TOS PROTOCOL AND PROCEDURE: 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 Locality labels attached to catch cups have been replaced by sampleID labels SOP B: Text added to recommend that traps not be hung over or near water (FOPS-1099). Locality labels attached to catch cups have been replaced by sampleID labels.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 Locality labels inserted into sample vials have been replaced by modified sample ID labels SOP E: deleted as content related to post-identification processing of mosquitoes at domain labs has been moved to the TOS Protocol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	1/29/2016	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

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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.

1.2.1 NEON Science Requirements and Data Products

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

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

1.3 Acknowledgments

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.000727	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[05]	NEON.DOC.000910	NEON Science Design for Mosquito Abundance, Diversity, and Phenology
AD[06]	NEON.DOC.000911	NEON Science Design for Vectors and Pathogens
AD[07]	NEON.DOC.004104	NEON Science Data Quality Plan

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Level 1, Level 2 and Level 3 Data Products Catalog
RD[04]	NEON.DOC.001271	AOS/TOS Protocol and Procedure: Data Management
RD[05]	NEON.DOC.001581	Datasheets for TOS Protocol and Procedure: Mosquito Sampling
RD[06]	NEON.DOC.001025	TOS Protocol and Procedure: Plot Establishment
RD[07]	Available via download of data from NEON portal	NEON Raw Data Ingest Workbook for TOS Mosquito Abundance, Diversity, and Phenology

2.3 Acronyms

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

2.4 Definitions

N/A

3 METHOD

Mosquito sampling involves preparing to sample (Standard Operating Procedures (SOP) A), collection in the field (SOP A), minor laboratory processing (SOP B), data handling (SOP C), and shipping to external facilities (SOP D). Field collection of live mosquitoes is conducted using Centers for Disease Control and Prevention (CDC) CO_2 light traps. A CO_2 light trap consists of (listed from the top to the bottom of the assembled trap): a cylindrical insulated cooler 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 CO_2 as it sublimates, and this gas attracts mosquitoes to the vicinity of the trap. The battery-powered fan sucks these mosquitoes into the mesh collection cup, where they remain alive until the trap is collected.

Sampling plots will be randomly located in each of the major vegetation types ($\geq$5% of total cover), with the number of plots per vegetation type proportional to the percent cover of that type at the site. Plots will be located within 30m of a road to facilitate expedient sampling.

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.

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.

Additional quality assurance will be performed on data collected via these procedures according to the NEON Science Data Quality Plan (AD[07]).

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 **Appendix D** 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 relocatable site**, with sampling alternating between relocatable sites, where applicable (**Figure 1**).

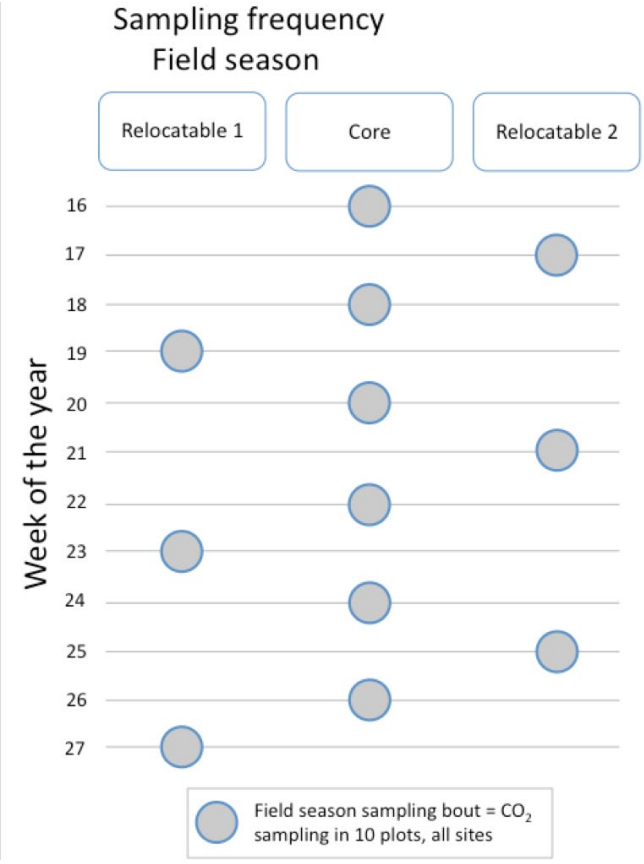


Figure 1 Frequency of sampling bouts at a domain; weekly sampling alternating between core and relocatable sites.

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 D). 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 involves only a single night of trapping.

   Note: Time windows for Alaska and sites with summer day length in excess of 17 hours will use 6:00 PM and 8:00 AM as their target time points in lieu of dusk and dawn, respectively. Traps should be deployed or checked within 2 hours of the target time (between 4:00PM – 8:00PM for the dusk time point and 6:00AM – 10:00 AM for the dawn time point).

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 2**, described below). If delays in sampling occur due to contingent events (see **Table 1**), 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.

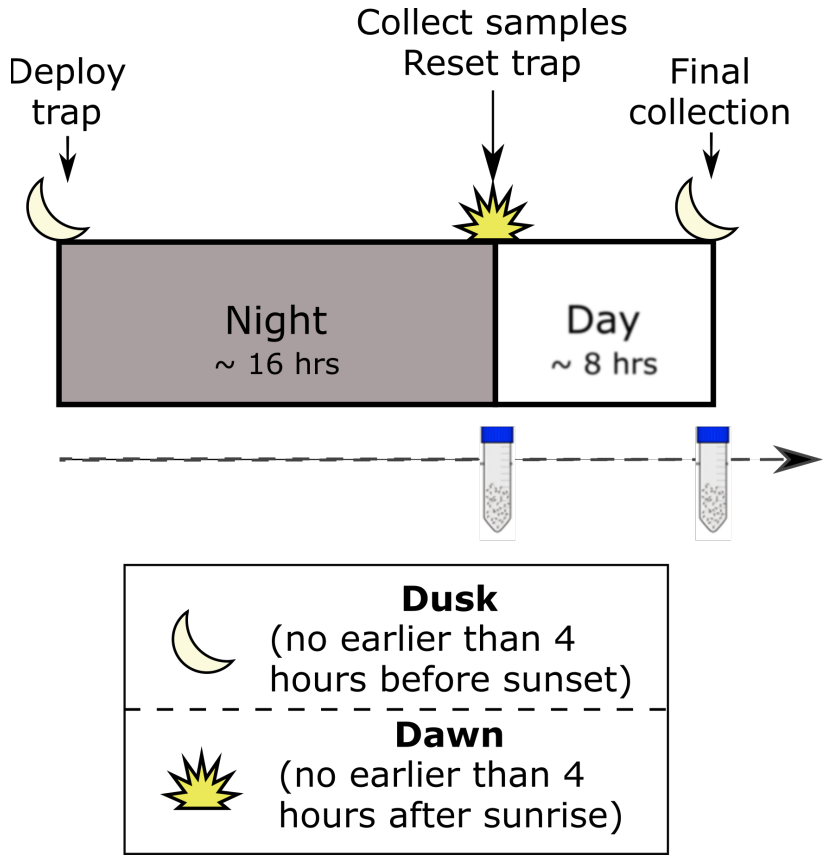


Figure 2 Timing of a mosquito sampling bout that generates two samples (designated by blue-capped vials), one night and one day of trapping. (Daytime and night-time durations shown are for illustrative purposes only.)

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 2**). Traps will be checked (full catch cups retrieved and replaced with new/empty catch cups) and reset (coolers refilled with dry ice) as close as possible to dawn (but no later than 4 hours after sunrise) on the second day of the bout. Traps will be 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.

4.2 Criteria for Determining Onset and Cessation of Sampling

Sampling will be conducted until specified collection thresholds that designate the end of field season sampling and beginning of off-season sampling are met (Figure 3). Off-season sampling initiates when 3 consecutive zero captures are observed at the core site. Until mosquitoes are confirmed as absent, field season bouts at the relocatable sites should continue as scheduled (Figure 3). Note that in some cases, the timing of sampling at a relocatable site will be based on conditions at a core site in a different domain. Please see **Appendix D** for details about these exceptions.

Off-season sampling will only occur if the mean daily high temperature at the core 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). As shown in Figure 3, off-season bouts are cancelled when low temperatures below the threshold are observed, 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 5, Figure 6).

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 3). 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 relocatable 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 relocatable 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.

Note: Although it is acceptable to cancel off-season bouts due to persistent low temperatures (previous 5 days below the domain-specific temperature threshold), it is NEVER acceptable to cancel field season bouts for this reason.

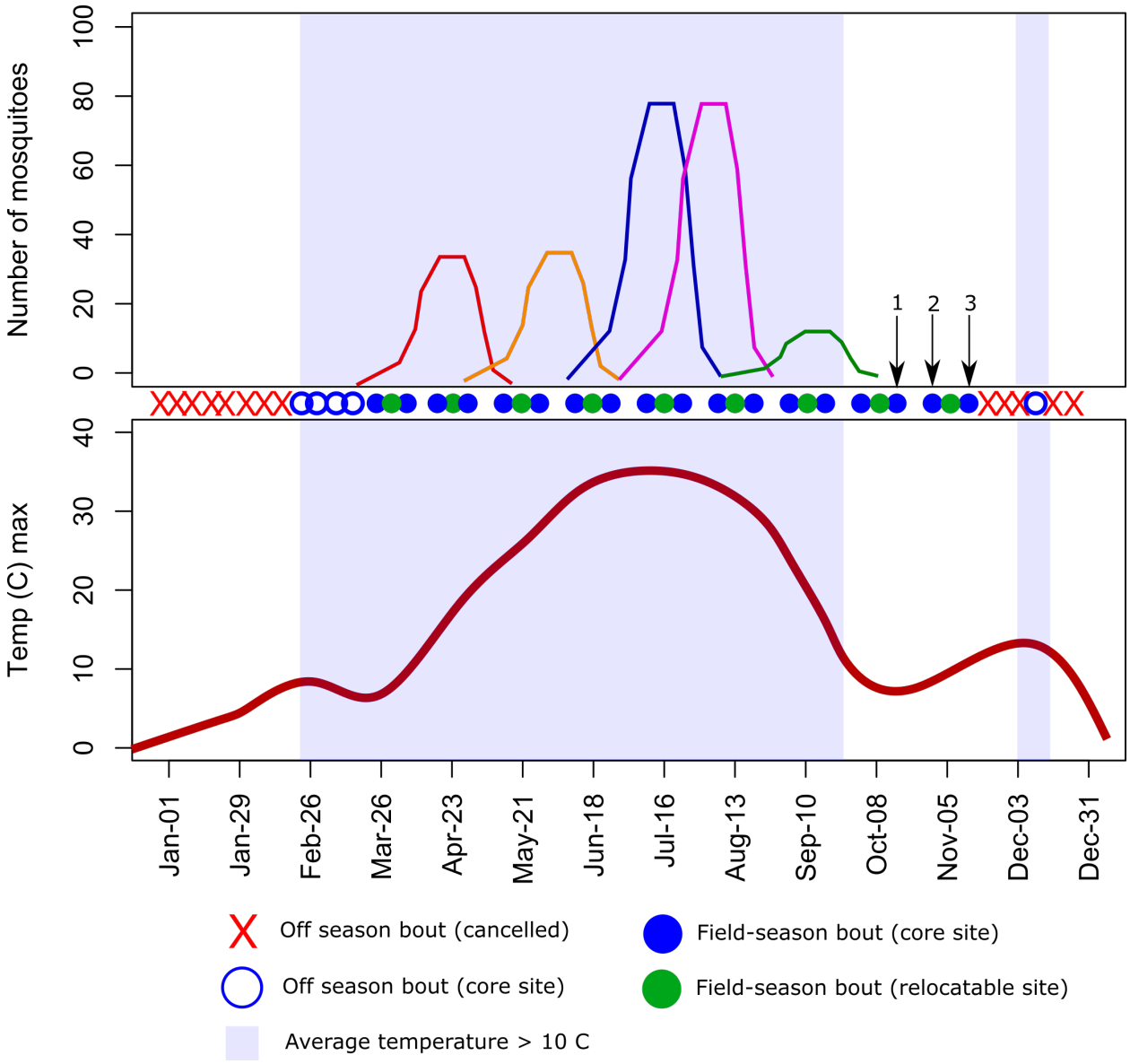


Figure 3 An example domain sampling schedule highlighting the transitions between field season and off-season mosquito sampling. At domains with three sites, this results in weekly mosquito sampling across the domain. Domains with fewer sites will have weeks where no sampling occurs; however, weeks without sampling will not count toward the three consecutive zero catches. [Note: in Alaska, the temperature threshold is 4°C due to early season activities of cold-adapted mosquitoes]

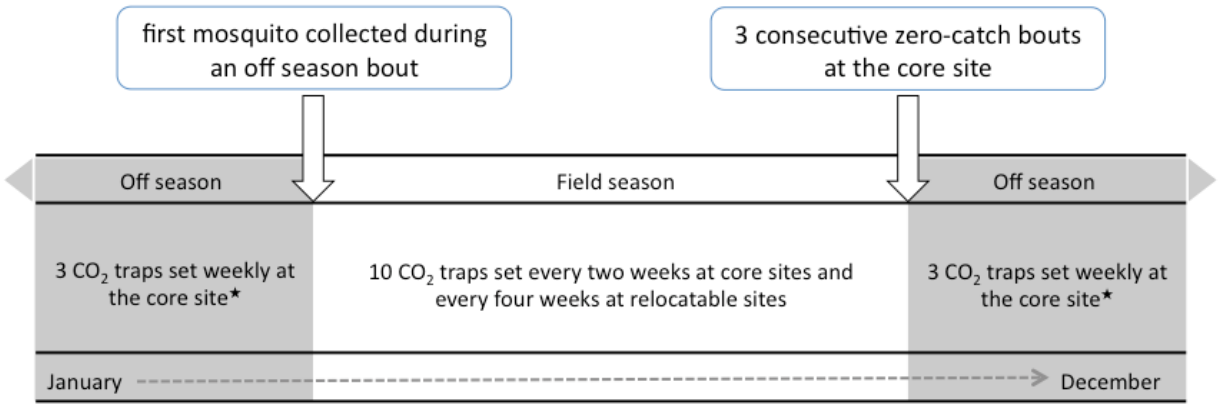


Figure 4 Annual summary of mosquito sampling intensity including transitions from off-season to field season and back to off-season sampling. *Off-season bouts occur only when the mean daily high temperature for the previous five days is above the temperature threshold (4°C in Alaska, 10°C elsewhere)

When sampling cannot be performed according to this protocol due to insufficient staffing, the domain manager will enter a problem ticket for each cancelled bout and indicate a) the bout that was missed (or the manner in which implementation of the protocol was changed) and b) that this deviation from protocol was due to lack of personnel and not an ecological or environmental issue.

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 Criteria for reallocation of sampling within a site

Mosquito sampling will occur on the schedule described above at ten plots within every site (2 collections per plot per bout; up to 20 samples generated per site per bout). Ideally, sampling will occur at those ten plots for the lifetime of the observatory (core sites) or the duration of site's affiliation with the NEON project (relocatable sites). However, circumstances may arise requiring that mosquito sampling within a site be shifted from a particular plot to another location within the site. In general, sampling at a given plot is considered to be compromised when sampling at a location becomes so limited that data quality is significantly reduced. If sampling at a given plot becomes compromised, a problem ticket should be submitted by Field Operations to Science.

There are two main pathways by which sampling can be compromised. Plots can become inappropriately suited to answer meaningful biological questions (i.e., a terrestrial sampling plot becomes permanently aquatic). Alternatively, plots may be located in areas that are logistically impossible to sample on a schedule that 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 **APPENDIX D**

for the number of expected bouts) over a two-year period. Plots that cannot be sampled on this schedule should be considered compromised.

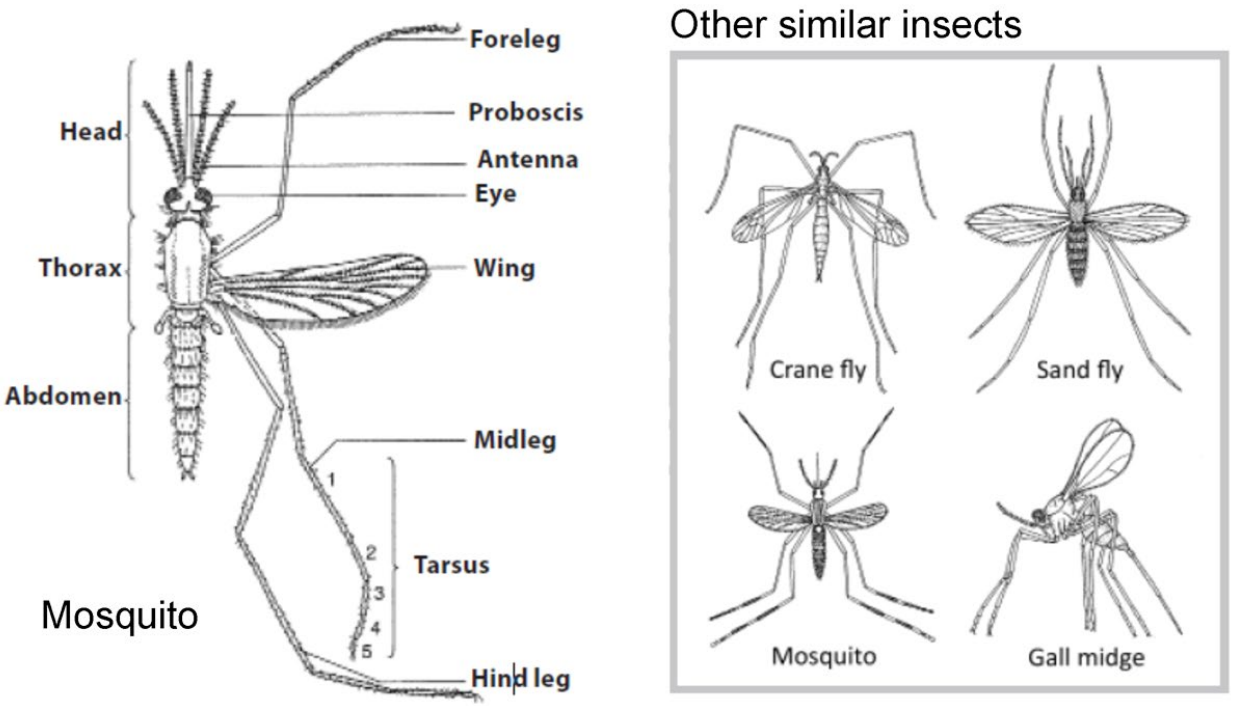


Figure 5 Basic morphology of a mosquito and comparison with other similar-looking insects

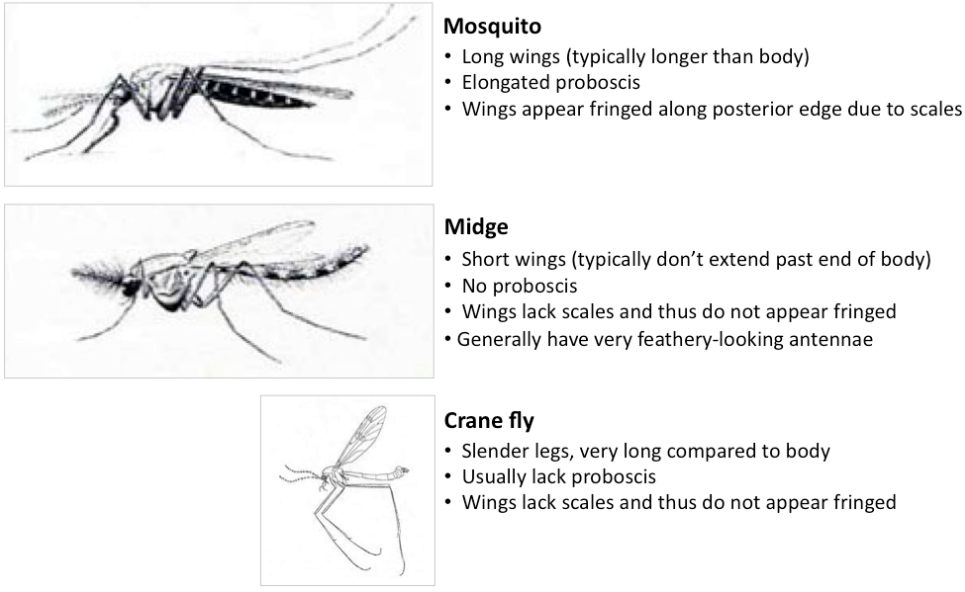


Figure 6 Morphological features that distinguish mosquitoes from midges and crane flies

4.5 Sampling Timing Contingencies

When field conditions require deviations from the protocol, follow the contingent decisions outlined in Table 1 below to maintain data quality. When indicated by Table 1, duration and cause of delays must be written in the remarks section of the data sheet and issue a problem ticket.

Table 1. Contingent decisions

Delay/Situation	Action	Outcome for Data Products
High winds >= 25mph	It is recommended that traps not be deployed if wind conditions have high potential to damage sampling equipment. This will occur when traps are being knocked around violently by the wind.	Sampling equipment could be damaged
< 3 hours	Resume/continue with normal sampling at conclusion of delay. (Note the duration and cause of the 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.
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, a) do not adjust (push back) dates for subsequent sampling bouts, and b) note the duration and cause of the delay	Brief interruption in consistent interval of time series data compromises statistical analysis of temporal trends in the data.
1-7 days (core), 1-14 days (relocatable)	**Scenario 1:** If the delay occurs prior to trap deployment and prior to the start of the sampling bout then push the start date for the bout back for the duration of the delay. **Scenario 2:** If the delay occurs after the initial deployment of traps during a bout but prior to the collection/resetting of traps, then repeat the entire sampling bout at the conclusion of the delay. In both cases, a) do not adjust (push back) dates for subsequent sampling bouts, and b) note the duration and cause of the delay	Moderate interruption in consistent interval of time series data compromises statistical analysis of temporal trends in the data.

> 7 days (core), > 14 days (relocatable)	Cancel the impacted sampling bout and stop sampling until next scheduled sampling bout. Contact associated TOS staff scientists. Note duration and cause of the delay.	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 on the second evening of the bout (result is a deviant collection over 24 hours). The following morning (originally last morning of the bout), traps should be reset to be checked in the evening in order to capture the missed day collection, then reset in the evening to capture the missed night collection. Any samples from the deviant collection can be processed as usual (i.e., not discarded), but the data product will indicate the extended duration of collection. Repeating collection to capture the correct intervals is pending staff availability.

5 SAFETY

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

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

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 field work.

Dry ice should be handled with extreme care. Refer to EHS Safety Policy and Program Manual (AD[01]), Section HM-01, Cryogenic Safety.

6 PERSONNEL AND EQUIPMENT

6.1 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 freezers, etc.

Table 2. Preparation for field sampling

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items						
Amazon	NOCO G4	R	Battery charger, 6V	Charge 6V batteries	10	N
REI	787680	S	Carabiner	Ease trap retrieval and deployment in field	10	N
Amazon	B00AVLQUHU	S	Key ring	Ease trap retrieval and deployment in field	10	N
Ben Meadows	150179	R	Secondary containment bin	Battery containment while charging	Variable	N
ULINE	H-998	S	X-acto knife	Cut sample labels	1	N
	Consumable items					
Ben Meadows Forestry Suppliers	010510-1 49247	R	All weather copy paper	Print datasheets and sampleID labels	5	N
		S	Masking tape	Cover vent holes in trap coolers	1 roll	N

R/S=Required/Suggested

Table 3. Equipment list – Trap deployment and sample retrieval during field season sampling

Supplier	Supplier ID	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items						
Batteries Plus	SLAA612F	R	Battery, 6V	Deploy traps, replace batteries during specimen collection	20	Y
John W. Hock	1012.NEON	R	CO2 light trap fan assembly	Deploy traps, spare fan assemblies	20	N
John W. Hock	1012.NEON	R	CO2 light trap rain cover	Deploy traps, protect trap from rain	10	N
John W. Hock	1.44	R	Collection cup with mesh sleeve	Deploy traps, replace collection cups during specimen collection	20	N
		R	Cooler	Chill perishable samples in field	1	N
Fisher	11394305	R	Cryogenic gloves	Protect hands while handling dry ice	1 pair	N
John W. Hock	1.1.NEON	R	Cylindrical insulated cooler	Deploy traps, sublimate dry ice bait	10	N
REI Cabela's	852554 IK532446 - 100Q	R	Dry ice cooler	Store/transport dry ice	1	N
		S	Container (32x20x17 Sterilite with wheels)	Container to transport fans into the field	1	N

Supplier	Supplier ID	R/S	Description	Purpose	Quantity*	Special Handling
		S	Container (Sterilite 30 ½ x 20 x 156; 27 gal capacity)	Container to transport coolers into the field	1	
Amazon Cabela's REI	IK270217 895022	R	GPS receiver, recreational accuracy	Navigate to sampling location	1 per team	N
VWR	15715152	S	Ice pack, -20°C	Chill perishable samples in field if dry ice is unavailable	Variable	N
		R	Rain cover screws	Spare screws for mosquito trap	30	N
		S	Large key ring	For more easily connecting mosquito trap insulated cooler to rain cover	10	N
		R	Ice scoop	For transferring dry ice into mosquito trap insulated coolers	1	N
		S	Scissors	Separate sampleID labels	1	N
Minuteman International / ACHLA Designs	TSW27	S	Shepherd's hook	Hang traps; alternative to natural structures	10	N
Thomas	1929M35	S	Small bottle brush	Remove debris from fan assemblies	1	N

Supplier	Supplier ID	R/S	Description	Purpose	Quantity*	Special Handling
Consumable items						
		R	AA battery	Spare battery for GPS receiver	1 set	N
Amazon Grainger	B004NG90X0 6CHG5 16W483	S	Aluminum foil	Deter cattle or other wildlife	As needed	N
		R	Cardboard or cloth	Protect catch cup and samples during transport	Variable	N
Varies by domain	Varies by vendor	R	Dry ice, pelletized	Bait traps and freeze collected samples	15 kg	Y
Grainger	4TKE5 5GUU1	R	Liquid laundry detergent, fragrance free	Wash mesh sleeves and collection cups	1 bottle	N
		R	Paper towel	Absorb excess moisture	1 roll	N
Grainger	5CNK5 8YAT5	R	Resealable plastic bag, 1 gal	Protect trap batteries from rain/moisture	10	N
		R	Rope	Hang traps from natural structures	Variable	N
		R	Safety pin	Attach sampleID labels	20	N

Supplier	Supplier ID	R/S	Description	Purpose	Quantity*	Special Handling
Resources						
		R	Charged & synced Mobile Data Entry Device	Enter data	1 per team of 2	N
RD[05]		R	Field datasheet	Record data		N
		R	sampleID label	Label samples	Variable	N

R/S=Required/Suggested

Table 4. Equipment list – Laboratory processing

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items						
Fisher	07200610	S	Centrifuge tube rack, foam	Organize samples	1	N
Fisher	1495949B (15 mL) 1443222 1495949A (50 mL)	R	Centrifuge tube, 15 mL or Falcon tube, 50 mL	Contain mosquitoes	25	N
Thomas Fisher	1217R63 03395450	R	Cryovial freezer storage box with dividers	Organize samples (small vials)	1	Y
Fisher	22269979		Plastic vial racks	Organize samples (larger vials)	1	Y
		S	Paintbrush	Transfer mosquitoes to tubes	2	N
		S	Wax paper	Transfer mosquitoes to tubes	Variable	
		R	Forceps	Transfer mosquitoes to tubes	3	N
		S	Funnel, paper or plastic	Transfer mosquitoes to tubes	1	N

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
		S	Dry ice	Setup chilling station	5	N
Consumable items						
Ben Meadows Forestry Supplier	010510-149247	R	All weather copy paper	Print additional sampleID labels	2	N
		S	Copy paper, white	Transfer mosquitoes to tubes		N
Fisher	15930C	S	Cryogenic label	Label sample vials	sheet	N
		R	Toilet paper	Cushion mosquitoes in sample vials	25	N
Bioquip	1154F	R	Permanent marker, archival ethanol-safe	Label sample vials	1	N
		R	Clear packing tape	Protect labels from falling off in the -80C	1	N
		S	Re-sealable freezer bag, 1 pint	Organize samples	Variable	N
		S	Rubber band	Organize samples	Variable	N
		R	Adhesive barcode labels (cryo, Type II)	Labeling sample containers with barcode-readable labels	1 sheet	N

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
Resources						
		R	sampleID label	Label samples	Variable	N

R/S=Required/Suggested

Table 5. Equipment list – Sample shipment

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
Consumable items						
ULINE	S-16478	S	Cardboard box or insulated shipper, UN packing group III	Package samples for shipment	Variable	N
		S	Cushioning material (i.e. bubble wrap, packing peanuts)	Package samples for shipment	Variable	N
		R	Dry ice shipping label	Label shipments containing dry ice	Variable	N

Supplier	Supplier Number	R/S	Description	Purpose	Quantity*	Special Handling
Varies by Domain	Varies by Vendor	R	Dry ice, pelletized	Keep samples frozen during shipment	Variable	N
		S	Styrofoam sheet	Insulate samples for shipment	Variable	N
Resources						
		S	Return label	Used if requesting materials be returned by the laboratory	1 per shipment	N

R/S=Required/Suggested

6.2 Training Requirements

All technicians must complete required field and lab safety as well as protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[04]).

6.3 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.

6.4 Estimated Time

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

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) 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.

SOP	Estimated total time	Suggested staff	Total person hours
A. Preparing for sampling	1 hr/bout	2	2 hrs/bout
B Field Sampling	0.25 – 0.5 hrs/plot	2	20 - 40 hrs/bout
C Laboratory processing and analyses	0.5-3 hrs/bout	2	0.5-3 hrs/bout
D Data entry and verification	1 hr/bout	1	1
E Sample shipment	0.5 hr/bout	1	0.5 hr/bout

Table 6. Estimated time required to complete field sampling and lab standard operating procedures

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

A.1 Prior to a sampling bout

1. 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 7**). On this setting, the trap fan remains on at all times.

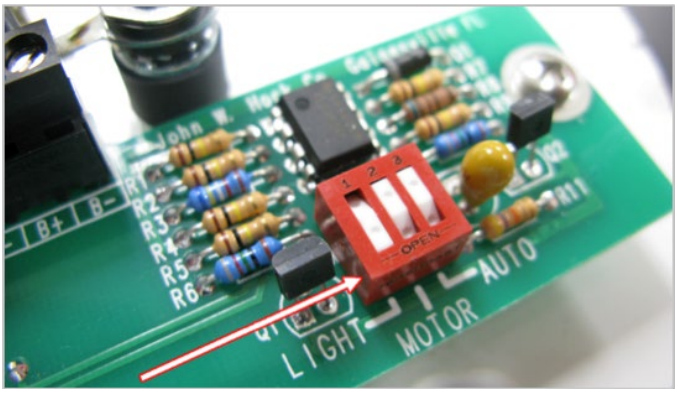


Figure 7 Switches inside circuit assembly of a CDC mosquito trap.

2. 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.

3. 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 carabiner to aid in removing/hanging traps in field.

4. 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 spin 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.

5. Mark the interior of the cooler 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.

6. 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).

7. If they will be used, make sure that reusable ice packs are frozen.

8. Identify the locations of plots used for mosquito sampling (use GPS and/or maps).

9. Print field datasheets (RD[05]) and one set of sampleID labels for each trap (total of 20 labels). The format of the sampleID is plotID.collectionDate.collectionTime (example **Figure 8**). 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.

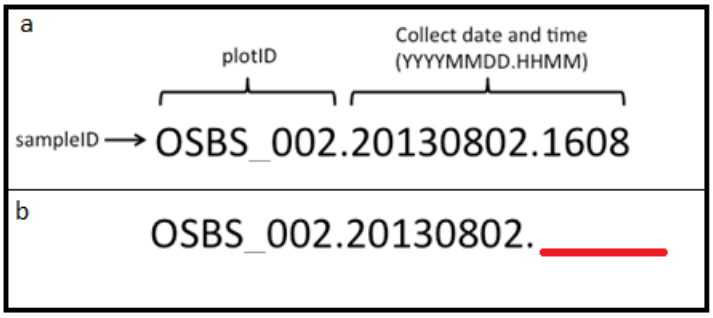


Figure 8 (a) Annotated sampleID example. (b) SampleIDs deployed in the field will have all but the last four-digits pre-filled.

10. Prepare final sample containers by affixing one adhesive barcode label to each vial and/or Ziploc bag used to contain each sample (Type II cryo-safe label, see **Figure 9**; **DO NOT** use other types on the vial).

    a. Adhesive barcode labels should be applied to dry, room temperature containers in advance of their use for sample storage (at least 30 minutes prior, but may be applied at the start of the season).
    b. If vials are used, 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.
    c. If your domain generates one (1) vial of mosquitoes per catch cup, affix the barcode label to each vial to be filled with a unique sample. If multiple vials are required to contain a sample from one trap, place the barcode on the Ziploc 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 Ziploc container that contains all ten vials, *not* each vial containing 1/10 the sample.

    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 you are encouraged to make these up in advance.

Figure 9 An example of a Type II barcode. These large-size, cryo-safe barcodes have a prefix of 'B' followed by 11 numbers.

A.2 Just prior to heading to the field for sampling:

1. Obtain enough dry ice to be able to fill the cylindrical insulated cooler of each trap (e.g., ~1.5kg of ice in pellet form) and freeze any samples during transport from the field to the lab (typically 2.5 – 4.5 kg is sufficient, but depends on drive time).

    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.

2. The use of insect repellent is recommended; see Section 5 (Safety) for details on application.

3. 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.

4. Use the checklist (**Appendix B**) to ensure that all required materials are in the field truck prior to sampling.

SOP B Field Sampling

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).

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 1.5 - 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 Shepard's hook may instead be placed into a 5 gallon bucket containing concrete. The concrete in the bucket will act as a weighted anchor to prevent the Shepard's hook from falling over in soils too unstable to use the T-post method or soils too compacted to insert the Shepard's hook alone.

    See **Appendix E** for a list of sites using either the T-post method of securing Shepard'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. 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. Ensure that the clip on the cooler does not cover the hole where the CO_2 escapes. 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).

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

5. 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 the 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.

6. ***Remove the tape covering the hole in the underside of the insulated cooler containing dry ice.*** **Easy to forget but critical step!

7. 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.

8. Record appropriate information about the visit to the sampling plot on the mobile data entry device 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).

    a. 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, make note of the blockage in the remarks and issue a problem ticket to NEON Science.

2. With the fan still running, gently tap flying mosquitoes down towards the bottom of the sleeve and into the cup. Tie the laces on the catch cup mesh sleeve 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.

3. Remove the collection cup by sliding the mesh sleeve off of the fan assembly, while keeping the fan running.

4. 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.

5. 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.

6. Redeploy trap as necessary. Remember to attach a new sampleID label to the new (empty) collection cup attached to the re-deployed trap.

7. In instances where mosquito abundance is exceedingly high, trap fans may become clogged with 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.

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

9. 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.**

10. 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 frozen reusable ice packs if logistics (e.g., duration of field visit, local availability of dry ice) preclude the use of dry ice.

11. Place a cardboard card or cloth between the catch cups and the ice so that they do not come into direct contact.

    a. 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.

12. 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. dryIceStatus (Present or Absent)

    f. "targetTaxaPresent" (may be revised during sample processing in SOP C)

        1) This field can be populated with values of Yes, No, or Maybe and can be filled out in the field (by circling Y, N, or M) or in the lab when the catch cup is processed.

        2) 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 sorted out when the catch cup contents are being processed in the lab. In this case (targetTaxaPresent=N), no sample vial is generated.

        3) Enter "Y" if the catch cup definitely contains mosquitoes. In this case (targetTaxaPresent=Y) one or more sample vials will be generated.

        4) 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)
   1) No known compromise – sample intact/good condition
   2) Cold chain broken – sample thawed at any point in the treatment of this sample
   3) 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
   4) Handling error – sample was damaged (i.e., catch cup dropped)
   5) Other (describe in remarks) – Use this option if multiple types of compromise occur or a compromise occurs not in this list

h. sampleFate (may be revised during sample processing in SOP C)
   1) active – a sample with mosquitoes (or insects that might be mosquitoes) was collected
   2) 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)

13. 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 samples into an ultra-low freezer (-80°C). Keep samples from different collection events separate (e.g., 1st collection samples should be in a different labeled bag than 2nd collection samples to aid in differentiating them during the transfer to vials in the lab).

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.

   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.
   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.
      1) 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 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 Laboratory Processing and Analysis

C.1 Transferring mosquitoes from catch cups to sample vials

1. Clear and clean off bench space prior to processing samples.
2. Obtain enough sample vials to hold samples from each catch cup. If available, use vials and/or bags that have been pre-labeled with barcodes (SOP A).
    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, 50 mL vials for sites with large numbers of mosquitoes). Preparing extra vials is recommended in the event that cup contents are higher than expected.
    c. Note: Each barcode-labelled 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 Ziploc bag that will contain all vials associated with that sample. Barcodes are unique, but are not initially associated with a particular sample, so you are encouraged to make these up 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 Ziploc container not each vial containing 1/10 the sample; the database cannot handle 10 barcodes mapping to the same catch cup.
    Labels must be adhered to each vial or Ziploc for 20-30 mins *before* introducing the container to dry ice or -80C.
3. Generate a sampleID label to be placed into each vial once it is full of mosquitoes (see step 10).
    a. For the sampleID labels already attached to each catch cup, make sure that the trap collection time is included.
    b. Print additional sampleID labels, if multiple vials are required, to insert one into each sample vial. Printing extra labels is recommended.
4. Prior to sample transfer, pull up the record of each trap in the mobile data entry application to be processed per labeled vial or container and scan the barcode that corresponds to that sample. Note: Data must be entered into Fulcrum prior to placing mosquitoes from the catch cup into one or more vials.
5. Fold a square of toilet paper (1-ply is sufficient) into quarters to create a small square.
6. 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. Note that for smaller vials,

the packing material will not form a pocket; for small vials merely make a tight plug (see **Figure 10**), using the back of a Sharpie marker to push the paper to the base of the vial in lieu of a thumb.

a. The paper padding will help absorb moisture, prevent mosquitoes from sticking to the bottom of the vial, and cushion the sample during transit.

7. Gather and/or prepare any equipment necessary for transferring mosquitoes from catch cups into sample vials (Table 4).

8. Set up a chilling station to keep sample vials cold following removal from freezer and during transfer.

a. A simple version of such a station involves a small cardboard box that has holes cut into the top into which chilled sample vials can be inserted (**Figure 9**). During sample transfer, the box is filled with ice or dry ice to keep the sample vials cold.

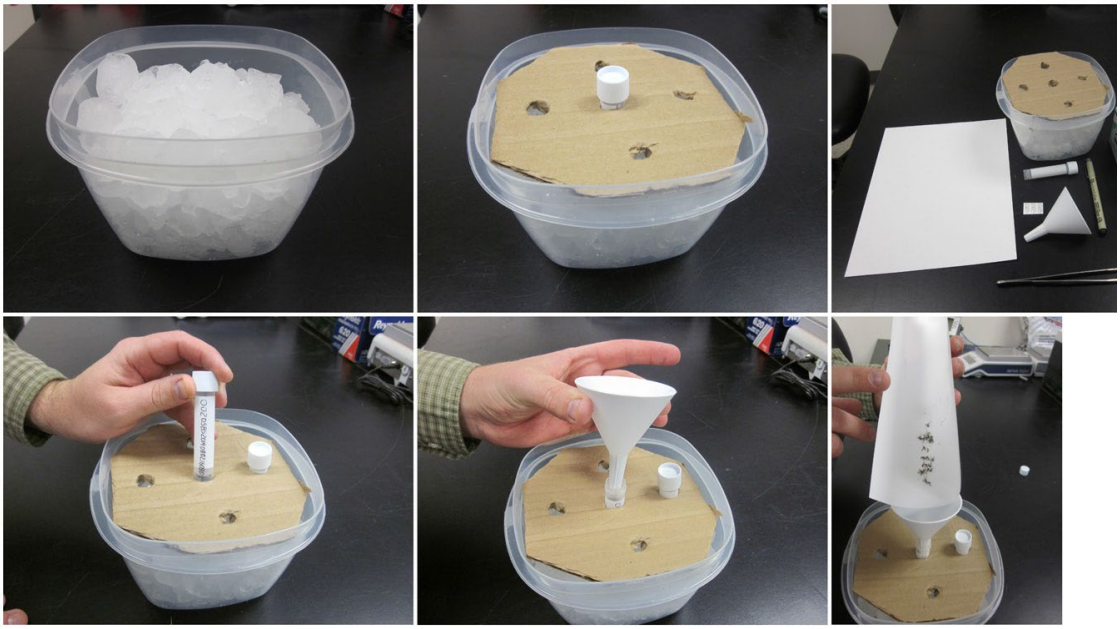


Figure 10 Example of a chilling station and associated laboratory setup for transferring mosquitoes from catch cups into sample vials.

9. Optional: If processing large numbers of samples, it is helpful to have an intermediate cooler filled with dry ice. If used, multiple traps can be placed into this intermediate cooler from the -

80°C freezer. This prevents having to repeatedly reopen the freezer. Prior to processing, all catch cups should be placed with the wire mesh facing up as mosquitoes can be damaged if they are in contact with the wire mesh.

10. 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.

11. 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.

    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.

        1) While by-catch removal can improve sample quality, prioritize keeping mosquito samples frozen (i.e., do not spend too much time removing by-catch).

        2) If by-catch is frozen to mosquitoes (and is of similar size to the mosquitoes), do not attempt to disentangle.

        3) 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.

        1) This field can be populated with values of Yes, No, or Maybe (choose one).

        2) 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.

        3) Enter "Y" if the catch cup definitely contains mosquitoes. In this case (targetTaxaPresent=Y) one or more sample vials will be generated.

        4) 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 10** a piece of paper is used for this purpose).

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: **Majority or many (>20% 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., 20% 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).

1) **Do not overfill** the sample vial. Overfilled samples with too many mosquitoes will results 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 11 for appropriate and inappropriate levels of mosquitoes.

g. Fold another square of toilet paper into quarters to form a square piece of padding.

h. Add an upper layer of toilet paper on top of the mosquito samples. Gently push the upper packing material 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.

1) Add additional packing material above the upper plug if the mosquito sample is small and there is empty space in the upper part of the vial.

2) 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.

12. Place a sampleID label into each vial above the upper plug of packing material. 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.

13. 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 plastic freezer bag or vial rack.

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

14. For each sample vial, record the number of vials used to contain the sample on the paper field datasheet (RD[05]) or record directly into the electronic data entry interface (SOP D).
15. 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 11 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 wadding and the lid of the vial.

C.2 Sample preservation

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

C.3 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

The importance of thorough, accurate data transcription cannot be overstated; the value of the efforts in the field is only manifested once the data are properly entered for delivery to NEON's end users. 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. For detailed instructions on protocol specific data entry into mobile devices, see the NEON Internal Sampling Support Library (SSL). 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 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). Data transcription must be completed 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 into the Mosquito mobile application or web user interface (WebUI), according to instructions in the AOS/TOS Protocol and Procedure: 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). However, if one or more traps for an entire bout is missed (e.g., only 8 of ten plots were visited during a bout) then no data need to be entered for the traps from that bout which were never set.

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: Data Management (RD[08]).

Sample Labels & Identifiers

By default each sample or subsample produced by this protocol is assigned a human-readable sample identifier which contains information about the location, date, and/or taxonomy of the collected sample. Each sample may also be associated with a scannable barcode, which will not contain information specific to sample provenance, but will reduce transcription errors associated with writing sample identifiers by hand.

Adhesive barcode labels should be applied to dry, room temperature containers in advance of their use in the field (at least 30 minutes prior, but may be applied at the start of the season). Barcodes are unique, but are not initially associated with a particular sample, it is encouraged to make these up in advance. Use the appropriate barcode label type with each container (i.e., cryo-safe barcode labels only used for samples that are stored at -80°C, etc).

Barcodes are scanned into the mobile application when the sample is placed into the container; only one barcode may be associated with a particular sample. Do not reuse barcodes. If a barcode is associated with multiple samples, the data ingest system will throw an error and refuse to pull in entered data. If multiple vials or containers are required to contain a sample from one trap, place the barcode on the outer container that will hold all vials associated with just that sample (i.e., if a catch cup from collection fills ten 50-mL falcon tubes, the single barcode is applied to the outer Ziploc container not each vial containing 1/10 the sample; the database cannot handle 10 barcodes mapping to the same sample).

Data and sample IDs must be entered digitally and quality checked prior to shipping samples to an external lab.

SOP E Sample Shipment

Information included in this SOP conveys science-based packaging, shipping, and handling requirements, not lab-specific or logistical demands. For that information, reference the Collection and Laboratory Analysis (CLA) shipping document on CLA's NEON intranet site.

E.1 Handling Hazardous Material

Dry ice is a Class 9 regulated material and must be shipped according to CFR 49 Subchapter C, Hazardous Materials Regulations.

Dry ice releases carbon dioxide gas, which can build up pressure and rupture packaging. Ensure the packaging used allows the release of this pressure to prevent rupturing the package. Dry ice must be packaged using **UN packing group III** compliant materials. The maximum amount of dry ice per package is **200 kg**. Refer to Chemical Hygiene Plan and Biosafety Manual (AD[03]) for additional requirements on commercial shipment of hazardous or dangerous materials.

Note: Some domains will need to comply with the Federal Aviation Administration (FAA) regulations and the International Air Transportation Association (IATA) guides.

E.2 Supplies/Containers

Use corrugated cardboard boxes that meet UN packing group III requirements. Add Styrofoam along the walls of the box as insulation. Ensure the Styrofoam IS NOT sealed to be airtight. Styrofoam must not be used as an outer packaging.

Put a layer of dry ice at the bottom of the insulated shipper, place samples to be shipped inside, then weigh the box containing samples. Add dry ice to surround the samples and reweigh the box to determine the amount of dry ice in each package. Be sure to include a hardcopy shipping manifest inside the box with the samples. At a minimum, each box should contain 10 pounds of dry ice.

When packing items in the container put dry ice and specimens as close together as possible with dry ice on top. Fill empty space with non-absorbent materials, such as Styrofoam peanuts or bubble wrap. Empty space will cause the dry ice to sublimate faster. As dry ice sublimates specimens will move around in packaging; cushioning provides additional protection for samples during shipment.

Note that this must be done quickly as it requires the samples be initially placed into the box without dry ice. Samples can thaw quickly and must remain frozen at all times.

Complete packaging and labeling for Class 9 dry ice hazard shipment.

E.3 Timelines

Avoid shipment on days that will require transit on a weekend or over a holiday period (Monday, Tuesday and Wednesday are typically best). Samples can be stored at -80°C for many months without loss of quality. Based on logistics timelines and domain storage capacity, samples should ideally be shipping out according to your domain shipping schedule (approx. once per month).

E.4 Conditions

Samples should be shipped frozen on dry ice via standard overnight delivery service to taxonomic ID facility. **Samples must remain frozen at all times.**

E.5 Grouping/Splitting Samples

All samples collected during each bout must be shipped together. Sample vials containing samples collected as part of the same bout combination can be taped or rubber-banded together, or placed in a separate bag, to allow them to be easily inventoried and sorted at the external facility.

E.6 Return of Materials or Containers

If using insulated shipper kits or other reusable containers include return ground shipping forms for the laboratory to return shipping materials.

E.7 Shipping Inventory

Each shipment must be accompanied by a hard-copy shipping manifest enclosed within the shipping container AND a corresponding electronic version of the manifest emailed to the taxonomic ID facility. Place the hard copy shipping manifest in resealable plastic bag on top of Styrofoam and send electronic manifest and shipper tracking information to CLA contact **and** the receiving laboratory using the Stork Shipment Verification Tool. The hard-copy shipping manifest lists every sample vial in the shipped batch.

The electronic manifest should be emailed to the taxonomic ID facility as soon as possible after a batch of samples has been shipped. The order of sample records in the electronic manifest should match the order in the corresponding hard-copy shipping manifest.

Procedure:

1. Navigate to the “Shipping Information for External Facilities” document on CLA’s NEON intranet site. Check whether there are items such as permits or cover letters required to include in the shipment. Check this document often as instructions are subject to change.
2. Print out required documents (if needed) to include in shipment box.

3. Prepare a shipping inventory detailing the contents of the shipment, using the Shipment Creation and Shipment Review applications. Include a printed copy of the inventory in the shipment box in resealable plastic bag on top of Styrofoam.
4. Complete packing slip, address shipment, and ship overnight to the destination(s) specified in the CLA "Shipping Information for External Facilities" document.

E.8 Laboratory Contact Information and Shipping/Receipt Days

See the CLA shipping document on CLA's NEON intranet site.

8 REFERENCES

N/A

APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 7. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.001581	Datasheets for TOS Protocol and Procedure: Mosquito Sampling

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

APPENDIX B QUICK REFERENCES

Quick Reference: Getting Ready for Sampling

STEP 1 – Gather all needed supplies (and extras).

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 datasheets (used only if there is a failure in the mobile data recorder).

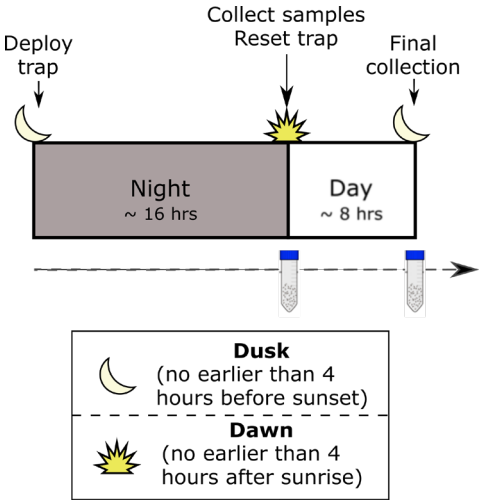
STEP 5 – Generate and print sampleID labels for each trap (plotID and collectDate pre-printed with space for collect time). You will need 20 labels per bout.

On the field day:

STEP 6 – Obtain enough dry ice to set (or 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 ice with cardboard or cloth.

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 – Fill cylinder cooler with dry ice pellets.

STEP 3 – Assemble trap components. Attach fan assembly to rain cover using screws. Attach catch cup (with mesh sleeve). Connect to battery. Remove tape from cylinder vent hole.

STEP 4 – Navigate to the plot location with all materials described above.

STEP 5 – Suspend trap.

STEP 6 – Record all metadata—especially plotID, set date, and set time—and any irregularities on mobile application (or paper data sheet if mobile application is unavailable).

Subsequent visits:

STEP 7 – After elapsed time, return to trap with replacement catch cup, dry ice and spare parts.

STEP 8 – Keep fan running. Tie off mesh sleeve and remove 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.

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.

Trap Setup:

- Suspend with hole in bottom of cylinder cooler approximately 1.5 to 1.8 m above the ground.
- Hang from tree or sturdy shrub.
- Shield from heavy wind, sunlight, and rain:
  - Hang on leeward side, away from wind.
  - Hang on west facing side, away from direct morning sunlight.
- Where tree or shrub is not available, secure trap to a shepherd's hook.
- Seal battery in resealable freezer bag and place on ground.

Quick Reference: Processing Mosquito Specimens

STEP 1 – Prepare chilling station and enough vials to contain the sample. Each vial should be pre-labeled on the exterior of the vial with the sampleID of the catch cup and have appropriate tissue in the bottom of the vial. Scan the barcode label before filling with the sample.

STEP 2 – Gently transfer contents of catch cup into sample vial(s). Remove any obvious bycatch during transfer, but prioritize keeping mosquitoes frozen.

STEP 3 –Place tissue paper and sampleID label into each sample vial and secure vial with cap. Make sure that the sampleID label is between the tissue and the vial cap, **not** mixed with the mosquitoes.

STEP 5 – Store vials in -80°C freezer.

STEP 6 – Record the number of vials used to contain the sample and any irregularities on mobile application (or paper data sheet if mobile application is unavailable).

STEP 7 – Repeat procedure with specimens in next catch cup.

> **Keep samples frozen. The genetic material detected during pathogen testing degrades when a sample thaws.**

APPENDIX C REMINDERS

Getting Ready for Sampling

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

- ☐ 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
- ☐ 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)
- ☐ Test fan by connecting battery
- ☐ Inspect fan wires for damage
- ☐ Charge batteries
- ☐ Charge and sync mobile data entry device
- ☐ Print datasheets (Rite in the Rain)
- ☐ Upload sample coordinates to GPS and obtain maps
- ☐ Check that sufficient dry ice is available
- ☐ Assemble mosquito traps

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)
- ☐ Taped the hole in the cylinder cooler (if it 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
- ☐ Freeze and store specimens on dry ice
- ☐ Record all required data and irregularities on mobile application (or paper data sheet if mobile application is unavailable).

Before leaving trap: Check that...

- ☑ Cylinder cooler has been refilled with dry ice if trap reset is required.
- ☑ Tape is removed from cylinder cooler and CO_2 is subliming.
- ☑ sample labels (with date) are attached to mesh sleeve.
- ☑ Battery is connected to fan housing and fan is running.

Transporting samples: Make sure...

- ☑ Mosquitoes remain frozen or as cold as possible
- ☑ Mosquitoes are not in direct contact with ice in transport cooler.
- ☑ Cooler is secure in vehicle and will not tip over during transport.
- ☑ Catch cups are transferred to -80C freezer in domain lab as soon as possible.

Note on transporting dry ice: Coolers should be in the truck bed not the cabin (due to dry ice sublimation)

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
- ☐ Provide sufficient padding for the mosquitoes on the bottom, tap the vial after filling to reduce space being specimens, and add a cushioning tissue to the top.
- ☐ Put sampleID label (plotID.collectDate.collectTime) in every sample vial between the cap and top tissue

APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

The dates in the table below are estimates for the start and stop dates of sampling based on NOAA weather station records from the most recent 10 years. Calculations are based on the 5-day running average of observed daily maximum temperatures (TMAX) from NOAA NCDC between 2006-2015 (Menne *et al.* 2012). 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 a date range of 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.

This table will be updated annually, as new data become available.

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.

		Average 5-day temp above 10°C	
Domain	**Site**	**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
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.

		Average 5-day temp above 4 °C	
Domain	**Site**	**Approx. Start Date (Field season)**	**Approx. End Date (Field season)**
18	TOOL	1-Jun	17-Sep
19	BONA	13-Mar	05-Oct

Note that in some cases, the timing of sampling at a relocatable site will be based on conditions at a core site in a different domain. The following sites are affected:

1. MOAB: this relocatable site is officially associated with D13 but will be sampled according to a timeline set by activities in D15 and its core site, ONAQ, due to shared personnel.

For D17, the core site is at a significantly lower elevation than its associated relocatable 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 relocatable 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 inaccessable from November to April. SOAP will follow the standard relocatable 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 limited site access.

References

Menne, Matthew J., Imke Durre, Bryant Korzeniewski, Shelley McNeal, Kristy Thomas, Xungang Yin, Steven Anthony, Ron Ray, Russell S. Vose, Byron E. Gleason, and Tamara G. Houston (2012): Global Historical Climatology Network - Daily (GHCN-Daily), Version 3. [subset: 2006-01-01 to 2015-12-31]. NOAA National Climatic Data Center. doi:10.7289/V5D21VHZ

APPENDIX E SITE-SPECIFIC PROTOCOL MODIFICATIONS

DOMAIN 04

At Lajas Experimental Station (relocatable 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 Shepard's hook is placed into a 5 gallon bucket containing concrete. The concrete in the bucket acts as a weighted anchor to prevent the Shepard's hook from falling over the compacted soil (soil that otherwise prevents insertion of the Shepard's hook alone).

DOMAIN 14

At Santa Rita Experimental Range (core site) and Jornada LTER (relocatable 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.