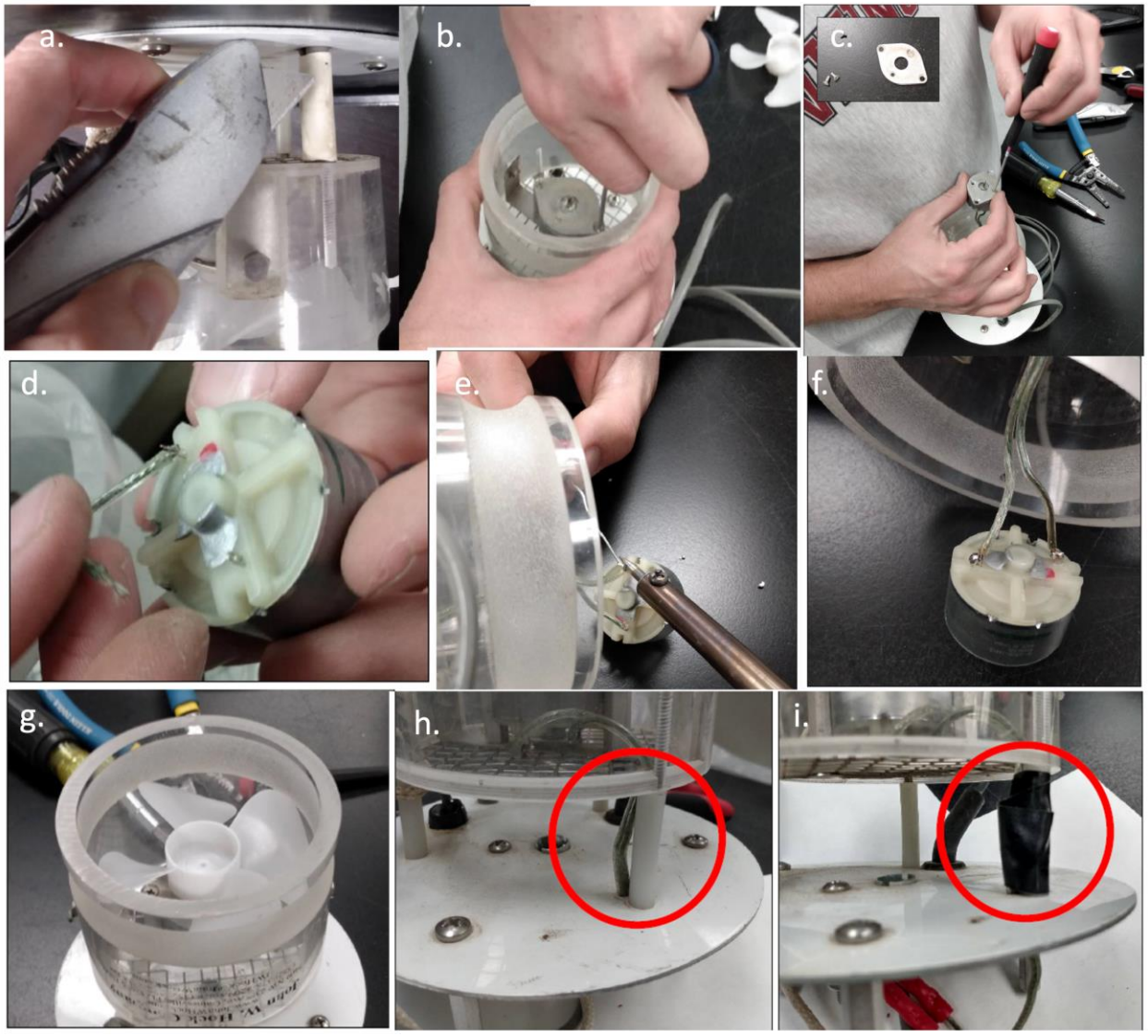


Figure 36. (a) Cut tape holding wire to support post; (b) remove motor from mount; (c) remove screws holding plate to the motor; (d) thread wire through hole in new motor terminal; (e) solder the motor terminals – positive copper wire gets soldered to the terminal with the red dot; (f) completed soldering work; (g) proper orientation of fan in the motor; (h) wire and support mount with tape removed; (i) and with electrical tape added.

APPENDIX H EQUIPMENT

The following equipment 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.

Table 11. Equipment list – Preparation for field sampling at one site.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
	N	Battery charger, 6V	Charge 6V batteries	10
	N	Carabineer	Ease trap retrieval and deployment in field	10
	N	Key ring	Ease trap retrieval and deployment in field	10
	N	Secondary containment bin	Battery containment while charging	Variable
	N	All weather copy paper	Print datasheets and sampleID labels	5
	N	Masking tape	Cover vent holes in trap coolers	1 roll
	N	50 lb CO_2 **siphon** canister (industrial grade)	Filling 5lb canisters	2-3
	N	Teflon tape	Place over the threads of the brass regulator fitting	1 roll
	N	0-30 PSI gauge replacement	Optional – replace 0-100 PSI gauge with a 0-30 PSI gauge to make regulator adjustments easier	11
	N	10” adjustable wrench	Attaching/removing regulator	1
	N	Liquid CO_2 transfer unit, CG320-CGA320 connection fittings	Transfer CO_2 from 50lb siphon canister to 5lb canisters	1
	N	Scale (0-25 lb)	Used when transferring CO2 from the 50 lb canisters to the 5 lb canisters	1
	N	Ratchet strap	Secure canisters upright in truck, during deployment, and in domain facility	4

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
Amazon #B0821RWTZC	N	Carry Handle for Luxfer style tanks	Safety handle to carry field tanks during deployment and reduce hazards if tank is dropped	10
	N	5/16" hex key/allen wrench	Attach safety handle to tank	1
Xpress Tags #LB-1960	N	Carbon Dioxide - Caution High Pressure Liquid And Gas Can Cause Rapid Suffocation Label - UN 1013	DOT compliance	1/pack of 25
	N	Heavy duty stainless steel key ring	Drop cable support loop	10
	N	Electrical tape	Drop cable support loop and fuse	1
	N	UV resistant cable ties	Drop cable support loop	30-50
	N	Heat shrink	Fuse	20-30 inches
	N	Fuse	Fuse	10
	N	Fuse holder	Fuse	10
	N	Spade connectors	Fuse	20
	N	Steel spring snap carabiner (60mm)	Trap leash	10
	N	Crimp sleeves (1/8”)	Trap leash	20
	N	Vinyl coated steel cable (3/32”)	Trap leash	varies
	N	Tape measure	Measure trap leash	1
	N	Wire cutters	Cut cable for trap leash	1

Table 12. Equipment list – Trap deployment and sample retrieval during field season sampling for one bout at one site.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
	N	Battery, 6V	Deploy traps, replace batteries during specimen collection	20
John W. Hock 1012.NEON	Y	CO_2 light trap fan assembly	Deploy traps, spare fan assemblies	20
John W. Hock 1012.NEON	Y	CO_2 light trap rain cover	Deploy traps, protect trap from rain	20
John W. Hock 1.44	Y	Collection cup with mesh sleeve (camo print)	Deploy traps, replace collection cups during specimen collection	20
	N	Cooler	Chill perishable samples in field	1
	N	Cryogenic gloves	Protect hands while handling dry ice	1 pair
	N	Cylindrical insulated cooler	Deploy traps using dry ice, sublimate dry ice bait, spare coolers	20
	N	Dry ice cooler	Store/transport dry ice	1
	N	Container (32x20x17 Sterilite with wheels)	Container to transport fans into the field	1
	N	Container (Sterilite 30 ½ x 20 x 156; 27 gal capacity)	Container to transport coolers into the field	1
	N	GPS receiver, recreational accuracy	Navigate to sampling location	1 per team
	N	Ice pack, -20°C	Chill perishable samples in field if dry ice is unavailable	Variable
	N	Rain cover screws	Spare screws for mosquito trap	30
	N	Large key ring	For more easily connecting mosquito trap insulated cooler to rain cover	10
	N	Ice scoop	For transferring dry ice into mosquito trap insulated coolers	1
	N	Scissors	Separate sampleID labels	1

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
Minuteman International / ACHLA Designs TSW27	Y	Shepherd's hook	Hang traps; alternative to natural structures	10
	N	Small bottle brush	Remove debris from fan assemblies	1
	N	AA battery	Spare battery for GPS receiver	1 set
	N	Aluminum foil	Deter cattle or other wildlife	As needed
	N	Cardboard or cloth	Protect catch cup and samples during transport	Variable
	N	5 lb compressed CO_2 canister with CGA 320 valve. Luxfer style preferred to fit carry handle	Store industrial grade CO_2 gas in the field	10
	N	Saint Gobain ACF00007 S3 E-3603 Tygon Non-DEHP Tubing, 1/8" Inner Diameter x ¼" Outer Diameter x 50'.	Output CO_2 in field	10ft/ canister
	N	CO_2 canister washers (nylon or fiber or material approved for CO_2 gas regulators)	Create tight seals between canister valves and regulators	20
	N	1" binder/book ring	Store / secure washers to regulator	1/pack of 25
	N	Hook and loop cable tie	Secure supply tube and power cable to Shepherd's hook post	20-25
Air-Logic or Universal Power Conversion #F2815071B85	Y	0.007" inline orifice restrictor with 1/8" hose barbs	Keep flow at $0.54 < x < 0.75$ l/min at delivery pressures 15 – 25 psi	20
	N	250 mL spray bottle	Check for leaks by spraying soap and water mixture	
	N	Flow meter 0.1-1.5 LPM	Measure flow in the field	1
Harris #301-100-320	Y	Single-stage regulator with low pressure delivery	Set pressure for CO_2 output	11

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
	N	1/8" hose barb x ¼" Male NPT adaptor	replace existing outlet fitting on regulator	11
	N	Velcro Cable Ties or zip ties	Secure 1/8" tubing to shepherds hook. 2 per delivery system	20-40
	N	¾" x 25' Velcro strap	Secure CO_2 Tank to Shepherds Hook	1
	N	Small parts organizer box	Store spare parts (orifice restrictors, washers etc.)	1
	N	Cable tie gun	Installing ties on supply tube fittings	1
	N	4" multi-purpose UV resistant cable tie	Secure supply tubing to barb fittings	1
	N	Loctite Extreme glue, 0.7 oz, No drip gel	Secure supply tubing to barb fittings	1
Creative Fabrications (place order through D07 manager Bill Martin)	Y	CO_2 tank storage/transport rack made of 3/16" aluminum. 5-canister capacity with carry handle	Store and transport 5lb CO2 field canisters according to DOT and Compressed Gas Safety	2
	N	Insulated backflow winter protection pouch 30"W X 26"H (white)	For use in high temperature regions to protect canister from direct sunlight and keep canister at a low temperature	11
	N	Dry ice, pelletized	Bait traps and freeze collected samples	15 kg
	N	Liquid laundry detergent, fragrance free	Wash mesh sleeves and collection cups	1 bottle
	N	Paper towel	Absorb excess moisture	1 roll
	N	Resealable plastic bag, 1 gal	Protect trap batteries from rain/moisture	10
	N	Rope	Hang traps from natural structures	Variable
	N	Safety pin	Attach sampleID labels	20
	N	Charged & synced Mobile Data Entry Device	Enter data	1 per team of 2

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
RD[05]	Y	Field datasheet	Record data	
	N	sampleID label	Label samples	Variable
	N	5 x 4 x 21" 1 Mil Gusseted Poly Bags	Optional plastic shield to protect mesh catch cup in rain	Up to 10
	N	Rubberband	Used with optional plastic shield	Up to 10

Table 13. Equipment list – Laboratory processing of collected mosquitoes (per bout per site).

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
	N	Centrifuge tube rack, foam	Organize samples	1
	N	Centrifuge tube, 15 mL or Falcon tube, 50 mL	Contain mosquitoes	25
	N	Cryosafe freezer bags (must be rated to -80C)	Contain mosquitoes; used for large quantity mosquito captures	up to 20
	N	Cryovial freezer storage box with dividers	Organize samples (small vials)	1
	N	Plastic vial racks	Organize samples (larger vials)	1
	N	Paintbrush	Transfer mosquitoes to tubes	2
	N	Wax paper	Transfer mosquitoes to tubes	Variable
	N	Featherweight forceps	Transfer mosquitoes to tubes	3
	N	Funnel, copy paper or plastic	Transfer mosquitoes to tubes	1 sheet
	N	Dry ice	Setup chilling station	5
	N	All weather copy paper	Print additional sampleID labels	2
	N	Copy paper, white	Transfer mosquitoes to tubes	1 sheet
	N	Toilet paper	Cushion mosquitoes in sample vials, create plugs	1 roll

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
	N	Permanent marker, archival ethanol-safe	Label sample vials	1
	N	Clear packing tape	Protect labels from falling off in the -80C	1
	N	Re-sealable freezer bag, 1 pint	Organize samples	Variable
	N	Rubber band	Organize samples	Variable
	N	Adhesive barcode labels (cryo, Type II)	Labeling sample containers with barcode-readable labels	Up to 20
	N	sampleID label	Label samples	Up to 20

Table 14. Equipment list – Troubleshooting and fixing mosquito fan issues.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
	N	Ohm meter	Check fan connections	1
	N	Wire cutter / stripper	Remove plastic from wires for battery terminal replacement	1
	N	Needle nose pliers	Crimp end where wires attach to new terminal	1
	N	Waterproof battery terminals size 22-18	Replace battery terminal connector	1
	N	Waterproof butt splices size 22-18	Connect wires	1
	N	6V battery	Connect to Ohm meter	1
	N	Screw drivers (1 flat, 1 philips head)	Remove screws in fan	1
	N	Soldering gun	Solder wires to terminal	1
	N	Box cutter	remove white tape holding wire to support posts	1