

<i>Title:</i> Draft NEON FSU Field and Laboratory Protocol for Operations Central Plains Experimental Range 2011: Mosquito Abundance, Diversity and Phenology	<i>Author:</i> C Gibson	<i>Date:</i> 09/23/2011
<i>NEON Doc. #:</i> NEON.DOC.014049		<i>Rrevision:</i> A_DRAFT

Draft NEON FSU Field and Laboratory Protocol for Operations Central Plains Experimental Range 2011:  
Mosquito Abundance, Diversity and Phenology

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See Configuration Management System for approval history

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### Change Record

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DRAFT

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## 1 INTRODUCTION

### 1.1 Purpose

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

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

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

### 1.2 Scope

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

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

- general safety practices (i.e. how to drive a boat)
- site-specific safety practices (e.g. how to safely walk in a stream)
- general maintenance (i.e. fill the car with gas)

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

### 1.3 Acknowledgements

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

*If a protocol is based closely on the work of another program or author, note that here.*

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## 2 RELATED DOCUMENTS AND ACRONYMS

### 2.1 Reference Documents

*(If you want to reference other procedural documents (e.g. associated Protocol document), drawings, etc. then include filenames in the following sections.)*

RD[01]	NEON.DOC.000008 NEON Acronym List
RD[02}	EHS Safety Policy and Program Manual
RD]03...]	<primary science design docs explaining/justifying this protocol/these procedures>
RD[04]	NEON Sampling Design Document
RD[05]	Training Plan
	QA/PA Plan
	DOORS requirements
	ATBD
AD[01]	FSU Science Requirements
AD[02]	FSU Field Operations Plan
AD[03]	Data Products Level 1-3 Catalog
	NEON.DOC.000243 NEON Glossary of Terms

### 2.2 Acronyms

*Insert table for definitions of acronyms used in this document.*

NEON	National Ecological Observatory Network
FSU	The NEON Fundamental Science Unit at Headquarters
P&P	Procedure and Protocol

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### 3 BACKGROUND AND OBJECTIVES

#### 3.1 Background

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

The purpose of this draft protocol is to inform the activities involved with planning and scheduling mosquito diversity and phenology sampling for the Field Operations Prototype. This section of the document outlines the field and laboratory procedures to collect, process, and maintain the integrity of the mosquito diversity and phenology samples.

The purpose of the Mosquito Diversity and Phenology sampling design is to capture inter- and intra-annual variation of mosquitoes (Diptera: Culicidae) in the NEON purview. Mosquitoes were chosen as a focal terrestrial invertebrate taxon because they are widespread and important vectors of disease. They are also relatively easy to sample and populations are sensitive to climate variation. Mosquito species' range expansions as well as invasions of non-native species are presently occurring in North America (e.g., *Aedes albopictus*). These shifts are expected to increase with warming climates in temperate latitudes, which can impact the spread of disease.

The following sub-sections contain draft protocols that provide detailed guidance for setting mosquito traps in a subset of the Biodiversity Plots, collecting insects and associated metadata, laboratory processing of the mosquitoes, and storage and shipment of samples to contracted experts (Taxonomists) for morphological identifications, to Genetic Analytical Laboratories for DNA barcoding, and/or to Archives (voucher collections). Other invertebrates (considered "bycatch") will be preserved and grouped by collection date and shipped to Archive facilities (for the 2011 sampling year this will be the Support Facility at 1635 38<sup>th</sup> St., Suite 100, Boulder, CO 80301).

A CO<sub>2</sub> light trap is baited with CO<sub>2</sub> (carbon dioxide) (also called dry ice pellets). Carbon dioxide acts an attractant for mosquitoes as they use it as a cue for vertebrate hosts. Mosquitoes display seasonal (phenological) patterns with maximum abundances and activity periods in spring, summer and/or fall (depending on the species).

*This document describes the required protocols for conducting field sampling, making a human-mediated field observation, or operating an instrument to make measurements in the field, or any other activity that generates a Level 0 data product.*

*Briefly describe science rationale for selecting protocol. Specific details of methodology are described in standard operating procedures (SOPs) included as appendices. Recommended length <1 page.*

#### 3.2 Metadata Collection

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

Data about the samples will be compiled into fields outlined in a spreadsheet to be supplied by FSU called 'NEON Mosquito Phenology & Diversity Datasheet'. These fields follow the Barcode of Life

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Datasystems (BOLD) standards and include some additional fields in order to be compliant with other global biodiversity projects.

*This document describes the required protocols for conducting field sampling, making a human-mediated field observation, or operating an instrument to make measurements in the field, or any other activity that generates a Level 0 data product.*

*Briefly describe science rationale for selecting protocol. Specific details of methodology are described in standard operating procedures (SOPs) included as appendices. Recommended length <1 page.*

### 3.3 Science Requirements

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

This protocol fulfills the following Observatory science requirements:  
*List science requirements from DOORS that are met by this protocol.*

### 3.4 Data Products

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

*List Level 0 data products measured by protocol.*

**Table 1** A summary of field and related lab measurements and the associated NEON Data Products

Measurement	Data Product

## 4 PROTOCOL

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

### 4.1.1 Plot Location

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

FSU is responsible for determining plot locations.

*Mosquito Phenology Trap Location:* CO<sub>2</sub> light traps will be set in routinely traveled areas near other Biodiversity Plots that will be / or were previously sampled for Mosquito Diversity. (2 total traps)

*Mosquito Diversity Trap Location:* CO<sub>2</sub> light traps will be placed adjacent to ten Biodiversity Plots (within

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the destructive sampling buffer zone) (10 traps per site). Two additional CO<sub>2</sub> light traps shall be placed at sites near the aquatic array (2 additional traps per site)

*Summarize the science rationale (e.g. experiment design), include key citations. Briefly summarize the procedure included in this document and variations in how NEON is implementing this protocol in different locations throughout Observatory (e.g. above ground tree biomass for temperate vs tropical zones). If the protocol is based on existing published procedures, reference those here (e.g. “These methods are based on the amazing work of John Updike (1964). No one has come up with anything better since then.”).*

*A protocol is a formal summary description of a procedure and its related rationale. A protocol includes information on knowledge and resources needed to implement the procedure. A procedure is a set of prescribed actions that must take place to achieve a certain result; can also be called a method.*

Read more:

[http://wiki.answers.com/Q/What\\_is\\_the\\_difference\\_between\\_a\\_method\\_and\\_a\\_procedure#ixzz1Flnc5Na](http://wiki.answers.com/Q/What_is_the_difference_between_a_method_and_a_procedure#ixzz1Flnc5Na)

*Identify assumptions or known-unknowns of the chosen protocol.*

*Identify and summarize quantitative aspects of the procedure (timing, # plots, # samples, location of sensors).*

*Provide a simple timeline diagram or table if pre-field activities occur the day prior to the field day, or if the field procedure is a multi-day task.*

## 5 QUALITY ASSURANCE AND QUALITY CONTROL

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

See QA/QC plan and reference QA/QC document.

*Summarize QA/QC plan and reference QA/QC document.*

## 6 DECISION TREE

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

See Decision Tree document

Mosquito Phenology

Delay	Action	Adverse Outcome	Outcome for Data Products
< 3 hours	Collect traps asap; note duration & cause; resume standardized sampling (1 week)	Specimens may not be morphologically identifiable	May not be readily able to obtain morph. ID; affects

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			<p>phenology measurements</p>
<p>3 hours - 1 day</p>	<p>Collect traps asap. Note duration and cause of the delay. (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. Do not adjust (push back) dates for subsequent sampling bouts. (2) If the delay occurs prior to the deployment of traps but during the sampling bout (e.g., prior to the deployment of traps on the second day of sampling), then push that sampling day, and any subsequent days in the bout, back the duration of the delay. Do not adjust (push back) dates for subsequent sampling bouts. (3) If the delay occurs after the deployment of traps but prior to the checking of traps, then repeat that day of sampling the same day (i.e., redeploy trap that night) or the subsequent day. Do not adjust (push back) dates for subsequent sampling bouts.</p>	<p>Specimens may not be morphologically identifiable</p>	<p>May not be readily able to obtain morph. ID; affects Phenology measurements</p>
<p>1 - 2 days</p>	<p>Collect traps asap. Note duration and cause of the delay. (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. Do not adjust (push back) dates for subsequent sampling bouts. (2) If the delay occurs prior to the deployment of traps but during the sampling bout (e.g., prior to the deployment of traps on the second day of sampling), then push that sampling day, and any subsequent days in the bout, back the duration of the delay. Do not adjust (push back) dates for subsequent sampling bouts. (3) If the delay occurs after the deployment of traps but prior to the checking of traps, then repeat the entire sampling bout at the conclusion of the delay. Do not adjust (push back) dates for subsequent sampling bouts.</p>	<p>Specimens may not be morphologically identifiable</p>	<p>May not be readily able to obtain morph. ID; affects Phenology measurements</p>

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3 days - 2 or more weeks	Note duration and cause of the delay. Contact appropriate scientific lead(s) on the FSU team for guidance. Cancel the impacted sampling bout and stop sampling until next scheduled sampling bout	Specimens may not be morphologically identifiable; true phenology of some species may be lost for this collection year	May not be readily able to obtain morph. ID; affects Phenology measurements
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### Mosquito Abundance and Diversity

*Summarize for the field technician or manager any implementation decisions regarding the protocol.*

<b>Delay</b>	<b>Action</b>	<b>Adverse Outcome</b>	<b>Outcome for Data Products</b>
< 3 hours	Collect traps asap; note duration & cause; resume standardized sampling (1 week)	Specimens may not be morphologically identifiable	May not be readily able to obtain morph. ID; affects Abundance&Diversity measurements
3 hours - 1 day	Collect traps asap. Note duration and cause of the delay. (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. Do not adjust (push back) dates for subsequent sampling bouts. (2) If the delay occurs prior to the deployment of traps but during the sampling bout (e.g., prior to the deployment of traps on the second day of sampling), then push that sampling day, and any subsequent days in the bout, back the duration of the delay. Do not adjust (push back) dates for subsequent sampling bouts. (3) If the delay occurs after the deployment of traps but prior to the checking of traps, then repeat that day of sampling the same day (i.e., redeploy trap that night) or the subsequent day. Do not adjust (push back) dates for subsequent sampling bouts.	Specimens may not be morphologically identifiable	May not be readily able to obtain morph. ID; affects Abundance&Diversity measurements

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1 - 2 days	Collect traps asap. Note duration and cause of the delay. (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. Do not adjust (push back) dates for subsequent sampling bouts. (2) If the delay occurs prior to the deployment of traps but during the sampling bout (e.g., prior to the deployment of traps on the second day of sampling), then push that sampling day, and any subsequent days in the bout, back the duration of the delay. Do not adjust (push back) dates for subsequent sampling bouts. (3) If the delay occurs after the deployment of traps but prior to the checking of traps, then repeat the entire sampling bout at the conclusion of the delay. Do not adjust (push back) dates for subsequent sampling bouts.	Specimens may not be morphologically identifiable	May not be readily able to obtain morph. ID; affects Abundance&Diversity measurements
3 days - 2 or more weeks	Note duration and cause of the delay. Contact appropriate scientific lead(s) on the FSU team for guidance. Cancel the impacted sampling bout and stop sampling until next scheduled sampling bout	Specimens may not be morphologically identifiable	May not be readily able to obtain morph. ID; affects Abundance&Diversity measurements

## 7 SAFETY

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

Materials Safety Datasheets are provided at training and are available in the lab for the following chemicals used in this work: carbon dioxide pellets, ethanol and paradichlorobenzene (moth crystals).

Care must be taken when handling batteries as improper handling can cause burns or acid leaks.

Personnel working at a NEON site should be familiar with and practice safe field work as outlined in the EHS Safety Policy and Program Manual. Additional safety issues associated with this field procedure are outlined below. The Field Operations Manager and the Lead Field Technician have primary authority to

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stop work activities based on unsafe field conditions; however, all employees have the responsibility and right to stop their work in unsafe conditions.

*Any safety issues specific to the procedure should be detailed here, along with references to any pertinent safety standards.*

## 8 PERSONNEL REQUIREMENTS

*Include special skills or subject matter expertise required (e.g. able to identify regionally specific plants by visual inspection, through use of dissecting microscope and through use of dichotomous key)*

### 8.1 Field Personnel

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

A minimum of two field technicians are required for Mosquito Diversity sampling in the field. The Mosquito Phenology sampling can be accomplished with available personnel and does not require a designated crew.

### 8.2 Laboratory Personnel

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

A minimum of one laboratory technician is required to process Mosquito Diversity samples within a week of receipt from the Taxonomist.

- Fine manual coordination (in order to properly handle pinned specimens)
- Small, neat handwriting
- Knowledge and ability to work with a dissecting microscope

## 9 TRAINING REQUIREMENTS

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

See NEON Training Plan document relevant to this method.  
 Reference NEON Training Plan document relevant to this method.

## 10 FIELD STANDARD OPERATING PROCEDURE

### 10.1 Sampling Frequency and Timing

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*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

There are two different sampling regimes.

*Mosquito Phenology Sampling Schedule:* This is two total CO<sub>2</sub> light traps being collected twice per week after 24 h. These traps are collected by whomever is available (field or lab technicians or domain staff) and then all collected invertebrates are shipped directly to a Taxonomist. Normally this activity would start around April 2 in Domain 10. This year, however, due to timing of hiring in 2011, the phenology sampling schedule will occur only at the end of the sampling period from Monday Aug 1 to Friday Sept 30, 2011.

This Mosquito Phenology sampling schedule is meant to be less intensive, but the data are meant to indicate seasonality of particular species and their anticipated changes over time.

*Mosquito Diversity Sampling Schedule:* This is ten total CO<sub>2</sub> light traps being collected twice per week within 24 h of trap set up in a subset of the Biodiversity Plots. These traps are to be collected by the Mosquito Diversity field crew. The field crew then sorts the samples and sends mosquitoes to a Taxonomist and the invertebrate bycatch to the Archive. The first actual sampling should occur on Monday May 16 and continue through to the week ending Friday July 29, 2011. (Training during Monday May 2 – Friday May 13)

**Table 2** The approximate sample dates for mosquito sampling at all NEON sites

Domain	Date	Frequency
10 (Diversity)	May16-July31	Weekly (10 traps)
10 (Phenology)	April2- May15 and Aug1-Sept30	Weekly (2 traps)

Mosquito sampling occurs for two nights with 7 days between the first sampling day of one bout and the first sampling night of the next bout and within 3 hours of standard time each week. We estimate Diversity sampling requires 2 technicians for 12min per trap x 10 traps = 120 min = 2 hours each sampling day plus hiking time and travel to and from site. We estimate Phenology sampling requires 2 technicians for 12min per trap x 2 traps = 24 min = 0.5 hour each sampling day plus hiking time and travel to and from site.

*Based on sampling frequency information, list estimated dates that correspond with timing rules or estimated dates when timing rules are fulfilled for each NEON site where this procedure is implemented. Include range of scientifically acceptable sampling timing. If the procedure involves multiple sampling events, include the sampling frequency and timing for each measurement. You may wish to summarize in a table.*

## 10.2 Contingent decisions

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

See Section 6.

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Summarize what-if decisions (how to accommodate site-specific or changing conditions).

*Example: If it starts raining halfway through the microbe sampling, work must stop for the day. 24 hours after the rain stops, work can continue from the previous stopping point (i.e. work does not need to be repeated).*

*Example: If you are unable to begin checking small mammal traps prior to 9am, field technicians should open all traps to release animals and avoid heat-induced mortality. Work should start over that night by resetting the traps.*

### 10.3 Field Procedure

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

Write the procedure as if a PDA were not available. This way, when the asteroid hits the earth and disrupts all electrical equipment, the field personnel can reference the “old school” procedure and collect data. These non-PDA procedures also help CI to define PDA and data ingest requirements, and provide sufficient information for an external reviewer to assess the procedure without the need of a PDA.

#### 10.3.1 Plot Location

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

FSU is responsible for determining plot locations.

*Mosquito Phenology Trap Location:* CO<sub>2</sub> light traps will be set in routinely traveled areas near other Biodiversity Plots that will be / or were previously sampled for Mosquito Diversity. (2 total traps)

*Mosquito Diversity Trap Location:* CO<sub>2</sub> light traps will be placed adjacent to ten Biodiversity Plots (within the destructive sampling buffer zone) (10 traps per site). Two additional CO<sub>2</sub> light traps shall be placed at sites near the aquatic array (2 additional traps per site)

#### 10.3.2 Plot Establishment

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

Field crews are responsible for setting up traps at designated locations (e.g. for mounting posts where no trees exists to hang CO<sub>2</sub> light traps)

*Mosquito Phenology & Diversity:*

All CO<sub>2</sub> light traps need to be suspended ~2 m above the ground. These traps should be hung in trees or sturdy shrubs where possible. Where trees are not available, a T post will need to be secured in the ground. Traps should be shielded from heavy wind, rainfall and direct sunlight. Each CO<sub>2</sub> light trap is equipped with a cylinder cooler filled with CO<sub>2</sub> that attracts mosquitoes to the vicinity of the trap. A

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battery-powered fan pulls the mosquitoes into the catch cup for live storage until the trap is collected by a field technician. Traps are set in the morning (or upon visitation) and then visited the following day as early as possible to collect trapped invertebrates.

#### Trap assembly and setting:

The CO<sub>2</sub> trap consist of the following parts (listed from the top of the assembled trap to the bottom),

- A cylinder cooler (to be stocked with dry ice pellets).
- A plastic rain guard.
- A plexi-glass fan housing.
- A catch cup (made of mesh and plastic).

#### To prepare the trap in the lab:

1. If the trap comes with a solenoid switch it must be removed so that the sensor is responsive to light to control the fan and the incandescent bulb, but no longer attached to the gas flow valve. Efforts have been made to order the correct trap with the solenoid already removed, however:
2. To remove solenoid: Unscrew the two nuts the side on the side of plexi-glass fan housing and on top of the metal housing where the clear tubes from the solenoid connect. Loosen L+, L- on the electronic circuit board. Detach the gray wires from the solenoid.
3. Cover the hole in the bottom of the cylinder cooler with duct tape to reduce the rate of dry ice loss. Fill the cylinder cooler ½ full with dry ice pellets (~1 kg).

#### While in the field:

##### [Figure 1]

1. Screw the rain guard onto the plexi-glass fan housing (red screws).
2. Clip the cylinder cooler onto the rain guard with the brass clasp.
3. Fit the elasticized mesh part of the catch cup over the lip of the fan housing.
4. Attach the battery so that the fan will turn on at dusk. Note the battery leads are color coded: the red-insulated lead goes to the (+) and the black- insulated lead goes to the (-) terminal on the battery. Reversing the polarity of the battery leads will not harm the trap and depending on the positions of the switches, the trap will either not run (all switches open) or run backwards (fan blowing upward) with the light on (all switches closed). The photoswitch on each trap has '1', '2', and '3' all in the closed position. The fan and light both switch on at dusk. The following morning, the light will go off and the fan motor continues, saving the catch in the catch cup.

##### [Figure 2]

5. Place the battery in a large ziplock bag to keep it dry.

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When the trap is set in the tree/post and the battery connected:

1. Remove the duct tape from the bottom of the cylinder cooler. Dry ice should be obviously subliming from the hole.

### 10.3.3 Equipment and Materials

Include all standard and unique equipment and capabilities required to execute the procedures in this document, including:

- A detailed list of materials (e.g. equipment, sampling gear, sample containers, chemical preservatives) used in the field.
- Do not include materials used for separate but related activity; that will be included in the Procedure for that activity (i.e. lab vs. field materials).
- Describe the chemicals being used or as a preservative when samples are immediately returned to the field – exact chemical constituents and strength, and bottle size. Leave a place-marker in the draft if unknown.
- Illustrations of materials (e.g. sampling gear) – all in jpeg format, 3 inches wide
- Can be in bullet point or table format, but be consistent throughout the document.

[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]

**Table 3** Materials and supplies required for the Mosquito Sampling Procedure.

Item Description	Quantity per sampling event	Hazardous Chemical
CO <sub>2</sub> trap locality labels		
Twist ties or safety pins to affix labels to traps		
CO <sub>2</sub> traps (rain guard, cylinder cooler, fan housing)		
Roll of extra string for hanging traps		
6V sealed gelled-electrolyte batteries (12, 10 for active field work, two for replacements)		
T posts (if no trees to hang traps)		
Shovel to dig T post		
CO <sub>2</sub> pellets		
Catch cups		
Large chest cooler with frozen ice packs (to be left in field vehicle; for catch cup transport)		
medium chest cooler dry ice sufficient for day of sampling (~15-20 kg; must include more due to loss)		
Ice packs		
Small cylinder coolers (12, one for each trap,		

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plus two replacements)		
Replacement catch cups		
Sharpies		
Archival, ethanol-safe pens (Pigma brand, size 01)		
Scissors		
Forceps		
Bin to keep some materials at plot		
Carry-all craft tote bag or other field pack		
Large ziplock bags		
Latex gloves		
Mosquito protective clothing		
knife or Leatherman (Optional)		
Handwipes		
Duct tape		
Flagging		
Bug spray		
Sun block		
First-aid kit		
Camera		
GPS/Trimble		
PDA		
Datasheets		

**10.3.4 Preparation**

**10.3.4.1 Plot Establishment**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

Field crews are responsible for setting up traps at designated locations (e.g. for mounting posts where no trees exists to hang CO2 light traps)

*Mosquito Phenology & Diversity:*

All CO<sub>2</sub> light traps need to be suspended ~2 m above the ground. These traps should be hung in trees or sturdy shrubs where possible. Where trees are not available, a T post will need to be secured in the ground. Traps should be shielded from heavy wind, rainfall and direct sunlight. Each CO<sub>2</sub> light trap is equipped with a cylinder cooler filled with CO<sub>2</sub> that attracts mosquitoes to the vicinity of the trap. A battery-powered fan pulls the mosquitoes into the catch cup for live storage until the trap is collected by a field technician. Traps are set in the morning (or upon visitation) and then visited the following day as early as possible to collect trapped invertebrates.

Trap assembly and setting:

The CO<sub>2</sub> trap consist of the following parts (listed from the top of the assembled trap to the bottom),

- A cylinder cooler (to be stocked with dry ice pellets).

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- A plastic rain guard.
- A plexi-glass fan housing.
- A catch cup (made of mesh and plastic).

To prepare the trap in the lab:

4. If the trap comes with a solenoid switch it must be removed so that the sensor is responsive to light to control the fan and the incandescent bulb, but no longer attached to the gas flow valve. Efforts have been made to order the correct trap with the solenoid already removed, however:
5. To remove solenoid: Unscrew the two nuts the side on the side of plexi-glass fan housing and on top of the metal housing where the clear tubes from the solenoid connect. Loosen L+, L- on the electronic circuit board. Detach the gray wires from the solenoid.
6. Cover the hole in the bottom of the cylinder cooler with duct tape to reduce the rate of dry ice loss. Fill the cylinder cooler ½ full with dry ice pellets (~1 kg).

While in the field:

[Figure 1]

6. Screw the rain guard onto the plexi-glass fan housing (red screws).
7. Clip the cylinder cooler onto the rain guard with the brass clasp.
8. Fit the elasticized mesh part of the catch cup over the lip of the fan housing.
9. Attach the battery so that the fan will turn on at dusk. Note the battery leads are color coded: the red-insulated lead goes to the (+) and the black- insulated lead goes to the (-) terminal on the battery. Reversing the polarity of the battery leads will not harm the trap and depending on the positions of the switches, the trap will either not run (all switches open) or run backwards (fan blowing upward) with the light on (all switches closed). The photoswitch on each trap has '1', '2', and '3' all in the closed position. The fan and light both switch on at dusk. The following morning, the light will go off and the fan motor continues, saving the catch in the catch cup.

[Figure 2]

10. Place the battery in a large ziplock bag to keep it dry.

When the trap is set in the tree/post and the battery connected:

2. Remove the duct tape from the bottom of the cylinder cooler. Dry ice should be obviously subliming from the hole.
  - Describe all activities that must occur prior to arrival in the field, for example equipment calibration or preparation.
  - A detailed list of tasks, using the numbering format shown below. Be consistent.

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- Break each step down into actionable steps
- Described and list the tasks in chronological order
- If pre-field tasks occur over multiple days, break Section 7.1 down further (e.g.:
  - One week prior to sample collection, do this
  - Two days prior to sample collection, do this
- Be explicit and use language geared toward 3<sup>rd</sup> yr undergraduate student. Assume the user has no previous knowledge of the activity.
- Include a description of what sampling gear, equipment, etc. they need to verify are in working order and properly packaged for the field day.
- Define safe practices (e.g. use gloves to mix chemicals in the fume hood), where necessary at each step. Do not assume this information is written elsewhere – include all relevant safety procedures in every document. Each document should be written as a stand-alone document.
- Illustrations or photographs of tasks that are complex or would benefit from an illustration – jpeg format, 3” wide.
- 

#### 10.3.4.2 Weekly Sampling Preparation

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

All necessary field equipment will be assembled at least one day prior to field sampling to ensure spreadsheets are uploaded or printed and that all consumables are available. PDA must be loaded with correct map and database information. Laboratory space and appropriate consumables must be available for use within 24h of sample collection.

The Mosquito Diversity Field Crew is responsible for transferring the appropriate files to the handheld PDA before field sampling and / or printing out appropriate datasheets. These files will include mapping tools to find the Biodiversity Plots and fields for entering GPS waypoints, metadata, and comments during sample collection.

The following must be prepared prior to field work:

1. Bench space (2.4 m) to spread out trap components and check all required parts.
2. Storage for dry ice – delivered before field sampling for trapping (dry ice or CO<sub>2</sub>pellet freezer).
3. Cabinet space for trap storage.
4. Space to store 12 batteries (12, 6 V – 10 for active field work and two for backup) with overflow secondary containment bins.
5. Access to outlets to charge 12, 6 V batteries with overflow secondary containment.

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6. Clear freezer space.

### 10.3.5 Sample Collection in the Field

- A detailed list of tasks:
  - Break each step down
  - Describe and list the tasks in chronological order
  - List decision criteria used to implement procedure based on plot/sampling location conditions (i.e. sampling plot composition, stream width, etc.).
  - If field tasks occur over multiple days or are complex, break this section down further (as shown in previous Section)
  - Be explicit and use language geared toward 3<sup>rd</sup> yr undergraduate student
  - Include detailed instructions on assembly of sampling gear, if gear is assembled in the field. If gear is assembled/disassembled in the Domain Office (e.g. not in the field), include that detail in the Pre- or Post-Field Task sections
  - Include safety issues and practices
  - Illustrations or photographs of tasks that are complex or would benefit from an illustration
    - jpeg format, 3” wide.

#### 10.3.5.1 Trap Collection

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

Upon arrival at the trap:

1. Attach three copies of locality labels (as a long strip, date filled in) to the catch cup using a twist tie or safety pin (see section generating labels). This procedure is vital to reducing error. [Figure 3]
2. Do not switch off the fan while removing the catch cup. The fan running ensures that the mosquitoes cannot escape from the catch cup as it is being removed.
3. Tie off the string around the mesh part of the catch cup, ensuring that all mosquitoes are clear of the mesh that is being tied into the knot.
4. Place catch cup into chest cooler (with ice packs).
5. Record in the ‘NEON Mosquito Phenology & Diversity Datasheet’ the number of mosquitoes in the trap using the scale 0/1/2/3 (numbers increasing with catch size) and any other relevant information (e.g. trap damaged by wind).

If a second night of sampling is occurring:

1. Refill the hanging cylinder cooler about ½ full with dry ice (~1 kg).
2. Attach a fresh (empty) catch cup to the trap.
3. Pull the battery leads off and then reattach them. This resets the trap and will ensure that the

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fan is off until dusk (reducing battery draw).

- Place the battery in a large ziplock bag to keep it dry.

If sampling is complete:

- Bring in batteries and all trap components to the lab

### 10.3.6 Sample Preservation

- Detail the task in order from start to finish*
- Include how long (time/days/hrs/indefinitely) sample can be preserved in this mode.*
- Only include activities that relate to the Field Task.*
- If a sample is processed at the Domain Lab, that work is written in the next Section or as a separate document if complex.*

#### 10.3.6.1 Sample transport and Storage Prior to Processing

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

- Catch cups should be placed with the mesh tucked gently into them in chest coolers (with ice packs).  
[Figure 4]
- Do not expose mosquitoes to direct sunlight or extreme temperatures (e.g., enclosed in vehicle for prolonged periods).
- The mosquitoes must be fresh, cool, and promptly handled. After several hours at warm temperatures the captured mosquitoes can dehydrate rendering their morphological identification impossible.

### 10.3.7 Sample Shipping (may not be applicable for all Field SOPs)

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

- Provide detail on shipping specifics (e.g. wrap the sample containers in a plastic bag, seal the top. Place containers upright in cooler labeled "Elephant Samples", include four reusable ice packs. Seal the container.*
- In addition to the shipping label, the following hazmat labels are required:*
- Check with EHS for label requirements*

### 10.3.8 Data Handling (may not applicable for all Field SOPs)

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

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*Briefly explain data upload steps (e.g. enter data into excel file name "XXXXX", plug in PDA, etc., etc.).*

Field data collected should be carefully entered into the 'NEON Mosquito Diversity and Phenology Datasheet' on the P drive within as short a time as possible from the field (preferable each day, required by the end of the week).

### 10.3.9 Refreshing the Field Sampling Kit

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

- *Provide detail on how to restock the sampling kit with non-perishable items. Best practice is to restock the sampling kit after the sample event, with a check at the start of each sample event that the kit is appropriately stocked*
- *Reference the materials list, above*
- *Be explicit and ensure information does not overlap or refute early info (ex. If preservatives used in the field have to remain cold, then the 'refreshment' of the preservative is a detailed in Section 7.2 and not here. )*

### 10.3.10 Equipment Maintenance, Cleaning, and Storage

#### 10.3.10.1 Battery handling

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

The batteries used are 6 V sealed lead acid. They pose little risk, but proper handling should be followed. Batteries must have their own charging area where they are situated in secondary containment bins. [Figure 5]

#### 10.3.10.2 Battery charging

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

CARE SHOULD BE EXERCISED WHEN CHARGING A DEPLETED BATTERY as chargers become HOT and the charger case can cause burns. Chargers should not be touched until after the GREEN light comes on. Warning signs should be placed around the batteries while charging.

The sequence of connections is very important for the charger's computer.

1. *Connecting the charger:*
  - a. Connect the color-coded leads to the battery.
  - b. Plug the charger into the AC outlet. If the battery is mistakenly connected backwards (negative to positive) or charger leads are shorted together, no damage or sparks occur—only a RED light comes on to indicate the possible mistake.
2. *Initial:*

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- a. When the charger is initially plugged in, the unit's computer sends intermittently, a small current into the battery and then monitors the battery's response to determine its charge state. A RED light may come on briefly if the battery was excessively discharged, but it should go off within the first few minutes.
3. *Bulk charge mode:*
  - a. When the charger determines that the battery can safely take a charge, it goes into the bulk mode where the full capacity of the charger is applied to the battery until it is 80% charged. In this mode, the YELLOW light comes on.
4. *Absorption charge mode:*
  - a. Once the battery is 80% charged, the computer within the charger tapers the current from 100% to 33% of charger capacity until the battery reaches full charge. The light will remain YELLOW during this phase.
5. *Float charge:*
  - a. After full charge has been reached, a GREEN light comes on, indicating the battery is ready for use. The unit is now in its maintenance mode, keeping the battery in its ideal charged state. Once the battery has reached this ideal state, the charger maintains this condition. 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.

*Estimating Battery run time:*

Maximum run time for a trap may be calculated by taking 75% of the battery's AmpHr rating divided by the traps current consumption. Note: as the battery ages and undergoes charge/discharge cycles, its AmpHr capacity when fully charged declines until finally the battery must be replaced. Cool storage prolongs battery life. The trapping nights during the summer are typically 8-9 h.

CO<sub>2</sub> light trap use:

$0.75 * 10 \text{ AmpHr} / 0.25 \text{ Amps per h consumption by the trap (fan and light bulb running together)} = 30 \text{ h total run time}$

*Estimating Battery charge time:*

CO<sub>2</sub> light trap use for 10 h of run time:

$10 \text{ h} * 0.25 \text{ Amps/hour} = 2.5 \text{ Amps}$

2.5 Amps drawn would take about 2 hours to recharge.

1.25 Amps drawn would take about 1 hour to recharge such a battery.

*Battery recovery after deep discharge:*

A battery that has been over-discharged requires a longer charging period than normal. PLEASE TAKE CARE TO NOT DEEP DISCHARGE THE BATTERIES. Often these batteries cannot be recharged and are not covered by the warranty. During the initial stage of charging an over-discharged battery the charger may give a false "full charge" indication, or may initiate charge at a float voltage. Quite often, once a battery has been over-discharged it cannot be recharged and must be replaced.

**11 LAB STANDARD OPERATING PROCEDURE**

*Write the lab procedures as if a PDA were not available. Use the same sections as the field protocol, above.*

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Separate the lab procedures into multiple sections and add on to the above title. For example:  
 11 Lab Standard Operating Procedure - Plant identification and drying  
 12 Lab Standard Operating Procedure - Plant mounting

**11.1 Timing**

[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]

This sample processing must be done within 24 h of collection of samples from the field. All of the samples from the week must be shipped to the Taxonomist by the end of the week.  
 Provide details on preferred timing of sample processing at the domain labs AND the maximum time between field collection and lab processing. If the procedure involves multiple sampling events, include the sampling frequency and timing for each measurement. You may wish to summarize in a table.

**11.2 Lab Procedure-Sorting**

**11.2.1 Equipment and Materials**

[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]

**Table 4** Materials and supplies required for the Mosquito Lab Procedure.

Item Description	Quantity per sampling event	Hazardous Chemical
Color Separation Guide and Gray Scale		
CO <sub>2</sub> freezer		
Facial tissues (cut in half)		
Scissors		
Sharpie marker		
Ice packs		
Paper plates		
Aluminum foil		
Chill table		
Plain white paper		
Small paint brush		
Ethanol		
Archival, ethanol-safe pens (Pigma brand, size 01)		
No. 3 pins archival quality		
50 mL nonsterile centrifuge tubes		
25 dram plastic tubes		
Schmitt boxes		
Point punch		

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Pinning block (anodized; height is better than wood)		
Fumigant (moth crystal) boxes		
Archival, ethanol-safe pinned specimen label paper		
Point paper (Strathmore archival quality bristol board)		
Jewelers forceps		
96 well microwell plates		
96 well microwell caps		
Hardware/software (i.e. Clemex Captiva)		
Microscope		
Canon Powershot camera		
Fumigant (paradichlorobenzene - moth crystals)		
Packing tape		
Shipping supplies (boxes, padded envelopes)		
Cardboard specimen mailers		
Self healing cut mat (30 cm x 46 cm)		
Scissors		
Exacto knife		
Metal ruler		
6V two at a time battery re-charger		
Unit trays for inside Cornell drawers (48 per cabinet)		
Cornell drawer(pine - hardboard bottom)		
Cornell cabinet (6 drawers, unassembled)		
Balsa wood for point preparation surface		
Secondary containment bins for charging batteries		
Latex gloves		
DNA barcode unique numbers		
Adhesive shipping labels		
Plain white paper (for pointing specimens under microscope)		
Tissue paper for photo box background		
LED lights (microscope, photos)		
Elmer's white glue		
Ethanol lamp (sterilizing)		
Lighter		
Drop down netting		

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## 11.2.2 Preparation

### 11.2.2.1 Generating Sample Labels

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

Six different kinds of sample labels are required. Each is dealt with in turn below.

1. **FIELD LOCALITY LABELS:** Locality labels for samples should be created before the field campaign begins in order to expedite processing and reduce opportunities for error.

Laboratory technicians are responsible for generating sample labels on archival, ethanol-safe paper. A template file will be provided from FSU for locality labels to be adjusted following the procedure below.

Locality labels include particular information in order to be consistent with collection records from other global campaigns.

- State
- County
- Town
- Elevation (m)
- GPS coordinates N and W with four degrees reported in WGS84
- trap type (e.g. pitfall or CO2)
- space left for the day, the month and the year are printed (e.g. \_\_\_Aug2008)
- collectors (e.g. KKBlevins)
- a collection code which is comprised of the following parts: NEON.FSU.Domain.Plot.Trap

To prepare labels:

- a. Cut labels into long columns using an exacto knife and ruler.
- b. Do this with as little white space on either side of the text as possible.
- c. These cuts result in many labels in a vertical strip.

[Figure 6]

- d. Store labels this way, and then when needed, cut individual labels across the row with scissors.

[Figure 7]

[Figure 8]

- e. Add date numbers with archival, ethanol-safe ink as needed. This is a key step, as this particular information and kind of ink is required. Prompts are found throughout the protocols for each time this is required.

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2. **LAB LOCALITY LABELS:** Storage containers for long term sample archive in voucher collections (e.g., 50 mL centrifuge tubes) will require locality labels inserted into them in triplicate (three individually cut labels).
3. **DNA BARCODE UNIQUE NUMBER LABELS:** A file will be provided from FSU with the reserved unique numbers for specimens that are to be DNA barcoded. These numbers are to be applied to pins below locality labels on each specimen to be DNA barcoded.
4. **TEMPORARY LABELS:** Consumables that only require temporary labels (e.g., waste ethanol cups, cardboard mailers) will be marked with laboratory tape and sharpie makers.
5. **MICROPLATE LABELS:** Ninety-six well microplates will have 'NEON#' written in sharpie on multiple sides of the plate. These numbers are in series and are to be designated by FSU.
6. **SHIPPING LABELS:** Shipping labels with the relevant details for addressor (Domain contact) and addressee (the external facility) should be pre-made to expedite shipping throughout the field season.

### 11.2.3 Sample Processing in the Lab

#### 11.2.4

##### 11.2.4.1 Establishing Workspace: Return from the Field

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

1. Space in -20°C freezer for killing collected invertebrates.
2. Clear 2.4 m lab bench for sorting purposes.
3. Drop down netting around lab bench to enclose space.

[Figure 9]

##### 11.2.4.2 Euthanizing invertebrates & sorting mosquitoes from bycatch

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

1. Put catch cups in freezer immediately upon arrival at lab.
2. Leave in freezer for a minimum of 30 min (to kill all invertebrates).
3. Place frozen invertebrates on a chilled surface. This can be either a foil-covered paper plate on a foil-covered ice pack or a chill table. In either case, use a piece of plain white paper on the chilled surface to assist in transferring samples into tubes.

[Figure 10]

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4. Drop down netting around work area. Carefully remove sample from a single trap (after ensuring all invertebrates are dead) onto chilled paper. Separate mosquitoes from other invertebrate bycatch by using a small paintbrush and/or forceps (being careful not to break off legs or other identifying parts).

#### 11.2.4.3 Establishing Workspace: Lab work to be completed within days of collection

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

1. Full lab bench space (4.6 m or equivalent) in clean lab with 2 microscopes (1 dissecting and compound) connected to computer and room for one additional dissection microscope.
2. Lab bench space (2.4 m or equivalent) in clean lab for preparation of pinning materials.
3. Clean lab bench space (no dust, wild air movement) for putting tissue samples into wells for genetic analysis (to be sent to external facility).
4. Access to sink for rinsing equipment.
5. Storage space in clean lab for pinning materials.
6. Storage space for pinned mosquitoes.
7. Cold storage space for preserved specimens from Taxonomists in 25 dram tubes.
8. Storage space for shipping materials.

#### 11.2.4.4 To distinguish mosquitoes from other similar-looking insects note these features

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

1. Antennae with 6 or more segments.
2. Male mosquito antennae are plumose (fluffy).
3. Female antennae are short-haired and appear like small wires.
4. Mosquitoes' wings have scales along the veins and the margins.
5. Mosquito mouthparts are long and tubular.

[Figure 11]

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#### 11.2.4.5 For each bycatch & mosquito sample

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

1. Record in the 'NEON Mosquito Phenology & Diversity Datasheet' the size of the catch using the scale 0/1/2/3 (numbers increasing with catch size) and any additional notes (e.g. 0 specimens because a battery failed).  
[Figure 12]
2. Record in the 'NEON Mosquito Phenology & Diversity Datasheet' an estimate of number of blood fed mosquitoes in the sample (if any).
3. Pad the bottom of a 50 mL tube with half of a piece of facial tissue.
4. Place mosquitoes in the tube.
5. Pad the top of the 50 mL tube with another half piece of facial tissue (use extra tubes for large samples).
6. Place three copies of the locality label (date filled in) on top of the topmost facial tissue.  
[Figure 13]
7. Double check that the sample has correct locality label.
8. Follow the above steps for the invertebrate bycatch also.
9. Label the lid of each tube with a sharpie as either 'mosq' for mosquitoes, or 'byc' for bycatch and the collection code and date.
10. Secure lid.

Mosquitoes will be sent to Taxonomists for identification (see Taxonomists section for details on shipping)

#### 11.2.1 Data Handling

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

At the end of each field and lab day, all information from field data sheets must be typed up and saved to the NEON server as directed by the Field Operations Manager.

#### 11.2.2 Sample Shipping

##### 11.2.2.1 Establishing Workspace: Shipping & Receiving

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

1. Storage space for shipping materials (50 mL centrifuge tubes, cardboard mailers, cardboard

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boxes, packing tape, etc.).

2. Space for preparing packages for shipping.

### 11.2.2.2 Taxonomists

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

Sorted mosquito specimens are shipped overnight to expert Taxonomists with a hard and digital copy of the 'NEON Mosquito Phenology & Diversity Datasheet' at the end of the sampling week.

1. Place 50 mL mosquito catch tubes in ziplock bags.
2. Label ziplock bags with the collection codes and date in sharpie.
3. Put ziplock bags in secure location (preferably refrigerated at 4°C) to be packed and shipped with all sampling bouts from the week.
4. Pack ziplock bags in cardboard boxes with ample packing material to ensure specimens are not jostled. These boxes should be clearly labeled with 'Fragile' and 'Dead Insects for Scientific Study'.

[Figure 24]

### 11.2.2.3 Genetic Analytical Laboratories

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

1. Ship 96 well microplates dry, overnight at room temperature (20°C) to external facilities along with a hard and digital copy of the 'NEON Mosquito Phenology & Diversity Datasheet'.

[Figure 25]

### 11.2.2.4 Archives

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

1. Archives are voucher collections of a variety of materials that represent many different kinds of storage (e.g. dry in cabinets, -80°C, specimens in ethanol). Each of these differing kinds of facilities should receive a hard and digital copy of the 'NEON Mosquito Phenology & Diversity Datasheet'.
2. Ship prepared dry material in cardboard mailers packed in outer boxes with ample packing material to ensure that the specimens are not jostled. These outer boxes should be clearly labeled with 'Fragile' and 'Dead Insects for Scientific Study'. Ship Genomic DNA extractions overnight to cryogenic facilities to be stored at -80°C. These facilities should aliquot and send sample location and storage parameter data to NEON. This information should be entered into the 'NEON Mosquito Phenology & Diversity Datasheet'.

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### 11.2.3 Sample Preservation

[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]

1. Specimens that arrive back from the Taxonomist in 25 dram plastic cap tubes are to be optimally stored at -20°C.
2. Failing this, they may be stored at 4°C.

[Figure 23]

### 11.2.4 Laboratory Maintenance, Cleaning, Storage

[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]

1. Wash out CO<sub>2</sub> catch cups and leave on lab bench to dry on paper towels.
2. Make sure all catch bags are clean and free of insect parts.
3. If the mesh of the cups becomes stained, wash them by hand with unperfumed soap to avoid odour contamination.
4. The residue from cleaning agents may have a repelling effect on the mosquitoes and consequently reduce the catch rate.
5. If the catch bag is damaged or torn it should be replaced, as the mosquitoes are able to escape.
6. All materials should be put away in clearly marked receptacles or cabinets after each bout of laboratory work.
7. All flammables must be returned to the appropriate cabinets.

## 11.3 Lab Procedure-Processing

### 11.3.1 Equipment and Materials

[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]

**Table 4** Materials and supplies required for the Mosquito Lab Procedure.

Item Description	Quantity per sampling event	Hazardous Chemical
Color Separation Guide and Gray Scale		
CO <sub>2</sub> freezer		
Facial tissues (cut in half)		
Scissors		
Sharpie marker		
Ice packs		
Paper plates		
Aluminum foil		
Chill table		
Plain white paper		
Small paint brush		

Ethanol		
Archival, ethanol-safe pens (Pigma brand, size 01)		
No. 3 pins archival quality		
50 mL nonsterile centrifuge tubes		
25 dram plastic tubes		
Schmitt boxes		
Point punch		
Pinning block (anodized; height is better than wood)		
Fumigant (moth crystal) boxes		
Archival, ethanol-safe pinned specimen label paper		
Point paper (Strathmore archival quality bristol board)		
Jewelers forceps		
96 well microwell plates		
96 well microwell caps		
Hardware/software (i.e. Clemex Captiva)		
Microscope		
Canon Powershot camera		
Fumigant (paradichlorobenzene - moth crystals)		
Packing tape		
Shipping supplies (boxes, padded envelopes)		
Cardboard specimen mailers		
Self healing cut mat (30 cm x 46 cm)		
Scissors		
Exacto knife		
Metal ruler		
6V two at a time battery re-charger		
Unit trays for inside Cornell drawers (48 per cabinet)		
Cornell drawer(pine - hardboard bottom)		
Cornell cabinet (6 drawers, unassembled)		
Balsa wood for point preparation surface		
Secondary containment bins for charging batteries		
Latex gloves		
DNA barcode unique numbers		
Adhesive shipping labels		
Plain white paper (for pointing specimens under microscope)		
Tissue paper for photo box background		
LED lights (microscope, photos)		

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Elmer’s white glue		
Ethanol lamp (sterilizing)		
Lighter		
Drop down netting		

**11.3.2 Preparation**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

Prior to pointing:

1. Prepare a batch of triangles using the point punch over a balsa wood (or other malleable) surface.  
[Figure 14]
2. Press No. 3 archival quality pins into the points equidistant from the three sides at the back of the triangle base.  
[Figure 15]
3. Push the point up the pin until it is 15 mm from the top of the pin.  
[Figure 16]
4. Check the height using the highest hole on a pinning block (for ease of using the collection at a later date).

**11.3.3 Sample Processing in the Lab**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

1. Twenty individuals of each species (representing a range of plots and dates) should be pointed upon return from the Taxonomist.
2. Pointing is probably the most widely used method of mounting small dried insects.
3. This method consists of attaching the specimen with adhesive (Elmer’s white glue) to the tip of a small triangle (archival quality cardstock) (known as a ‘point’).
4. Points are transfixed by a No. 3 entomological archival pins (number refers to thickness of pin).
5. Points are cut with a special, standardized triangle-shaped punch.
6. A good example video of pinning and pointing specimens resides at: <http://www.nhm.ac.uk/nature-online/collections-at-the-museum/making-part-collection/making-pinning/index.html>

NEON shall follow the convention of the Walter Reed Biosystematics Unit. This results in mosquitoes prepared with their heads facing left and the legs on the point toward the pin. This can protect the legs from falling off. Pointing is best done under the microscope.

[Figure 17]

**11.3.3.1 Pointing mosquitoes**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

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To mount a specimen on a point:

1. Using forceps, put the mosquito or mosquitoes (it may be easier to do them in batches) on their right side on a slip of plain white paper so that their heads are facing the left and their legs are toward the edge of the bench.
2. Slide the slip of paper with the mosquitoes to the center of the microscope stage.
3. Take the prepared point and touch the tip of the point in a bit of glue. (Use the minimum amount of glue that will attach the specimen firmly to the point).
4. Lift and angle so that the weight of the specimen is leaning directly into the point.
5. Place the pin in a block of Styrofoam in a manner that ensures that gravity is holding the specimen to the point.
6. Adjust the specimen with forceps or a pin. Adjusting the specimen should cause it to bind firmly to the point. Specimen should be straightened as the glue thickens but before it completely hardens.

Finally, the locality label can protect the specimen. Pointed specimens bisect the type.

[Figure 18]

When all of the specimens are mounted:

1. Examine the specimens under the microscope to see that they are all attached securely to the points.
2. To test, place the specimens in a Schmitt box or unit tray.
3. Tap the box or tray.

The specimens should remain fixed. A specimen dissociated from its locality label is worthless. Re-attach any specimens (as long as it is clear which is its/their locality labels).

### **11.3.3.2 Removing a sub-sample for DNA barcoding**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

After all of the mosquitoes have been mounted and properly prepared:

1. Prepare the 'NEON Mosquito Phenology & Diversity Datasheet' and 96 well microplates so they are within easy reach.
2. Apply a unique DNA barcode number to each specimen.

The leg priority for removal for DNA barcoding is as follows: insect's right side midleg, first priority (1), if missing or if opposite leg missing, then foreleg right side, second priority (2), if missing or if opposite leg

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missing, then right hindleg (3), if missing or opposite leg missing, left midleg (4), left foreleg (5), left hindleg (6).

1. Excise a leg sample from the first specimen.
2. Place the tissue sample in the well.  
[Figure 19]  
[Figure 20]
3. Record the unique number in the 'NEON Mosquito Phenology & Diversity Datasheet'.

DO NOT place any foreign objects (e.g. labels) into sampling wells. Before proceeding to the next sample, ensure that no residual tissue is present on the forceps by rinsing them in 95% ethanol and then flame sterilizing them with an ethanol lamp.

### 11.3.3.3 Digital Photography

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

One photograph of each specimen to be sent for DNA barcoding shall be archived to maintain visual records. This also creates a database of images to use for training. These photos will be taken with a standard Color Separation Guide and Gray Scale, measurement scale and standard camera. These specimens should also be measured and their length recorded (mm) in the 'NEON Mosquito Phenology & Diversity Datasheet'.

Photograph shall be taken with the microscope camera (Leica DM1000 microscope, Clemex Captiva 5.0 camera software).

1. Pin the mosquito to a small (approximately 3.5 x 5 cm) Styrofoam block covered with green tissue.
2. Pin a second copy of the specimen's unique number label next to the specimen at a height that it will be easily viewed in the photograph.
3. Place the 'Color Separation Guide and Gray Scale' near the specimen.
4. Orient the specimen under the microscope to get a lateral shot with the head of the specimen facing to the left.  
[Figure 21]
5. Set the magnification as high as possible while still being able to view the whole specimen, its unique number, and the 'Color Separation Guide and Gray Scale'.
6. Adjust focus to the highest point at which part of the specimen is still in focus.
7. Using the camera software, capture a multi-layer image.
8. Prioritize the rear leg and proboscis in the image.

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9. Choose the 'Capture Multi-Layer' option in the 'Advanced' menu.
10. Manually focus through the entire specimen using the fine focus knob on the microscope.
11. Change focus as little as possible while still capturing the full depth range of the entire specimen. A progress bar appears in the lower left-hand corner of the image window when the 'Capture Multi-Layer' is initiated.
12. Manually click 'Done' once finished focusing through the specimen. This is recommended as the software shuts down if left idle for too long.

After the picture is taken but before it is saved:

1. Place a digital image of a scale bar on the bottom right of the image, parallel to long axis of the mosquito as close as possible without obscuring it.
2. Activate the 'Scale Line' icon in the task bar of the Image window.
3. Change the units used on the scale bar in the 'Options' menu under 'Preferences'.
4. Use millimeters.
5. Change the number of units that appear on the scale bar by adjusting the number in the 'Scale Line Object Attributes' window that appears when the scale bar is clicked on.
6. Change the number of units that appear on the scale bar to 5.

[Figure 22]

Saving the Image:

1. Open a viewer in the Clemex window.
2. Click and drag the image into this viewer. This is the only way to save the image with the scale bar.
3. Save the image in viewer window.
4. Click on the save icon and save the image.
5. Save images to the P drive at P:\FSU\Invertebrates\CPER 2011\Mosquito Pictures\unedited. The raw file is named 'culicid' and the unique number of the specimen and then the letter 'r' to indicate it is the raw file (e.g. The uniquely numbered specimen '888', the file name is culicid888r.jpg). Raw files can be accumulated and periodically processed. This and all subsequent file paths should be entered into the 'NEON Mosquito Phenology & Diversity Datasheet'.

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### 11.3.3.4 Processing images

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

After the raw image has been saved it must be processed. When editing groups of pictures, copy the original raw files of the group of pictures to be edited and paste them to the edited pictures folder at P:\FSU\Invertebrates\CPER 2011\Mosquito Pictures\edited. Make sure to complete all of the steps below or delete any files that have not been edited from the folder before leaving or changing to a new task.

Batch renaming files, generate a list of current file names in 'edited' folder:

1. Open command prompt: Open Run & type in **cmd**.
2. Navigate to the P drive within the command prompt: Prompt will show which drive is in use (e.g. if in H drive will say H:\>). To change to a different drive, type the name of that drive (e.g. **P:**) and hit enter.
3. Navigate to desired folder.
4. Type in the command **cd** followed by a space and then the complete file path to the folder containing the pictures to be compressed. (e.g. P:\>**cd FSU\Invertebrates\CPER 2011\Mosquito Pictures\edited**). Note: to copy and paste into command prompt, short cuts will not work (e.g. Ctrl+V); right click must be used.
5. Use the command prompt to generate a list of the file names in the compressed pictures folder: type in the command **dir /b \*.jpg>list.txt** - this will create a text file with a list of the file names in the folder. (e.g. P:\>FSU\Invertebrates\CPER 2011\Mosquito Pictures\edited>**dir /b \*.jpg>list.txt**). Note: spaces are required after dir and b in the command.
6. The newly formed text file (list.txt) will be located in the edited pictures folder.

Create an excel spreadsheet to generate commands for new file names:

1. Create an excel spreadsheet and save it to the 'edited' folder under the name 'rename'. Label Column A "Original," Column B "New," and Column C "Command."
2. Copy the list of file names from the list.txt file into Column A.
3. In Column B fill in the new file names, which are made different from the old file names by replacing the r with an e (e.g. culicid2100r.jpg becomes culicid2100e.jpg). The easiest way to do this is to copy the original file names into the second column, highlight the second column, choose replace and replace r.jpg with e.jpg.
4. In Column C, paste this formula into C2: **=CONCATENATE("ren ",A2," ",B2)**. Fill this formula in for the remaining rows, it should automatically fill the correct values, but check to make sure the formula is filling correctly (A3,B3, A4,B4, etc.)
5. Select and copy the cells containing the commands that are desired (i.e. C2:C...)

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6. Right click in the command prompt, make certain that the correct folder is being used, and paste the commands in and then hit enter. The files should all be renamed
7. Open the pictures in Microsoft Office Picture Manager
8. Go to edit pictures and crop
9. Fix aspect ratio to '4x6'
10. Crop out empty space so the images include just the mosquito, the scale bar, color scale and number
11. edit all of the pictures and then choose File>Save All when they are finished

#### Compressing Pictures:

When compressing a group of edited pictures, copy the edited files of the group of pictures to compress and paste them to the compressed pictures folder at P:\FSU\Invertebrates\CPER 2011\Mosquito Pictures\compressed. Make sure to complete all of the steps below or delete any files that have not been compressed from the folder before leaving or changing to a new task.

To batch rename files, generate a list of current file names in 'compressed' folder:

1. Open command prompt: Open Run & type in **cmd**.
2. Navigate to the P drive within the command prompt: Prompt will show which drive currently in use (e.g. if in H drive will say H:\>). To change to a different drive, type the name of that drive (e.g. **P:**) and hit enter.
3. Navigate to desired folder: Type in the command **cd** followed by a space and then the complete file path to the folder containing the pictures to be compressed. (e.g. P:\>**cd FSU\Invertebrates\CPER 2011\Mosquito Pictures\compressed**). Note: to copy and paste into command prompt, short cuts will not work (e.g. Ctrl+V); right click must be used
4. Use the command prompt to generate a list of the file names in the compressed pictures folder: type in the command **dir /b \*.jpg>list.txt** - this will create a text file with a list of the file names in the folder. (e.g. P:\>FSU\Invertebrates\CPER 2011\Mosquito Pictures\compressed>**dir /b \*.jpg>list.txt**). Note: spaces are required after dir and b in the command.
5. The newly formed text file (list.txt) will be located in the compressed pictures folder.

Create an excel spreadsheet to generate commands for new file names:

1. Create an excel spreadsheet and save it to the 'compressed' folder under the name 'rename'. Label Column A "Original," Column B "New," and Column C "Command."
2. Copy the list of file names from the list.txt file into Column A.
3. In Column B, fill in the new file names, which are made different from the old file names by replacing the e at the end of the file name to c to the end (e.g. culicid2100e.jpg becomes culicid2100c.jpg). Copy the original file names into the second column, highlight the second column, choose replace and replace e.jpg with c.jpg.
4. In Column C, paste this formula into C2: **=CONCATENATE("ren ",A2," ",B2)**. Fill this formula in for the remaining rows, it should automatically fill the correct values
5. Check to make sure the formula is filling correctly (A3,B3, A4,B4, etc.)

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6. Select and copy the cells containing the commands that are desired (i.e. C2:C...)
7. Right click in the command prompt, making sure you are still operating in the correct folder, and paste the commands in and then hit enter. The files should all be renamed
8. Open the newly renamed pictures in Microsoft Office Picture Manager
9. Select all the pictures by going to the multiple picture view, clicking on the first one, then holding down shift and clicking on the last one. They should all be highlighted when selected
10. Go to edit pictures and then Compress Pictures
11. Under Compress Pictures, choose Web Pages, then click OK. Once compressed, the thumbnails will have an asterisk in front of the file name
12. Go to File & Click Save All. All the pictures should now be compressed. To check, look at the file sizes, they should all be less than 100 KB

#### 11.3.4 Sample Preservation

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

Prepared, pointed, mosquito specimens are to be stored in airtight Schmitt boxes or in unit trays in Cornell cabinet drawers with a small amount of moth crystals (~6.5 g) in a cardboard fumigant box. The fumigant box must be firmly secured in the corner of the Schmitt box or unit tray with pins.

[Figure 23]

#### 11.3.5 Data Handling

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

All data and files from the image processing must be backed up and loaded to the NEON server.

#### 11.3.6 Refreshing the Laboratory Supplies

#### 11.3.7 Laboratory Maintenance, Cleaning, Storage

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

1. All materials should be put away in clearly marked receptacles or cabinets after each bout of laboratory work.
2. All flammables must be returned to the appropriate cabinets.
3. Pinned material should be placed in the appropriate Schmitt box or Cornell cabinet.
4. Temporary waste holding containers must be emptied into proper (clearly labeled; no abbreviations) receptacles.
5. Pack equipment and consumables for subsequent field work and store neatly.

## 12 DEFINITIONS

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

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*Define all protocol specific technical terms in alphabetical format.*

**13 REFERENCES**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

*Use Ecology style*

DRAFT

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## APPENDIX A Field Data Sheets

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

The following field data sheets serve as a backup procedure for times when electronic data collection devices (PDA) are not available.

See 'NEON Mosquito Phenology & Diversity Datasheet' which includes several tabs (Field Data Collection, Field Data Entry Logistics). The columns in each tab are TBR but currently look as follows:

*Include copies of all data sheets – jpg format*

*(data sheets are useful for CI to define PDA and data ingest requirements)*

Activity  
 Names  
 Month  
 Day of the Month  
 Day of the Week  
 Year  
 Start Location  
 Start Time (in car, departing start location)  
 Arrival at site (in car, arrival on site)  
 Plot  
 GPS Location  
 Arrival at plot  
 Collection start (from first trap)/CO2 set  
 # specimens in each trap (0/few/many)  
 Collection end (from last trap)/CO2 pickup  
 Notes  
 Freezer time in  
 Freezer time out  
 Total mosquitoes (0/few/many)  
 Bloodfed mosquitoes (0/few/many)  
 Bycatch (0/few/many)  
 Time of ethanol change for each trap  
 Departure from site (in car, leaving)  
 End Time (arrival at end location)  
 End Location  
 Start Location  
 Start Time  
 Arrival at site  
 Plot # for 1st - 10th plot (order of visitation)  
 Arrival time at each plot  
 Collection time start each plot  
 Collection time end each plot  
 Departure time from site  
 End Location  
 End Time  
 Drive time to site  
 Drive time from site  
 Total time at site

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Average Collection time at each plot  
 Average Travel time between plots

**APPENDIX B Lab Data Sheets**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

The following data sheets serve as a backup procedure for times when electronic data collection devices (PDA) are not available.

See ‘NEON Mosquito Phenology & Diversity Datasheet’ which includes several tabs (Mosquito Trap Data Entry, Biodiversity, 96 well plate, DNA barcode). The columns in each tab are TBR but currently look as follows:

*Include copies of all data sheets – jpg format  
 (data sheets are useful for CI to define PDA and data ingest requirements)*

- Date
- Plot
- Trap
- Collectors Initials
- Freezer Time in
- Freezer Time out
- Total time in freezer
- # of insects in trap (0/few/many)
- # mosquitoes in trap (0/few/many)
- # bloodfed mosquitoes in trap (0/few/many)
- Unique #
- Collectors
- Collection Date
- Collection Method
- Plot #
- Trap #
- Continent
- Country
- State
- Region
- Exact Site
- Latitude
- Longitude
- Elevation (m)
- Other Info
- Phylum
- Class
- Order
- Family
- Subfamily
- Tribe

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Morphospecies  
 Genus  
 species  
 Species Authority  
 Basis of ID  
 Date of ID  
 Taxonomist Identifier  
 Technician Identifier  
 Length (mm)  
 Sex  
 Reproduction  
 Life Stage  
 Notes  
 Well positions  
 NEON Plate #  
 CCDB Plate #  
 Well  
 NEON Specimen #  
 Sample ID  
 Institution Storing  
 Collectors  
 Collection Date  
 Collection Method  
 Plot #  
 Trap #  
 Continent  
 Country  
 State  
 Region  
 Exact Site  
 Latitude  
 Longitude  
 Elevation (m)  
 Other Info  
 Phylum  
 Class  
 Order  
 Family  
 Subfamily  
 Tribe  
 Morphospecies  
 Genus  
 species  
 Species Authority  
 Basis of ID  
 Date of ID  
 Taxonomist Identifier  
 Technician Identifier  
 Length (mm)  
 Sex

DRAFT

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Reproduction  
 Life Stage  
 Notes

**APPENDIX C Considerations for implementation**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

*Indicate activities that could result in equipment damage, degradation of sample, or possible invalidation of results; listed here and at the critical steps in the procedure.*

*Describe any component of the process that may interfere with the accuracy of the final product.*

*Discuss how to avoid common errors in sampling or common ways samples can be contaminated.*

*Clearly flag things that might impact their work or the scientific data that aren't covered in the procedural pieces (stupid examples: "We're measuring nitrates, if you are exposed to or using nitrates at home on your lawn, trace amounts might contaminate our data"; "If it's raining, sky water getting into the samples before you seal them could alter results" )... i.e. call out weird issues and folklore explicitly. See: [http://en.wikipedia.org/wiki/Phantom\\_of\\_Heilbronn](http://en.wikipedia.org/wiki/Phantom_of_Heilbronn)*

**APPENDIX D Procedure Checklist**

**APPENDIX E [This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]**

**APPENDIX F Tables**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

**APPENDIX G Figures**

*[This section is a draft for the Field Operations Prototype at CPER 2011 and is TBR.]*

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<i>NEON Doc. #:</i> NEON.DOC.014049	<i>Revision:</i> A_DRAFT	



Figure 1

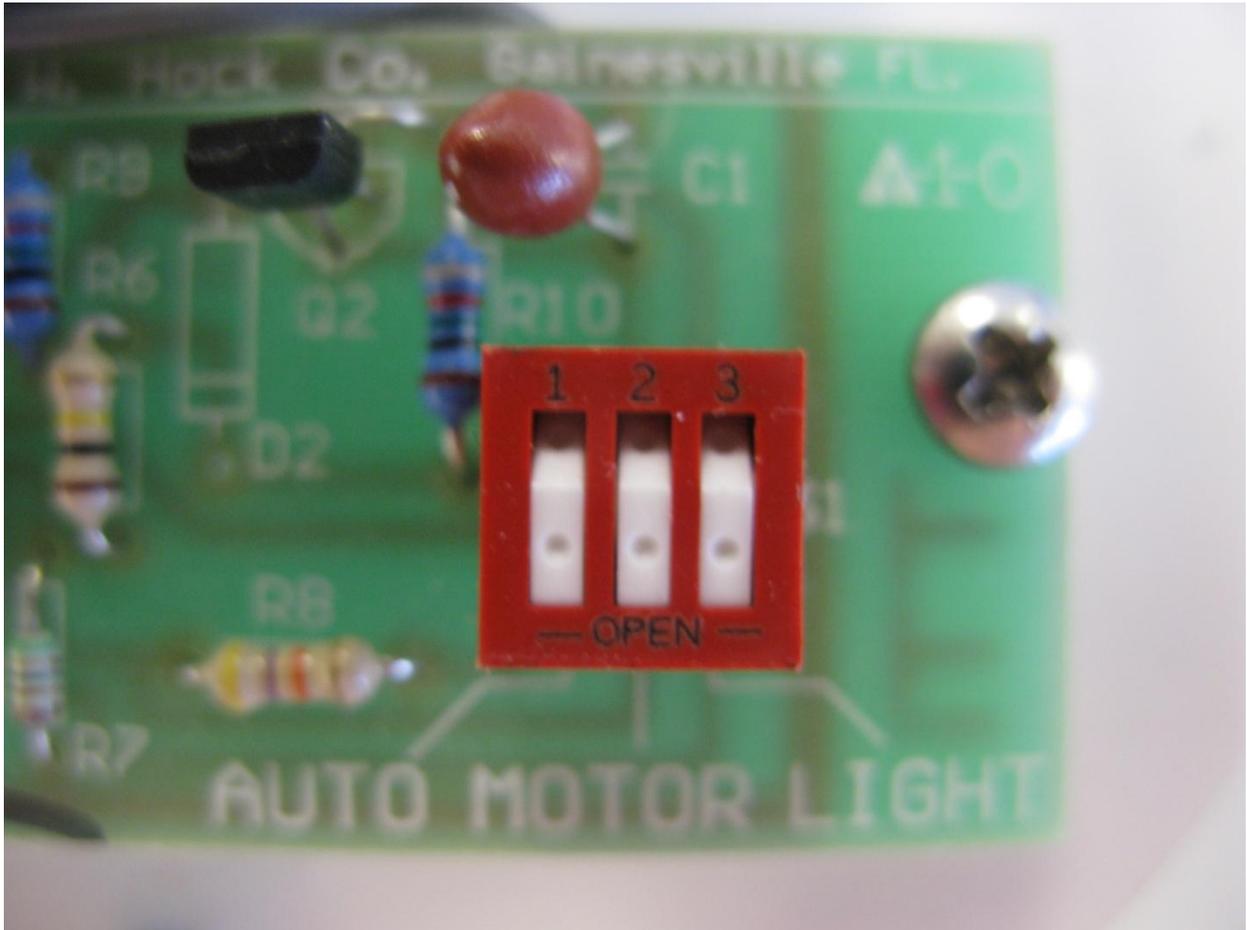


Figure 2

NEW image that depicts applying locality label to catch cup upon first arrival at trap

Figure 3

NEW image that depicts properly stowed mesh in catch cups in chest coolers for transport to car

Figure 4

NEW image of 6 V batteries charging in containment bins with warning signs.

Figure 5

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COLORADO Weld County.  
Central Plains EXPER RNG.  
1522m N40.8164, W104.7490.  
Pitfall trap. Jul2011. [KKBlevins](#).  
NEON.FSU.D10.

COLORADO Weld County.  
Central Plains EXPER RNG.  
1522m N40.8164, W104.7490.  
Pitfall trap. Jul2011. [KKBlevins](#).  
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NEON.FSU.D10.

COLORADO Weld County.  
Central Plains EXPER RNG.  
1522m N40.8164, W104.7490.  
Pitfall trap. Jul2011. [KKBlevins](#).  
NEON.FSU.D10.

Figure 6

COLORADO Weld County.  
Central Plains EXPER RNG.  
1522m N40.8164, W104.7490.  
Pitfall trap. Jul2011. [KKBlevins](#).  
NEON.FSU.D10.

Figure 7

COLORADO Weld County.  
Central Plains EXPER RNG.  
1522m N40.8164, W104.7490.  
Pitfall trap. Jul2011. [KKBlevins](#).  
NEON.FSU.D10.

Figure 8

NEW image which depicts workspace with drop down netting

Figure 9

NEW image that depicts general look of stunning invertebrates on a chilled surface.

Figure 10

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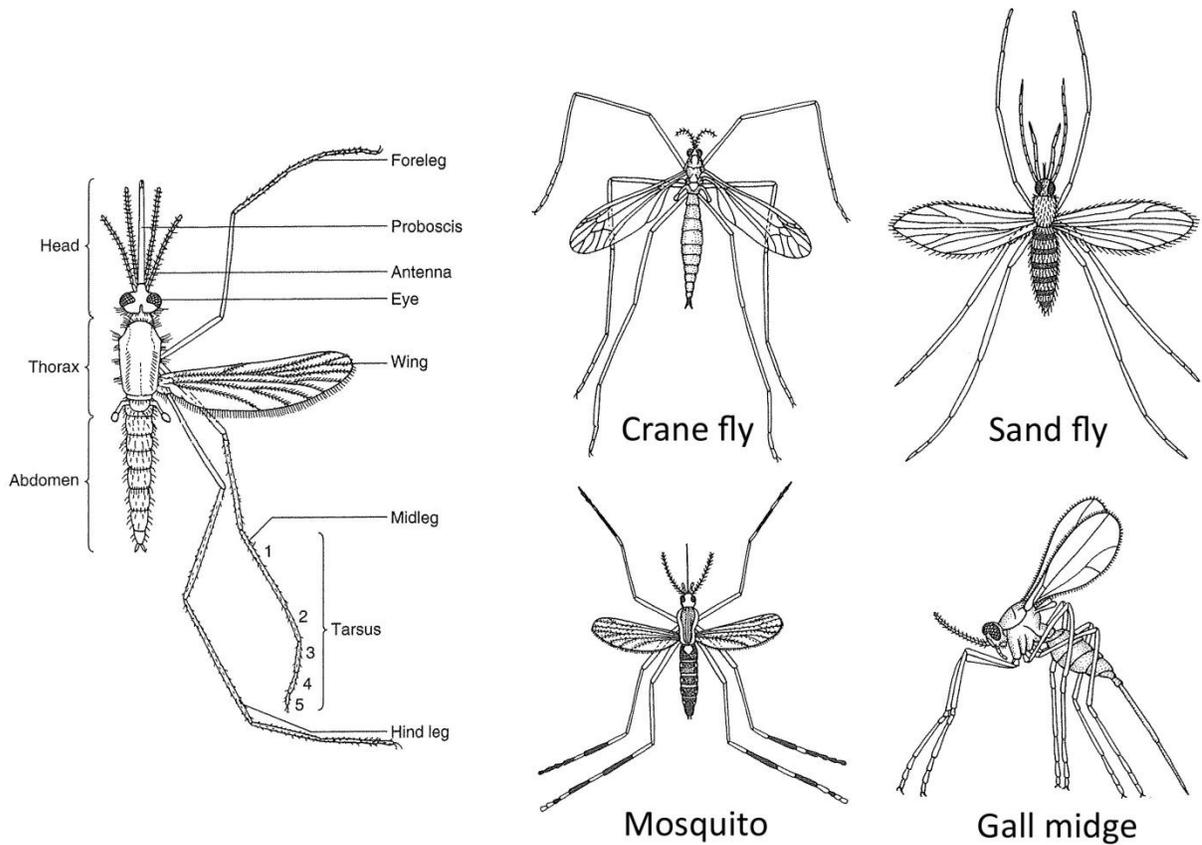


Figure 11

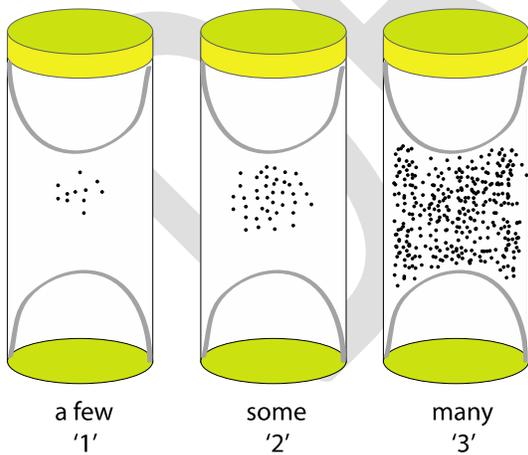


Figure 12

NEW image depicting proper look of mosquitoes packed in 50mL centrifuge tube  
Figure 13

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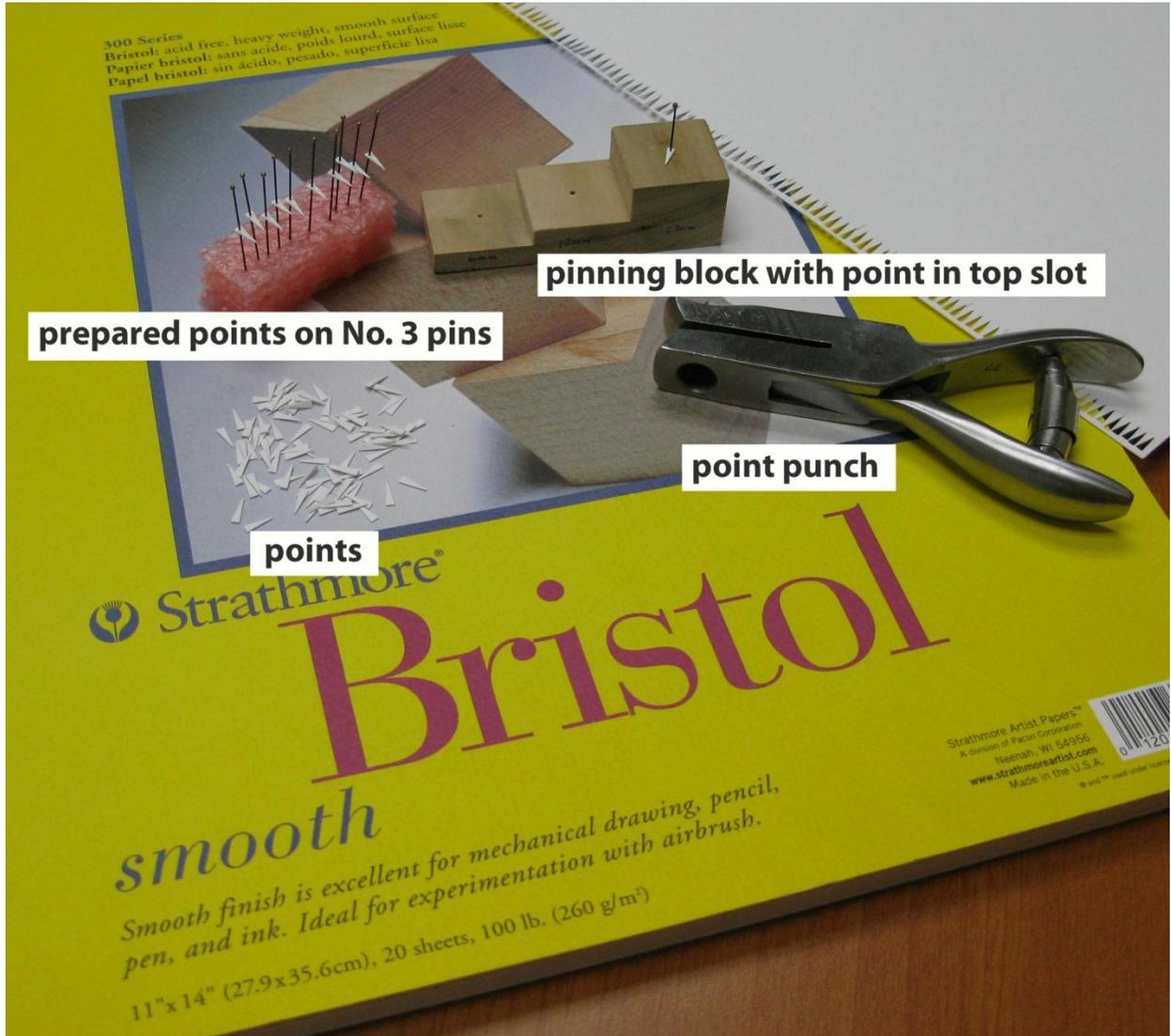


Figure 14

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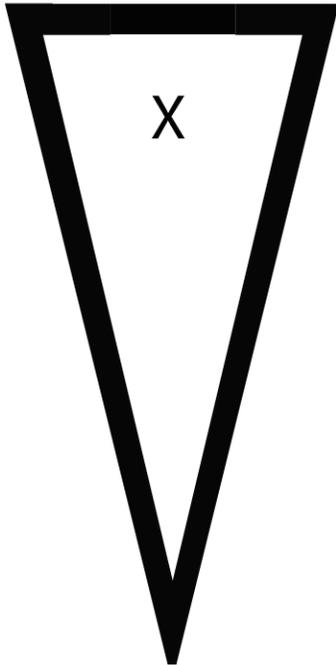


Figure 15

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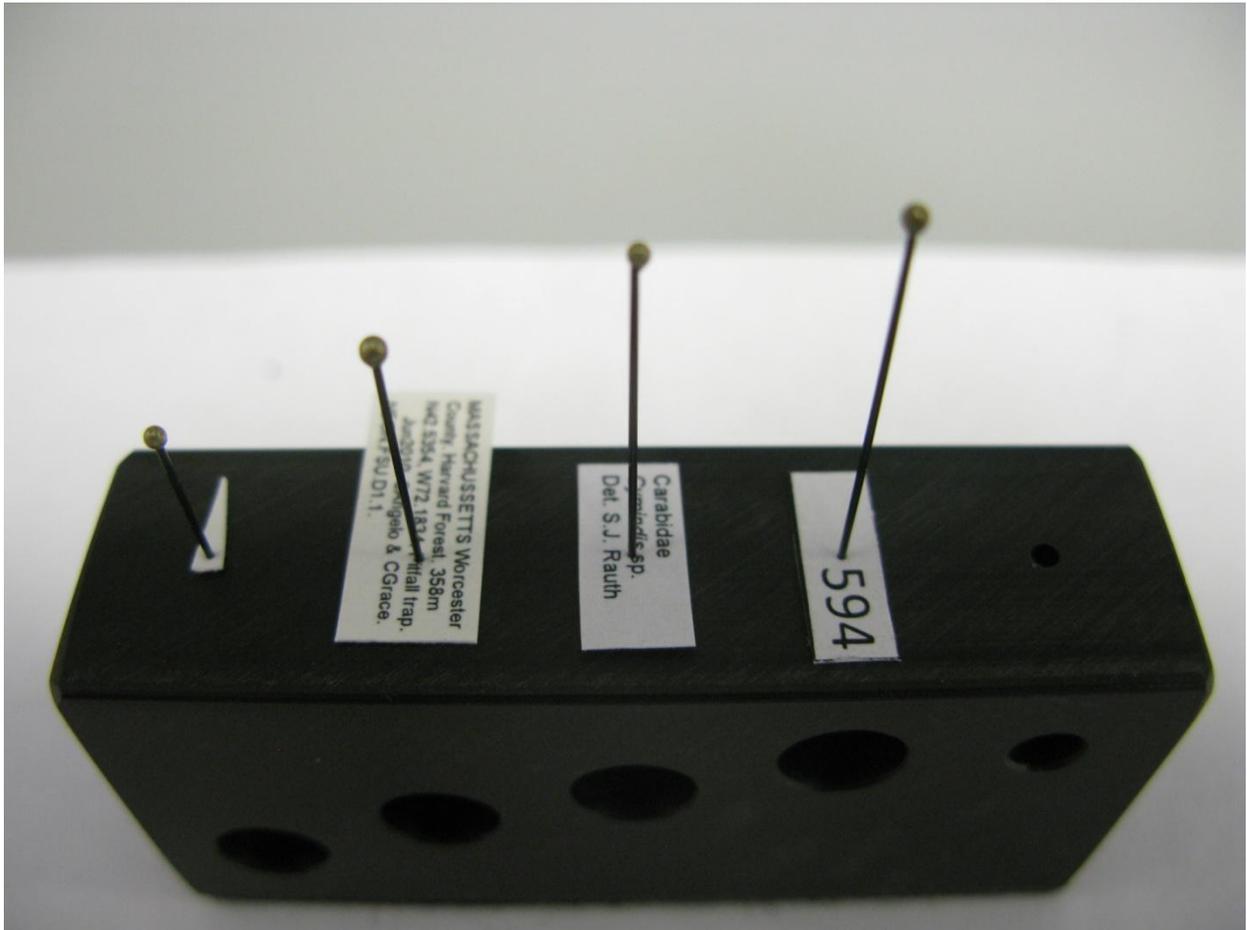


Figure 16

DRAFT

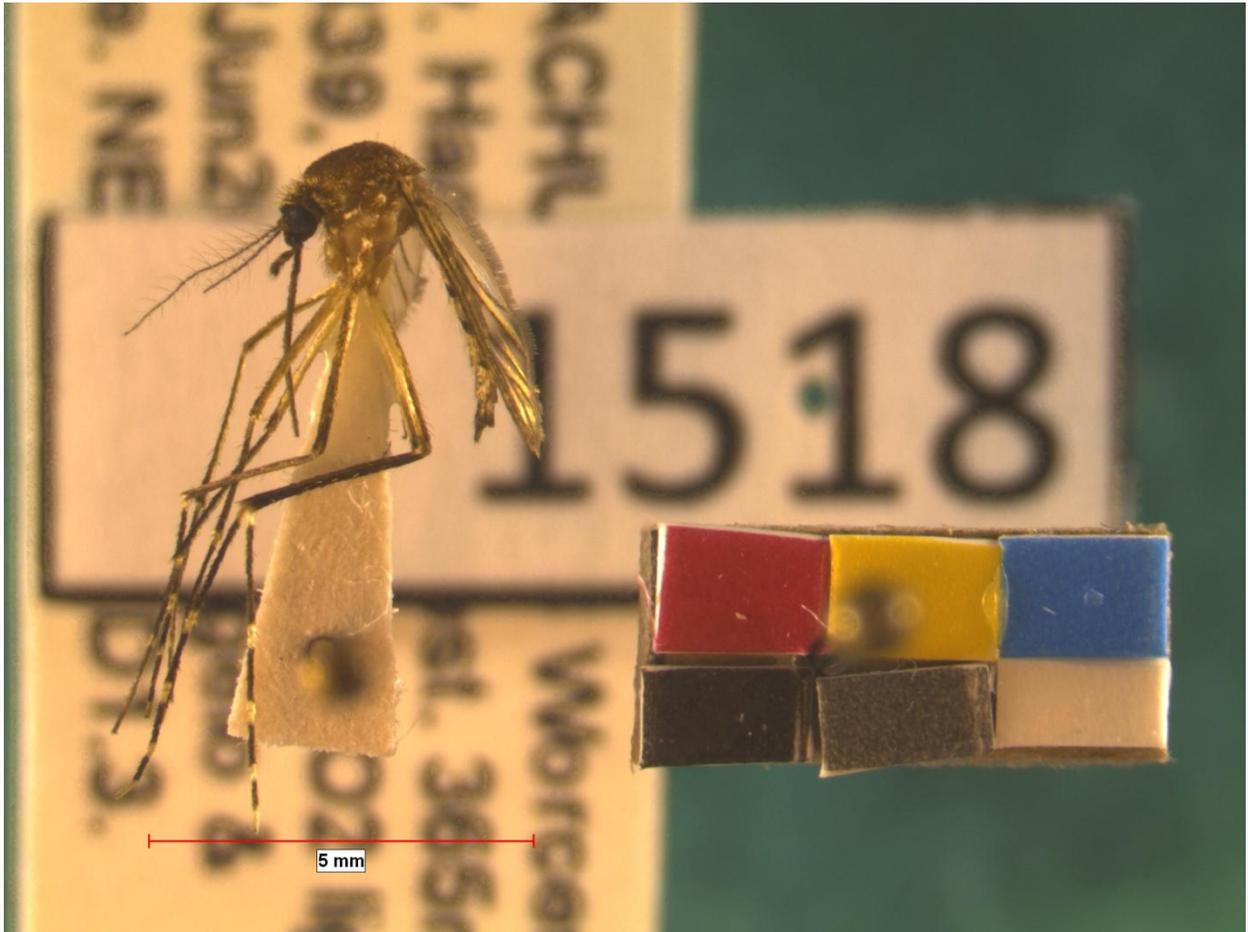


Figure 17

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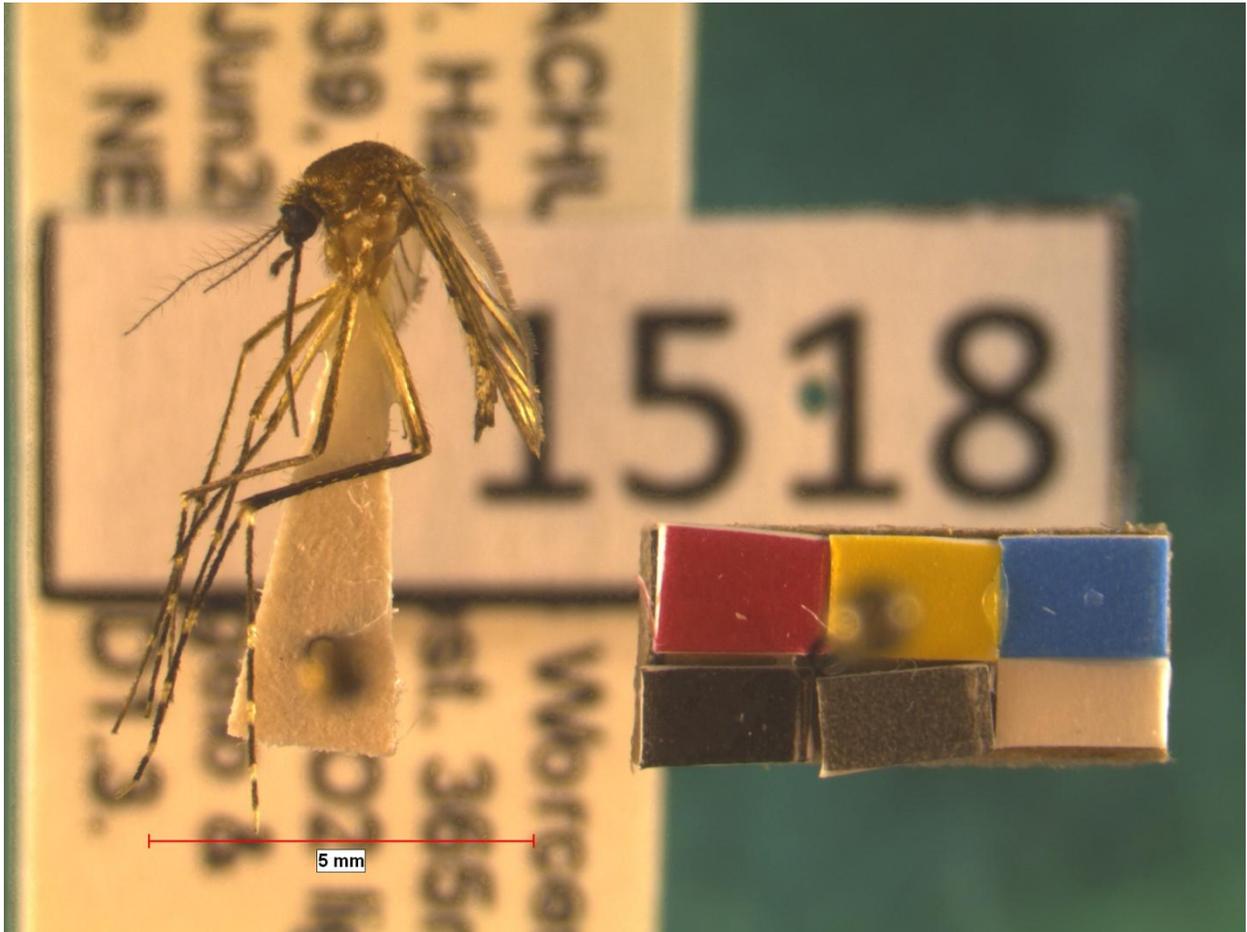


Figure 18

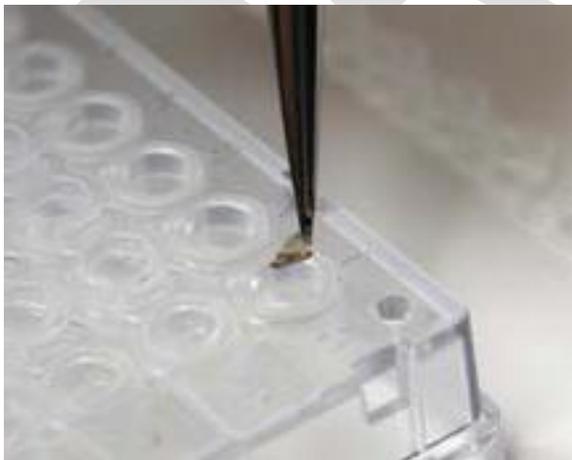


Figure 19

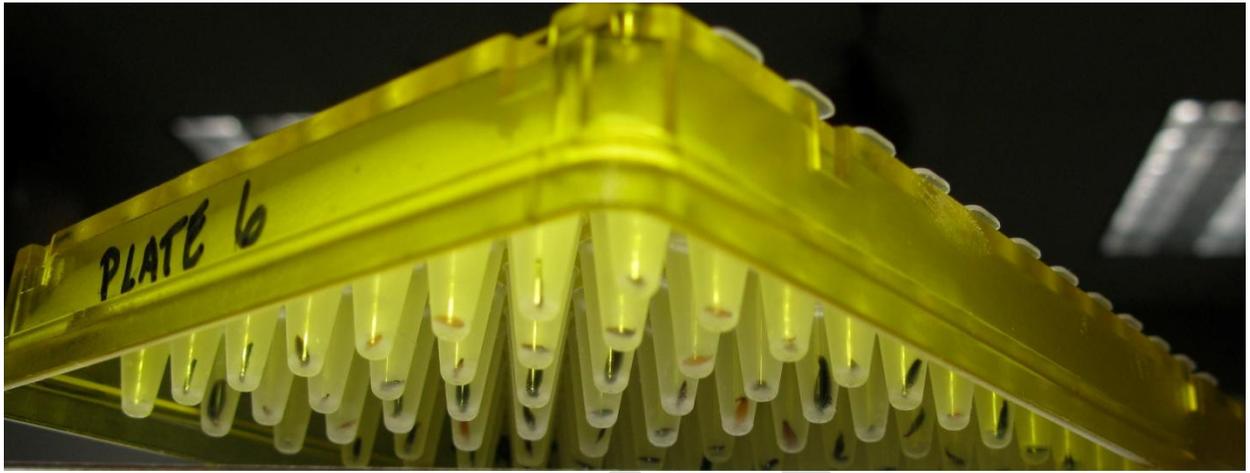


Figure 20

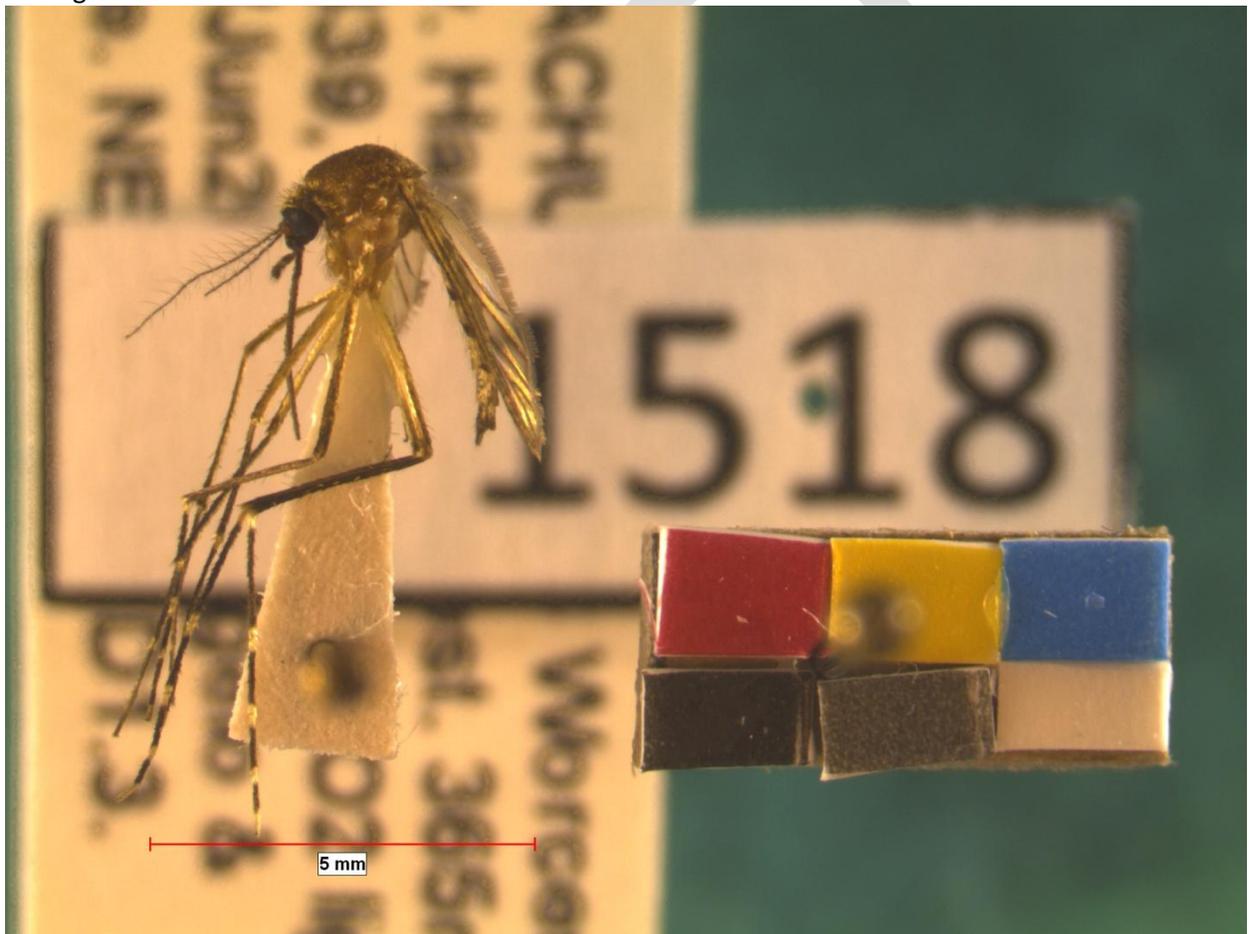


Figure 21

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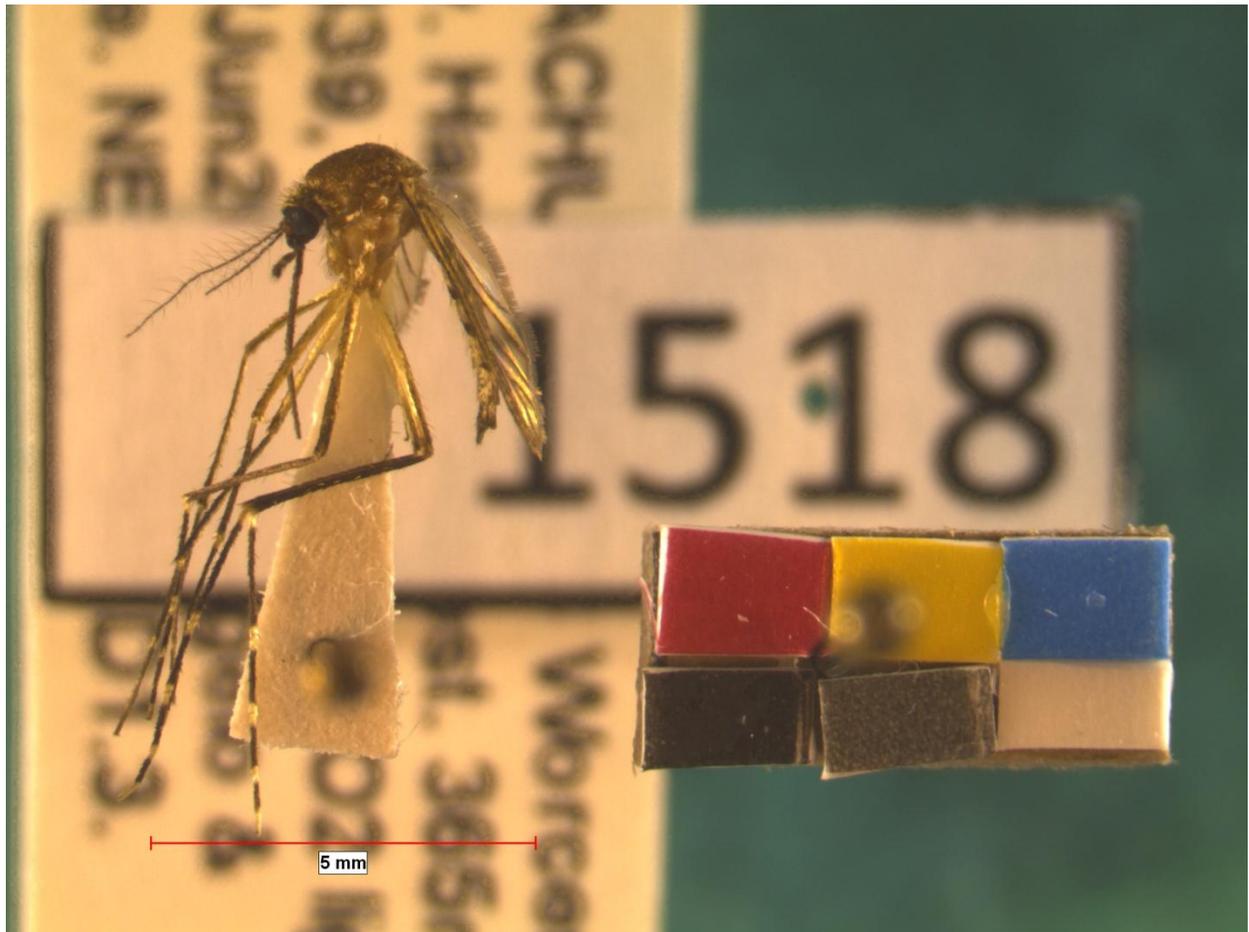


Figure 22

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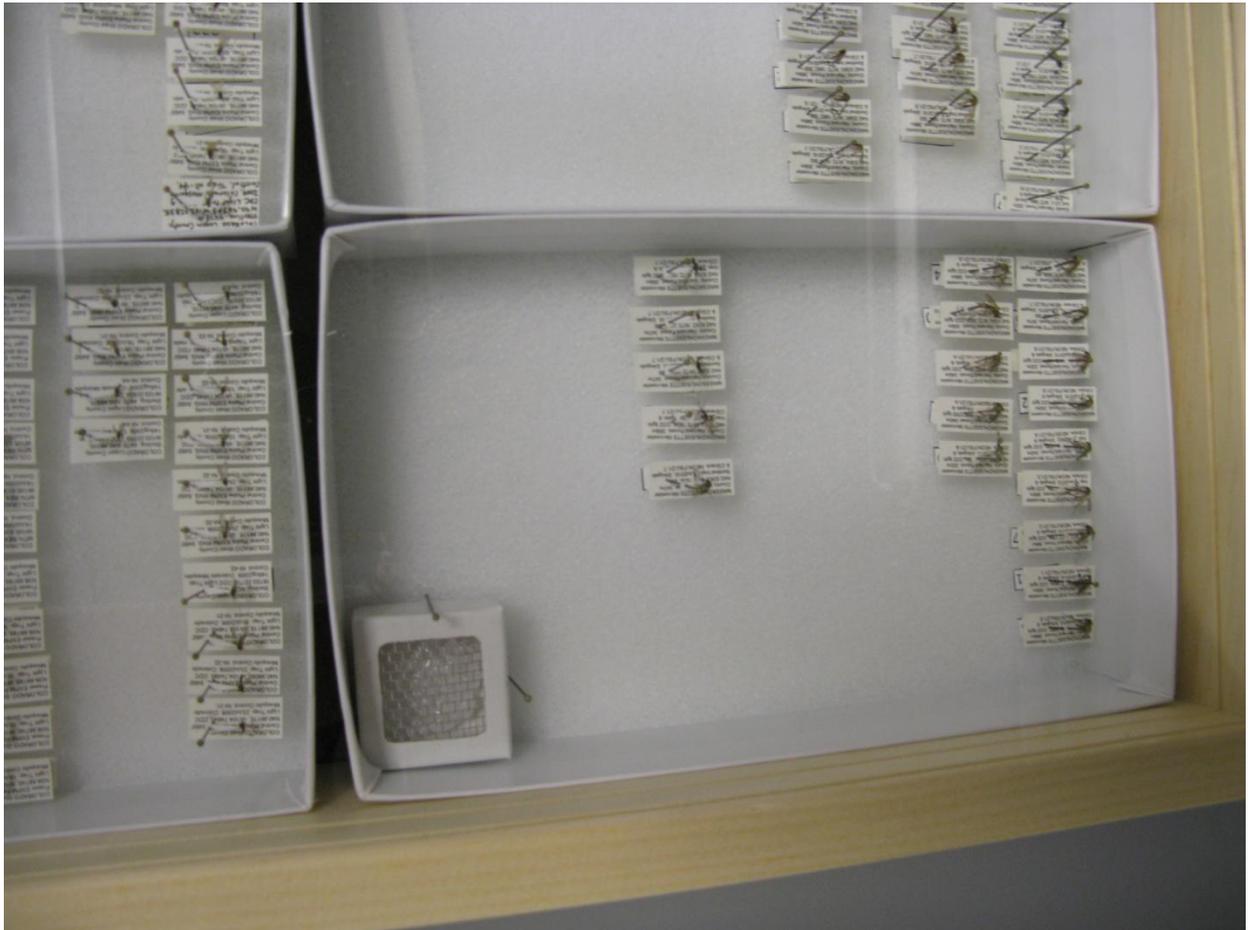


Figure 23

NEW image depicting ziplock bags with 50 mL centrifuge tubes well packed.

Figure 24

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Figure 25

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