

<i>Title:</i> NEON Field and Lab Procedure and Protocol: FSU Mosquito Protocol	<i>Author:</i> C Gibson	<i>Date:</i> 4/29/2012
<i>NEON Doc. #:</i> NEON.DOC.014049		<i>Revision:</i> B_DRAFT

NEON FSU Field and Lab Protocols for Field Operations 2012 Prototype in Domain 3: Mosquito Diversity (No Phenology data collection in 2012)

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See Configuration Management System for approval history.

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
-	03/30/2011	ECO-00159	Initial Draft Release
A_DRAFT	09/23/2011	ECO-00280	Update to new document number's/template throughout document.
B_DRAFT	07/30/2012	ECO-00442	Update Draft Release for Mosquito 2012.

DRAFT

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1 INTRODUCTION

1.1 Purpose

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

The primary purpose of this document is to provide a change controlled version of Observatory protocols and for external review by subject-matter experts. This document also 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 DOMAIN 3 2012 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 DOMAIN 3 2012 and is TBR.]

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2 RELATED DOCUMENTS AND ACRONYMS

2.1 Reference Documents

RD[01]	NEON.DOC.000008 NEON Glossary of Abbreviations
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/QC Plan
	DOORS requirements
	ATBD
AD[01]	FSU Science Requirements
AD[02]	FSU Field Operations Plan
AD[03]	Data Products Level 1-3 Catalog

2.2 Acronyms

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 DOMAIN 3 2012 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 for morphological identifications (Taxonomists), Genetic Analytical Laboratories to conduct the bi-directional DNA sequencing during the construction period of the observatory (during operations only uni-directional sequencing should be required for verifying well-circumscribed specimens), and/or to Archives.

For 2012, the expert taxonomist contract is with C. Roxanne (Rutledge) Connelly, PhD, Associate Professor, Extension Medical Entomology Specialist, UF/IFAS/FMEL. The Biodiversity Institute of Ontario is the current bid-winning contract for the Genetic Analyses. The NEON Support Facility at 1635 38th St., Suite 100, Boulder, CO 80301 will be the Archive for the 2012 sampling year.

A CO₂ light trap is baited with CO₂ (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).

3.2 Metadata Collection

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

Data about the samples will be compiled into fields outlined in a spreadsheet to be supplied by FSU called 'NEON Mosquito Diversity Data sheets'. These fields follow the Barcode of Life Datasystems (BOLD) standards and include some additional fields in order to be compliant with other global biodiversity projects.

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3.3 Science Requirements

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This protocol fulfills the following Observatory science requirements:

3.4 Data Products

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

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

Measurement	Data Product
	Revising catalogue with Sarah Elmendorf and Steve Berukoff; note added 20120517

4 PROTOCOL

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

4.1.1 Plot Location

This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.

FSU is responsible for determining plot locations.

Mosquito Phenology Trap Location: CO₂ 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)
There is no phenology sampling in the 2012 prototype.

Mosquito Diversity Trap Location: CO₂ light traps will be placed adjacent to ten Biodiversity Plots (within the destructive sampling buffer zone) (10 traps per site). Two additional CO₂ light traps shall be placed at sites near the aquatic array (2 additional traps per site)

5 QUALITY ASSURANCE AND QUALITY CONTROL

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

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

6 DECISION TREE

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

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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)	YES. Specimens may not be morphologically identifiable	May not be readily able to obtain morph. ID; affects phenology 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.	YES. Specimens may not be morphologically identifiable	May not be readily able to obtain morph. ID; affects Phenology measurements
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	YES. Specimens may not be morphologically identifiable	May not be readily able to obtain morph. ID; affects Phenology measurements

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	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.		
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	YES. 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

Mosquito Abundance and Diversity

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

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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.	YES. Specimens may not be morphologically identifiable	May not be readily able to obtain morph. ID; affects Abundance&Diversity measurements
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	YES. Specimens may not be morphologically identifiable	May not be readily able to obtain morph. ID; affects Abundance&Diversity measurements

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	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.		
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	YES. 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 DOMAIN 3 2012 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 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 stop work activities based on unsafe field conditions; however, all employees have the responsibility and right to stop their work in unsafe conditions.

1. Technicians should take steps to cover exposed skin and thereby reduce the probability of being bitten by mosquitoes. Chemical insecticides may be used according to the following guidelines. Insecticide, preferably containing DEET, may be applied prior to heading out to a field site. Application, particularly if the insecticide is in spray form, should be done outside in a well ventilated area and away from people and sampling equipment. Insecticide should be applied to clothing and around ankles, wrists, waistband, and neck. Note that applying repellent directly to skin may produce an allergic

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reaction in some individuals.

2. Extreme care must be taken to avoid contaminating experimental equipment with insecticide as this could bias collection results by repelling mosquitoes. The following steps should help reduce the chances of such contamination. Apply insecticide well in advance of arriving in the field to deploy or collect traps (at least 30 minutes). Do not apply insecticide in the vicinity of traps or associated hardware. Wash hands thoroughly after applying insecticide so as to avoid transfer to traps during handling. If repellent is mistakenly applied to any of the trap components in any manner the component(s) should be carefully cleaned with perfume-free soap.

3. In the laboratory, drop down netting is used to ensure that mosquitoes that are presumed dead, but in reality are still alive, cannot fly from the work space and bite any technicians. This is a safety precaution to prevent the risk of infectious disease transmission in the work place.

8 PERSONNEL REQUIREMENTS

8.1 Field Personnel

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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 DOMAIN 3 2012 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)

9 TRAINING REQUIREMENTS

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

See NEON Training Plan document relevant to this method.

10 FIELD STANDARD OPERATING PROCEDURE

10.1 Sampling Frequency and Timing

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

There are two different sampling regimes.

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Mosquito Phenology Sampling Schedule: This is two total CO₂ light traps being collected twice per week within 24 h of trap set up in a subset of the Biodiversity Plots that are being sampled for Mosquito Diversity during the rest of the season. These traps are collected by whoever is available (field or lab technicians or domain staff) and then all collected invertebrates are shipped directly to a Taxonomist.

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.

There will be no Mosquito Phenology sampling during the 2012 activity.

Mosquito Diversity Sampling Schedule: This is ten total CO₂ 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.

For Domain 3 in 2012, IDEALLY the first actual sampling should occur during the week of Monday July 9 and continue for 4 weekly sampling bouts per site. IDEALLY more than one site would be sampled per week.

The current draft plan for 2012 however is for 12 weeks of sampling (with one site per week) through to the week ending Friday, Sept 28 2012. (Training & Establishment Monday Jun 25 – Tuesday July 10).

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

Domains	Early Season Weeks - Prior to (inclusive)	Season - Prior DS - Prior to DS	No. Weeks - Prior to DS	Ending Season Weeks - After DS (inclusive)	No. weeks - After DS	Total No. Weeks sampling	Approx. Calendar Start Date	DS	Approx. Calendar Stop Date
1,6	16-20		5	36-42	8	13	16-Apr	21-May - 27-Aug	15-Oct
2	14-20		7	36-44	9	16	2-Apr	21-May - 27-Aug	29-Oct
3,11	12-20		9	36-45	10	19	19-Mar	21-May - 27-Aug	5-Nov
4,20	1-20		21	36-52	13	34	1-Jan	21-May - 27-Aug	24-Dec
5	19-20		2	36-40	5	7	7-May	21-May - 27-Aug	1-Oct
7,8	13-20		8	36-45	10	18	26-Mar	21-May - 27-Aug	5-Nov
9	19-21		3	36-38	3	6	7-May	28-May - 27-Aug	17-Sep
10,13	18-20		3	36-40	5	8	30-Apr	21-May - 27-Aug	1-Oct
12	23-24		2	37-38	2	4	4-Jun	18-Jun - 3-Sep	17-Sep
14,17	11-20		10	36-47	12	22	12-Mar	21-May - 27-Aug	19-Nov
15	19-20		3	36-41	6	9	7-May	21-May - 27-Aug	8-Oct

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16	18-20	3	36-42	8 11	30-Apr	21-May - 27-Aug	1-Oct
18,19	19-21	3	35-36	2 5	7-May	28-May - 27-Aug	3-Sep

Table 3 The approximate sample dates for mosquito diversity and abundance at all NEON sites.

Domains	Diversity Start Week	Diversity Stop Week (inclusive)	Total No. Weeks	Approx. Calendar Start Date	Approx. Calendar Stop Date
1,2,3,4,5,6,7,8,10,11,13,14,15,16,17,20	21	35	15	21-May	27-Aug
9,18,19	22	35	14	28-May	27-Aug
12	25	36	12	18-Jun	3-Sep

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. 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.

10.2 Contingent decisions

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

See Section 6.

10.3 Field Procedure

10.3.1 Equipment and Materials

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

Table 3 Materials and supplies required for the Mosquito Sampling Procedure for one site for one sampling day.

Item Description	Quantity per sampling event	Hazardous Chemical
CO ₂ trap locality labels	30, 3 per trap	N
Twist ties or safety pins to affix labels to traps	30	N
CO ₂ traps (rain guard, cylinder cooler, fan housing)	12	N
Roll of extra string for hanging traps	Variable	N
6V sealed gelled-electrolyte batteries (12, 10 for active field work, two for replacements)	12	N
Shepherd's hook (if no trees to hang traps)	10	N
Shovel to dig T post	1	N
CO ₂ pellets	1 kg per trap, 15-20 kg to	Y

	accommodate loss	
Catch cups	10	N
Large chest cooler with frozen ice packs (to be left in field vehicle; for catch cup transport)	1	N
medium chest cooler dry ice sufficient for day of sampling (~15-20 kg; must include more due to loss)	2	N
Ice packs	5	N
Small cylinder coolers (12, one for each trap, plus two replacements)	12	N
Replacement catch cups	2	N
Sharpies	6	N
Archival, ethanol-safe pens (Pigma brand, size 01)	6	N
Scissors	2	N
Forceps	4	N
Carry-all craft tote bag or other field pack	2	N
Large ziplock bags	15	N
Latex gloves	Variable	N
Mosquito protective clothing	2	N
Handwipes	Variable	N
Duct tape	Variable	N
Flagging	Variable	N
Bug spray	Variable	Y
Sun block	Variable	N
First-aid kit	1	N
Camera	1	N
GPS/Trimble	1	N
PDA	1	N
Datasheets	10	N

10.3.2 Preparation

10.3.2.1 Plot Establishment

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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 CO₂ 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 shepherd's hook will need to be secured in the ground. Traps should be shielded from heavy wind, rainfall and direct sunlight if possible. Each CO₂ light trap is equipped with a cylinder cooler filled with CO₂ 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.

Secure the trap 17.96 m south of the center plot marker. This location can be adjusted by 25 cm further south (outside of the biodiversity sampling ring) or by 25 cm to the left or right of the 17.96 m south of the center plot marker. The trap can be fastened to a tree if it is in this area or otherwise use a shepherd's hook. THE POSITIONING OF THE TRAPS MUST BE COMPARABLE ACROSS THE OBSERVATORY.

Trap assembly and setting:

The CO₂ 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 any holes 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. Ensure that the rain guard is secured to the plexi-glass fan housing.
2. Clip the cylinder cooler onto the rain guard with the brass clasp or carabiner.
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

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morning, the light will go off and the fan motor continues, saving the catch in the catch cup.

[Figure 2]

5. Tie the battery cord once around the shepherd's hook to secure it. 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:

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

10.3.2.2 Weekly Sampling Preparation

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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 CO₂pellet 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.
6. Clear freezer space.

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10.3.3 Sample Collection in the Field

10.3.3.1 Trap Collection

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

Upon arrival at the trap:

1. Attach six copies of locality labels (as a long strip, date filled in) to the catch cup using a twist tie or safety pin (see section on 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 lining the bottom) carefully to ensure that any residual water does not get into catch cup and damage specimens.
5. Record in the 'NEON Mosquito Diversity Data sheets' the number of mosquitoes in the trap using the scale 0/few/many 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 fan is off until dusk (reducing battery draw).
4. 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

10.3.4 Sample Preservation

10.3.4.1 Sample transport and Storage Prior to Processing

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

1. Catch cups should be placed with the mesh tucked gently into them in chest coolers (with ice packs lining the bottom).
[Figure 4]
2. Do not expose mosquitoes to direct sunlight or extreme temperatures (e.g., enclosed in vehicle for prolonged periods).

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3. 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.

10.3.5 Data Handling (may not applicable for all Field SOPs)

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

Field data collected should be carefully entered into the 'NEON Mosquito Diversity and Phenology Datasheets' on the P drive daily.

10.3.6 Refreshing the Field Sampling Kit

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

10.3.7 Equipment Maintenance, Cleaning, and Storage

10.3.7.1 Battery handling

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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]

10.3.7.2 Battery charging

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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.

Chargers should not melt bin. Report to Domain manager if there is an issue.

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:*
 - 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.

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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.

CO₂ 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:

CO₂ 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

11.1 Timing

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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.

11.2 Lab Procedure-Sorting

11.2.1 Equipment and Materials

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

Table 4 Materials and supplies required for the Mosquito Lab Procedure for one site for one sampling day.

Item Description	Quantity per sampling event	Hazardous Chemical
Color Separation Guide and Gray Scale	1	N
CO ₂ freezer	1	N
Facial tissues (cut in half)	many	N
Sharpie marker	6	N
Ice packs	6 (swap out when thaw)	N
Paper plates	2	N
Aluminum foil	Variable, to cover paper plates	N
Plain white paper	1	N
Small paint brush	3	N
Ethanol	Variable, for ethanol lamp	Y
Archival, ethanol-safe pens (Pigma brand, size 01)	3	N
No. 3 pins archival quality	Variable	N
50 mL nonsterile centrifuge tubes	Variable	N
25 dram plastic tubes	Variable	N
Schmitt boxes	4	N
Point punch	1	N
Pinning block (anodized; height is better than wood)	1	N
Fumigant (moth crystal) boxes	Variable, for each drawer or Schmitt box	N
Archival, ethanol-safe pinned specimen label paper	Variable	N
Point paper (Strathmore archival quality bristol board)	Variable	N
Jewelers forceps	3	N
96 well microwell plates	Variable	N
96 well microwell caps	To fit plates	N
Hardware/software (i.e. Clemex Captiva)	1	N
Microscope	1	N
Canon Powershot camera	1	N

Fumigant (paradichlorobenzene - moth crystals)	Variable, ~6.5 g per box	Y
Packing tape	Variable	N
Shipping supplies (boxes, padded envelopes)	Variable	N
Cardboard specimen mailers	Variable	N
Self healing cut mat (30 cm x 46 cm)	1	N
Scissors	2	N
Exacto knife	3	N
Metal ruler	1	N
6V two at a time battery re-charger	6	N
Unit trays for inside Cornell drawers (48 per cabinet)	48 per cabinet	N
Cornell drawer(pine - hardboard bottom)	6 drawers per cabinet	N
Cornell cabinet (6 drawers, unassembled)	2	N
Balsa wood for point preparation surface	4	N
Secondary containment bins for charging batteries	2	N
Latex gloves	variable	N
DNA barcode unique numbers	Variable	
Adhesive shipping labels	Variable	N
Plain white paper (for pointing specimens under microscope)	Variable	N
Tissue paper for photo box background	1	N
LED lights (microscope, photos)	3	N
Elmer's white glue	1	N
Ethanol lamp (sterilizing)	1	N
Lighter	1	N
Drop down netting	1	N

11.2.2 Preparation

11.2.2.1 Generating Sample Labels

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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.

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- 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)
- “collector” (e.g. Domain manager name, RNelson)
- a collection code which is comprised of the following parts: NEON.FSU.Domain.Plot, e.g. NEON.FSU.D10.BD01

To prepare labels:

- a. Do NOT change to template set of labels; instead, rename the file name with the appropriate month and year THEN in this new file, select 'Edit' > 'find' and 'replace' to replace the month in this new set of labels.
- b. Once labels have been printed, cut labels into long columns using a snap-off blade knife and ruler. There should be no obstructions on the cutting mat or the ruler while doing this. Using a snap-off blade is essential to making clean cuts that DO NOT ADD ANY additional whitespace to the edges of labels. Unfortunately, this outcome is inevitable with scissors when making a cut of this length.
- c. Cut labels with as little white space on all sides of the text as possible (see picture).
- d. These cuts result in many labels in a vertical strip.

[Figure 6]

- e. Store labels this way, and then when needed, cut individual labels across the row with scissors; again, leaving as little white space around as possible.
- f. Store labels this way, and then when needed, cut individual labels across the row with scissors.

[Figure 7]

[Figure 8]

- g. 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.

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).

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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 DOMAIN 3 2012 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 DOMAIN 3 2012 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]
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).

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Always use drop down netting for safety reasons.

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 DOMAIN 3 2012 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 DOMAIN 3 2012 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]

11.2.4.5 For each bycatch & mosquitoes sample

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1. Record in the 'NEON Mosquito Diversity Data sheets' the size of the catch of all specimens using the scale 0/few/many and any additional notes (e.g. 0 specimens because a battery failed).
[Figure 12]
2. Record in the 'NEON Mosquito Diversity Data sheets' 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. Use additional 50 mL tubes if it seems as though specimens might damage one another.
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.

All mosquitoes will be sent to Taxonomist for identification within a couple of days for morphological ID.

11.2.1 Data Handling

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

At the end of each field and lab day, all information (including any deviations or errors) from field data sheets must be entered into the digital system.

Make sure to note deviations or any other points of interest in the digital datasheet.

11.2.2 Sample Shipping

11.2.2.1 Establishing Workspace: Shipping & Receiving

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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 DOMAIN 3 2012 and is TBR.]

Sorted mosquito specimens are shipped overnight to expert Taxonomists with a hard and digital copy of the 'NEON Mosquito Diversity Data sheets' 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 DOMAIN 3 2012 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 Diversity Data sheets'.

[Figure 25]

11.2.2.4 Archives

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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 Diversity Data sheets'.
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 Diversity Data sheets'.
3. Only 3 specimens of each species should remain in the teaching collection at a Domain facility. All other specimens should be shipped to the Archive.

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11.2.3 Sample Preservation

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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. If there is no access to -20°C, specimens 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 DOMAIN 3 2012 and is TBR.]

1. Wash out CO₂ 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 DOMAIN 3 2012 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	1	N
CO ₂ freezer	1	N
Facial tissues (cut in half)	many	N
Sharpie marker	6	N
Ice packs	6 (swap out when thaw)	N
Paper plates	2	N
Aluminum foil	Variable, to cover paper plates	N
Plain white paper	1	N
Small paint brush	3	N
Ethanol	Variable, for ethanol lamp	Y

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Archival, ethanol-safe pens (Pigma brand, size 01)	3	N
No. 3 pins archival quality	Variable	N
50 mL nonsterile centrifuge tubes	Variable	N
25 dram plastic tubes	Variable	N
Schmitt boxes	4	N
Point punch	1	N
Pinning block (anodized; height is better than wood)	1	N
Fumigant (moth crystal) boxes	Variable, for each drawer or Schmitt box	N
Archival, ethanol-safe pinned specimen label paper	Variable	N
Point paper (Strathmore archival quality bristol board)	Variable	N
Jewelers forceps	3	N
96 well microwell plates	Variable	N
96 well microwell caps	To fit plates	N
Hardware/software (i.e. Clemex Captiva)	1	N
Microscope	1	N
Canon Powershot camera	1	N
Fumigant (paradichlorobenzene - moth crystals)	Variable, ~6.5 g per box	Y
Packing tape	Variable	N
Shipping supplies (boxes, padded envelopes)	Variable	N
Cardboard specimen mailers	Variable	N
Self healing cut mat (30 cm x 46 cm)	1	N
Scissors	2	N
Exacto knife	3	N
Metal ruler	1	N
6V two at a time battery re-charger	6	N
Unit trays for inside Cornell drawers (48 per cabinet)	48 per cabinet	N
Cornell drawer(pine - hardboard bottom)	6 drawers per cabinet	N
Cornell cabinet (6 drawers, unassembled)	2	N
Balsa wood for point preparation surface	4	N
Secondary containment bins for charging batteries	2	N
Latex gloves	Variable	N
DNA barcode unique numbers	Variable	
Adhesive shipping labels	Variable	N
Plain white paper (for pointing specimens under microscope)	Variable	N
Tissue paper for photo box background	1	N
LED lights (microscope, photos)	3	N
Elmer's white glue	1	N

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Ethanol lamp (sterilizing)	1	N
Lighter	1	N
Drop down netting	1	N

11.3.2 Preparation

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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. Ensure that the point is at a 90 degree angle from the pin and not angled up nor down. It is easiest to prepare these in batches of 40 or so.
[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 DOMAIN 3 2012 and is TBR.]

1. Twenty individuals of each species (representing a range of plots and dates to capture diversity through space and time) should be pointed upon return from the Taxonomist. The remainder should be stored in the freezer.
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]

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11.3.3.1 Pointing mosquitoes

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

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.
7. Attach the locality label. Ensure that the proper details are filled in appropriately.

Think of the locality label as protection for the specimen. Pointed specimens bisect the type.
[Figure 18]

8. Attach a unique number to each specimen.

When all of the specimens are mounted and labeled:

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 DOMAIN 3 2012 and is TBR.]

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

1. Prepare the 'NEON Mosquito Diversity Data sheets' and 96 well microplates so they are within easy reach.

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2. Wipe down the work area with 70% ethanol.
3. Apply a unique number to each specimen.
4. Use the appropriate spreadsheet in the 'NEON Mosquito Diversity Data sheets'.
5. Assess the total richness represented in the provided spreadsheet. The number will likely be on the order of 15 different types of specimens per site. At 3 specimens to be sequenced for each of 15 different morphospecies this would be 45 mosquito species per site. This gives 135 specimens per Domain for a season. The maximum number of mosquito specimens to be sent per site is 238 (based on budget) in general. Likely fewer than this will require sequencing in general. For the 2012 Field season the budget is for 95 mosquito specimens. The following is the rationale for selecting specimens:
 - o Mosquito specimens are selected first by checking whether there are 5 or more public sequences represented for the particular species of interest in the BOLD database.
 - Point your browser to: <http://www.boldsystems.org/views/login.php?&>
 - Select 'Taxonomy browser' at the top left of the page
 - Select 'Arthropoda' > 'Insecta' > 'Diptera' > 'Culicidae' > 'Anophelinae' for species in the genus 'Anopheles'
 - (you must also go back and check 'Culicinae' for species in other mosquito genera in North America)
 - Look for the genus of interest in the 'Genera' category and select it
 - The number in square brackets beside each species name is the number of sequences
 - Click on the species name and then click on the species progress link
 - Fill in the number of public sequences > 500 bp in the appropriate spreadsheet tab within the 'NEON Ground Beetle Diversity Datasheets'
 - If there are fewer than 10 public sequences > 500 bp for a species add at least 3 more specimens (if possible) from the season's field sampling
 - If there are more than 10 public sequences only one specimen is required from the season's field sampling

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

1. Flame sterilize the forceps with the ethanol lamp. (If there is difficulty with the wick cut a small piece off. If there is too much air movement in the room create an aluminum foil covered barrier to prevent the draft from affecting the flame).
2. Excise a leg sample from the first specimen.

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3. Place the tissue sample in the well.
[Figure 19]
[Figure 20]
4. Record the unique number in the 'NEON Mosquito Diversity Data sheets'.
5. Periodically sterilize gloves by spraying them with 70% ethanol – AWAY FROM THE ETHANOL LAMP. WAIT until gloves are air dry (not by waiving them, simply by waiting) before proceeding.

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.

If something accidentally falls into a well (e.g. eyelash), note it, do not place a specimen sub-sample into that well and move to the next well. TRY TO AVOID THIS.

Well caps can be applied to wells that are not in use. Note strip caps are numbered and correspond to numbering on plates.

Well plates can be stored at room temperature.

11.3.3.3 Digital Photography

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

Follow all directions that arrive with camera and microscope for proper set up. Specifically for these protocols, the scale bars should be matched between camera and microscope.

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 Diversity Data sheets'.

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]

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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.
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.

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5. Save images to the P drive at P:\FSU\Invertebrates\DOMAIN 3 2012\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 Diversity Data sheets'.

11.3.3.4 Processing images

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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\DOMAIN 3 2012\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. From Start menu select 'Run'
2. Open command prompt: Open Run & type in **cmd**.
3. 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.
4. Navigate to desired folder.
5. 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\DOMAIN 3 2012\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.
6. 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\DOMAIN 3 2012\Mosquito Pictures\edited>**dir /b *.jpg>list.txt**). Note: spaces are required after dir and b in the command.
7. 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

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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...)
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. Attempt to 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\DOMAIN 3 2012\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\DOMAIN 3 2012\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\DOMAIN 3 2012\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.

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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.)
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 DOMAIN 3 2012 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 DOMAIN 3 2012 and is TBR.]

All data and files from the image processing must be backed up and loaded to digital system daily.

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 DOMAIN 3 2012 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.

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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 DOMAIN 3 2012 and is TBR.]

13 REFERENCES

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

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APPENDIX A Field & Lab Data Sheets

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 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 Diversity Data sheets' which includes several sheets where 'P' indicates a version for print and 'D' indicates a digital version.

Sheet	Purpose
FieldSheet_P	Printable sheet for data entry in field. The 0/few/many estimates help to ensure that samples are sorted appropriately in the lab
FieldSheet_D	Digital sheet for entry of the field data
CO2TrapSorting_D	Digital sheet to track trap sorting
BiodiversityDataEntry_P	Printable sheet for entry of the biodiversity information required for upload to BOLD and analyses
BiodiversityDataEntry_D	Digital sheet for entry of the biodiversity information required for upload to BOLD and analyses
SpecimenSelection	Digital sheet for the selection of specimens to DNA barcode
ListInfo	Locked sheet with drop down list choices for ease of filling out sheets; includes coordinates and elevation for the plots and autofills them in other sheets
TaxInfo-Reference	Locked sheet with taxonomic information as an aid for spelling species names and for filling in higher level taxonomic information in the Biodiversity Sheet

The columns in each sheet are TBR but currently look as follows:

FieldSheet_P – See sheet, similar fields as digital sheet different layout

FieldSheet_D

Week

Date

Field sheet filled out by:

Site

Plot ID #

Landcover

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GPS lat

GPS long

Arrival at plot

Departure from plot

Specimens (0/Few/Many)

Notes

Additional notes

Data entered by

Date of data entry

Time elapsed before sorting (ex.10 h)

CO2TrapSorting_D

Plot

Week

Date of Collection (Month/Day)

Sorter's Initials

bloodfed (estimate)

mosquito tubes

bycatch tubes

BiodiversityDataEntry_P – See sheet, similar fields as digital sheet different layout

BiodiversityDataEntry_D

Unique #

Collector

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Collection Date

Collection Method

Plot #

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

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Date of ID

Taxonomist Identifier

Technician Identifier

Length (mm)

Sex

Reproduction

Life Stage

Notes

Date ready to ship to taxonomist

Date received back from taxonomist

Date ready to ship to barcoding institution

SpecimenSelection

Species List

of seq >500 bp in BOLD

at Jones

from Jones to sequence

at Disney

from Disney to sequence

at Ordway

from Ordway to sequence

TOTAL to sequence. Must not be over 95 for all sites in 2012.

ListInfo

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Site

Region

Landcover

Plot ID

Latitude

Longitude

Elevation (m)

specimens

Field Techs Names

Field Techs Initials

Basis of ID

TaxInfo-Reference

Order

Genus

Species

Species author and year

Subfamily

Tribe

Notes

Reference

Date

APPENDIX B Considerations for implementation

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

APPENDIX C Procedure Checklist

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APPENDIX D [This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

APPENDIX E Tables

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]

APPENDIX F Figures

[This section is a draft for the Field Operations Prototype at DOMAIN 3 2012 and is TBR.]



Figure 1

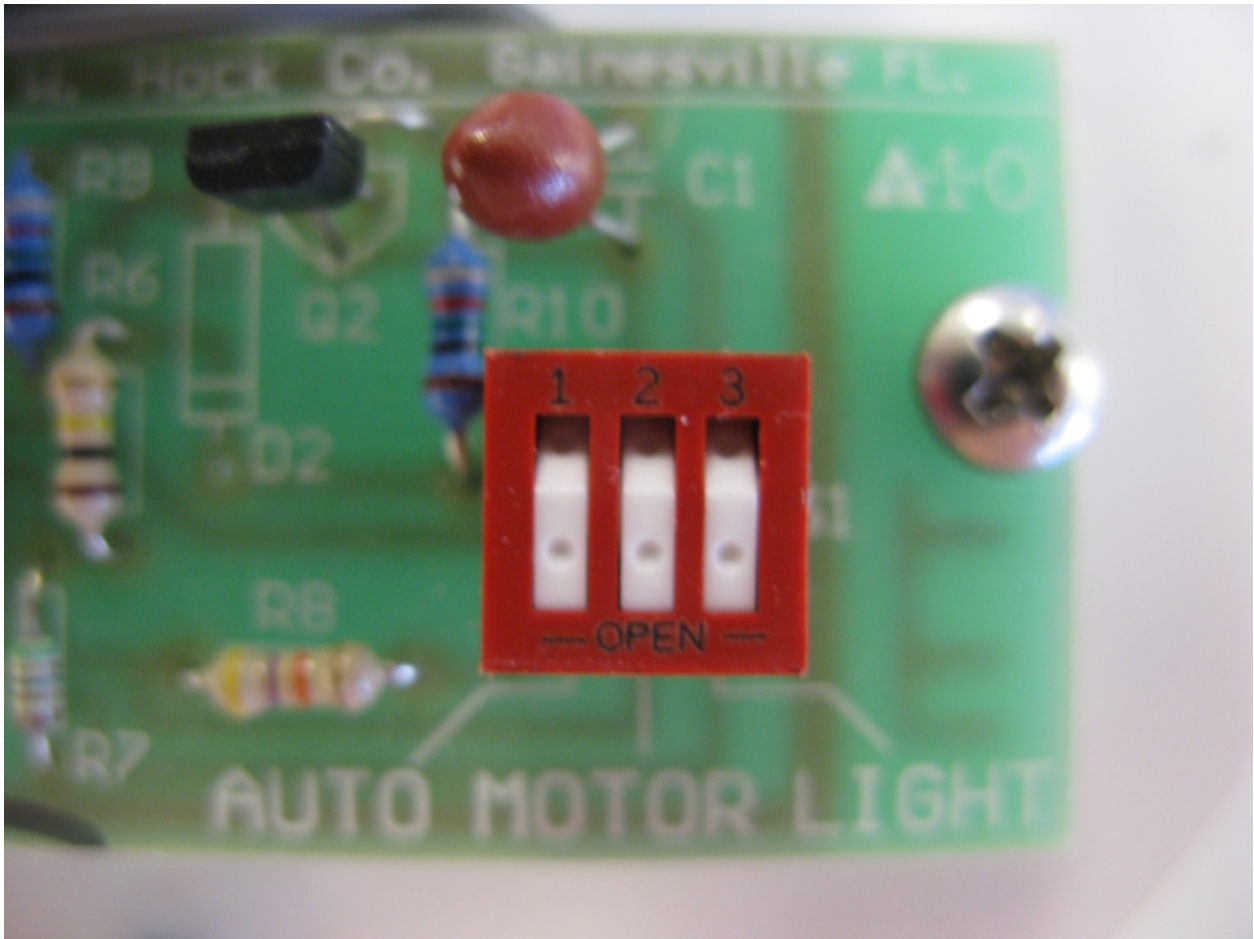


Figure 2

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](#).
NEON.FSU.D10.

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](#).
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

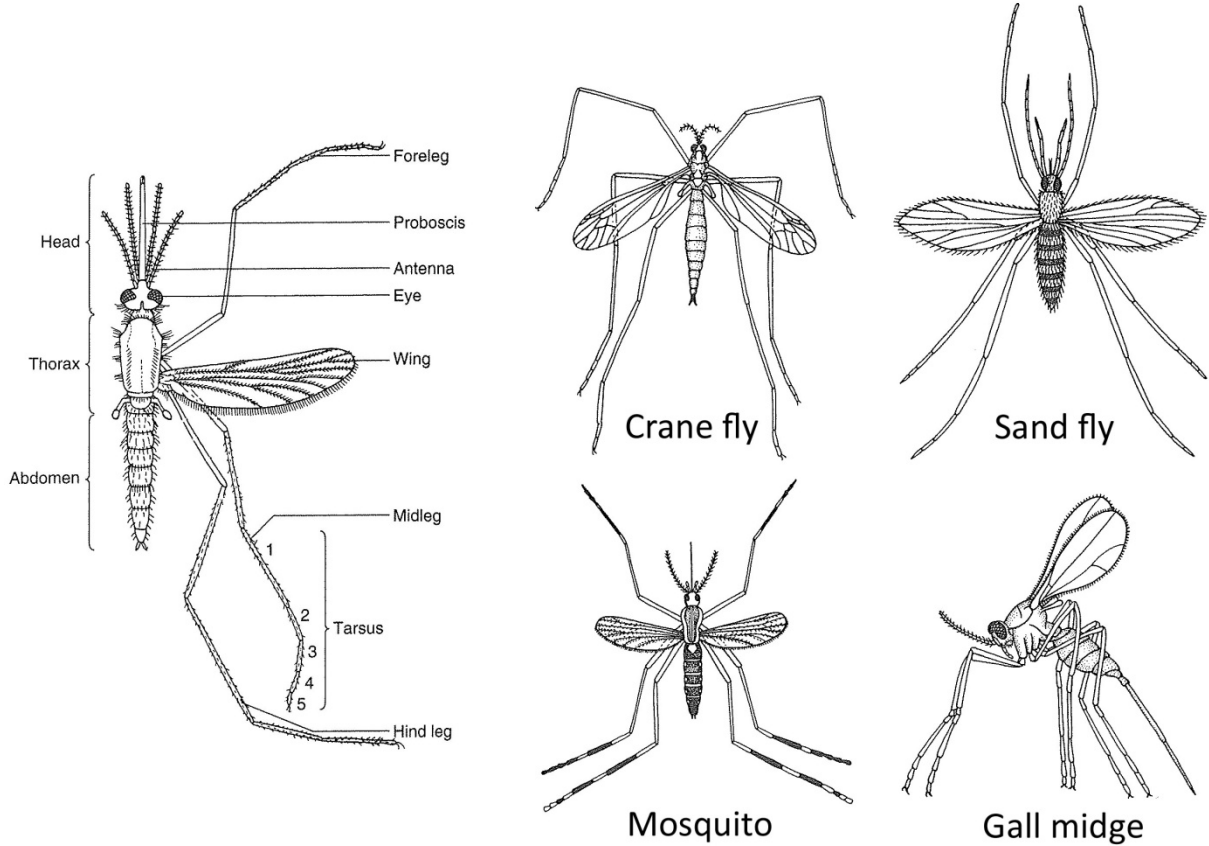


Figure 11

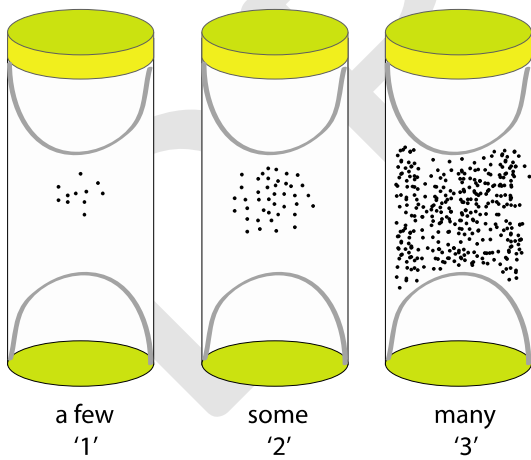


Figure 12

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

Figure 13

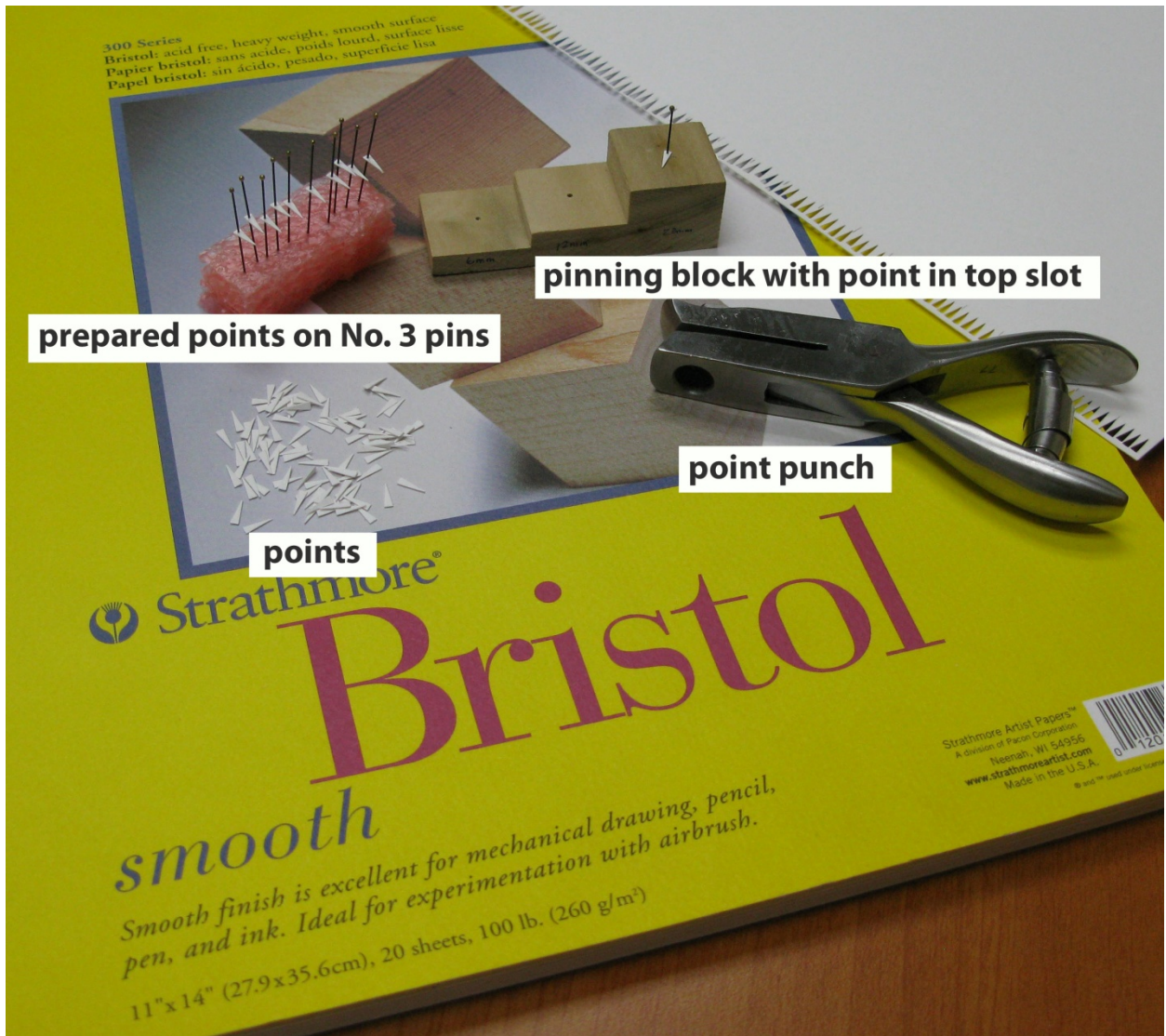


Figure 14

<i>Title:</i> NEON Field and Lab Procedure and Protocol: FSU Mosquito Protocol	<i>Author:</i> C Gibson	<i>Date:</i> 4/29/2012
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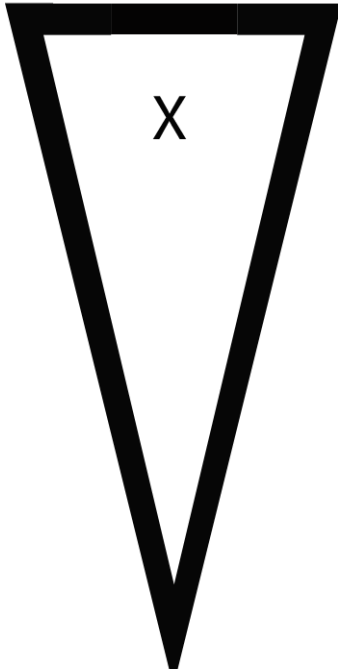


Figure 15

DRAFT

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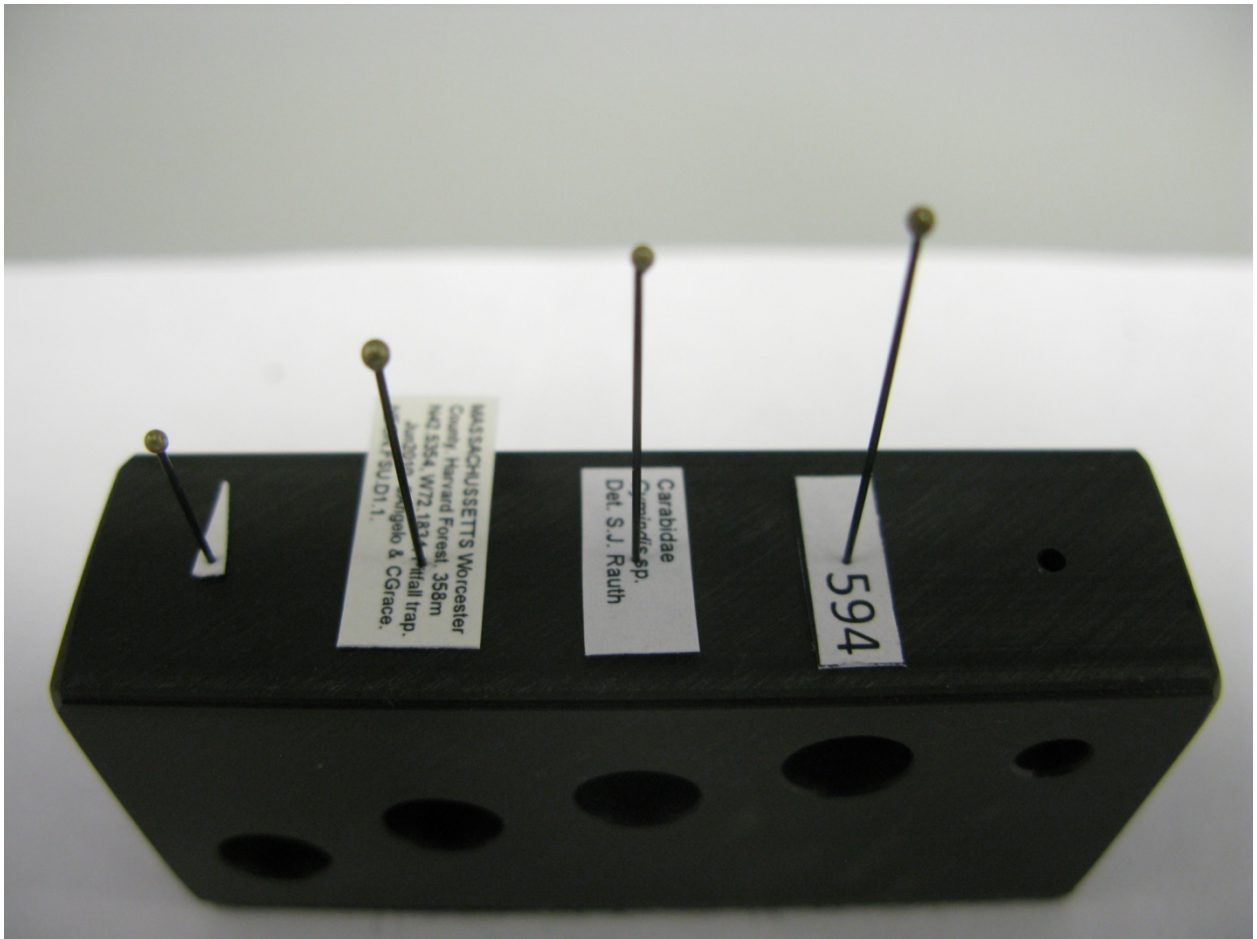


Figure 16

DRAFT

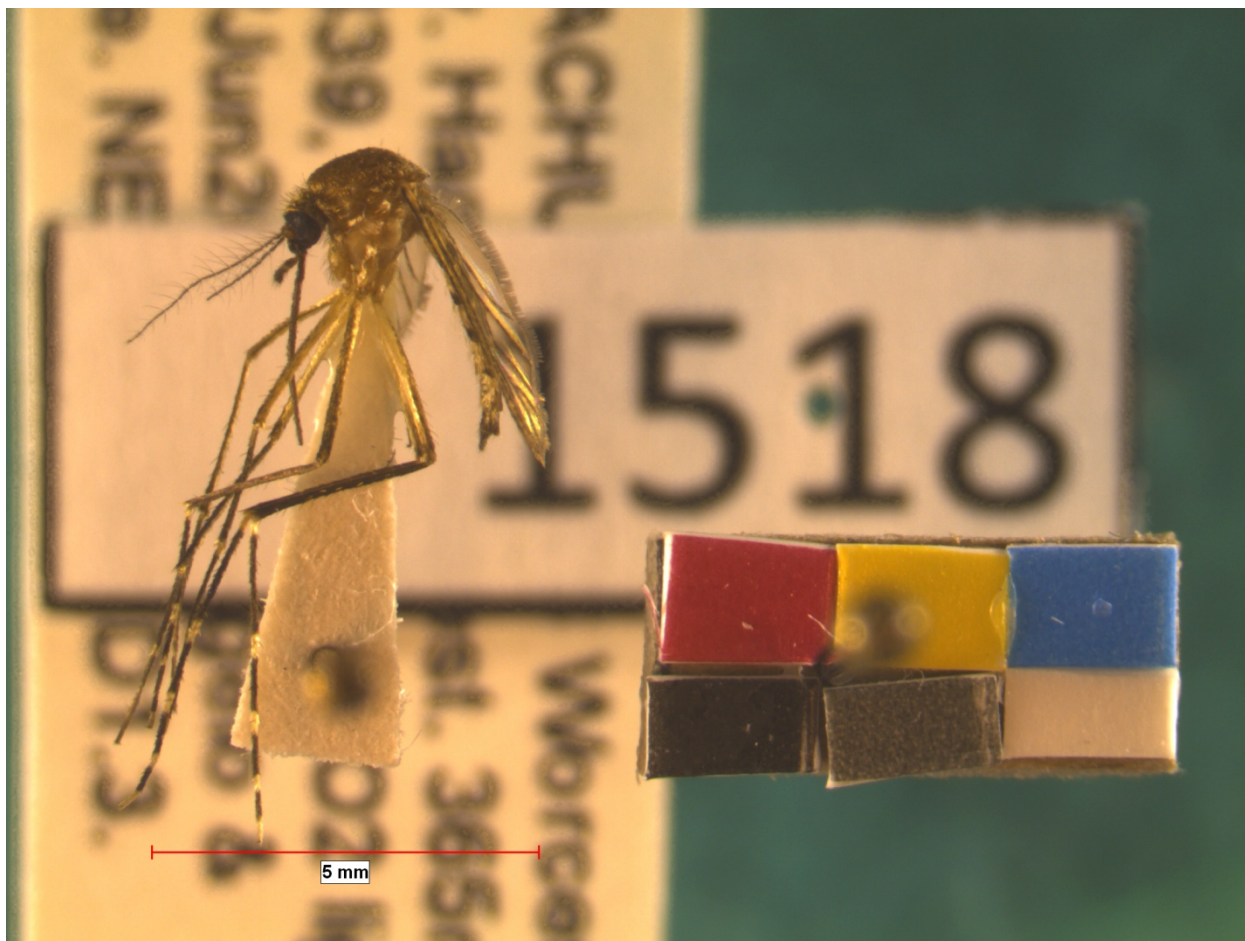


Figure 17

Title: NEON Field and Lab Procedure and Protocol: FSU Mosquito Protocol	Author: C Gibson	Date: 4/29/2012
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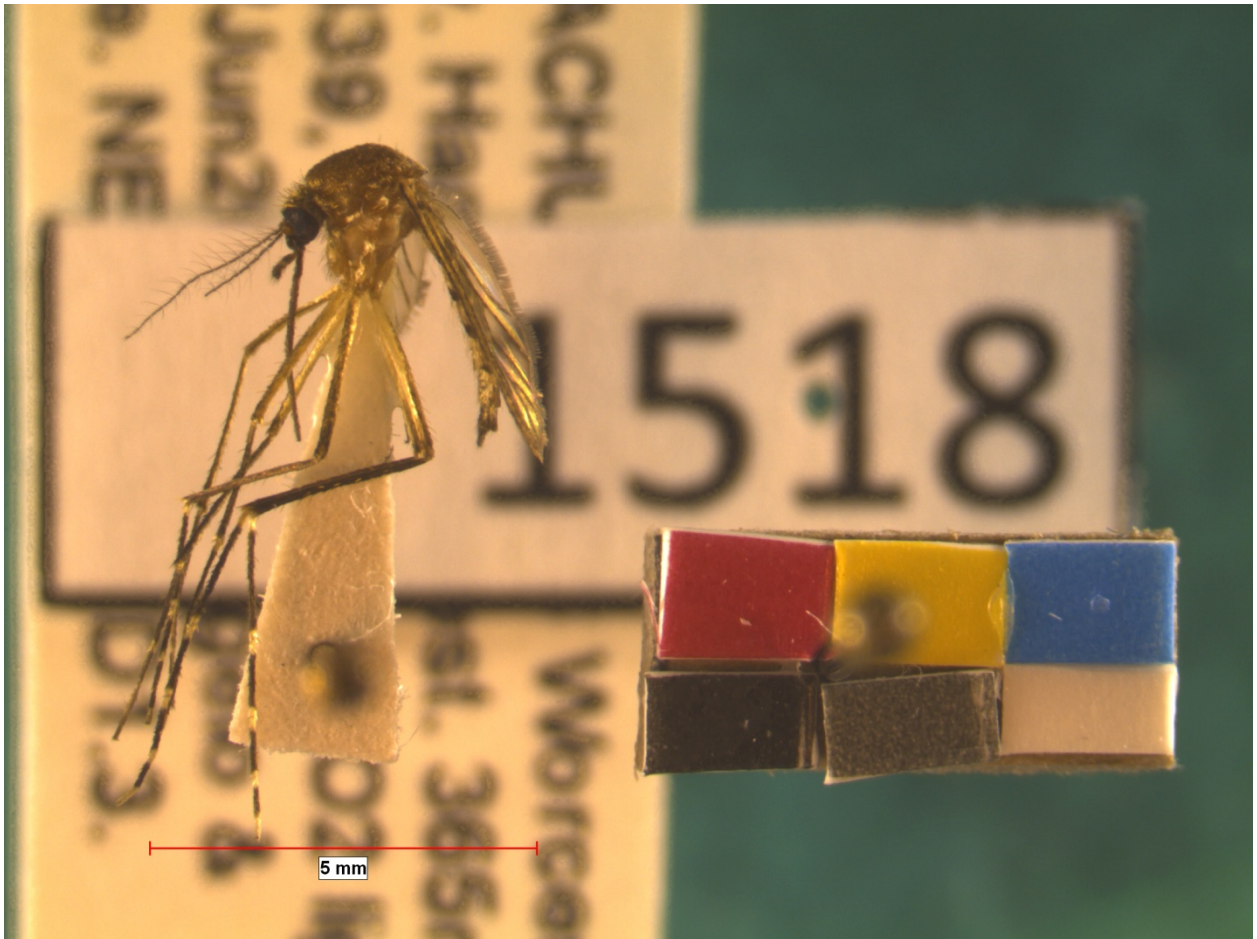


Figure 18

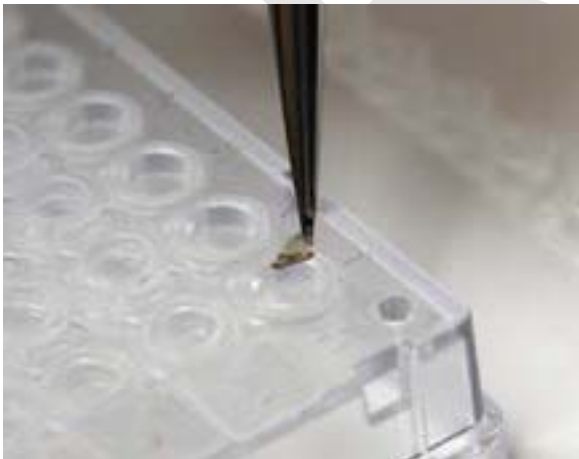


Figure 19

Title: NEON Field and Lab Procedure and Protocol: FSU Mosquito Protocol	Author: C Gibson	Date: 4/29/2012
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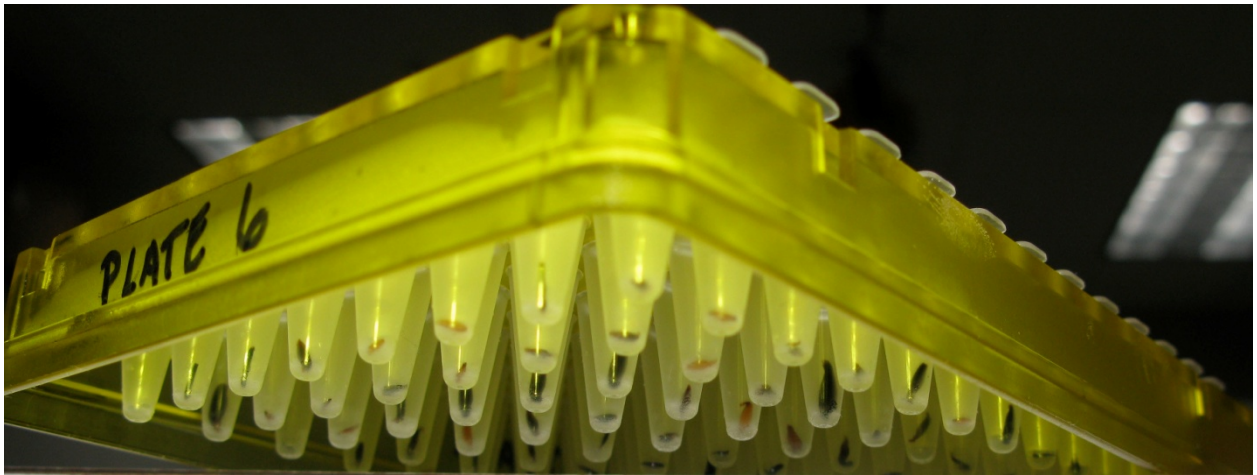


Figure 20

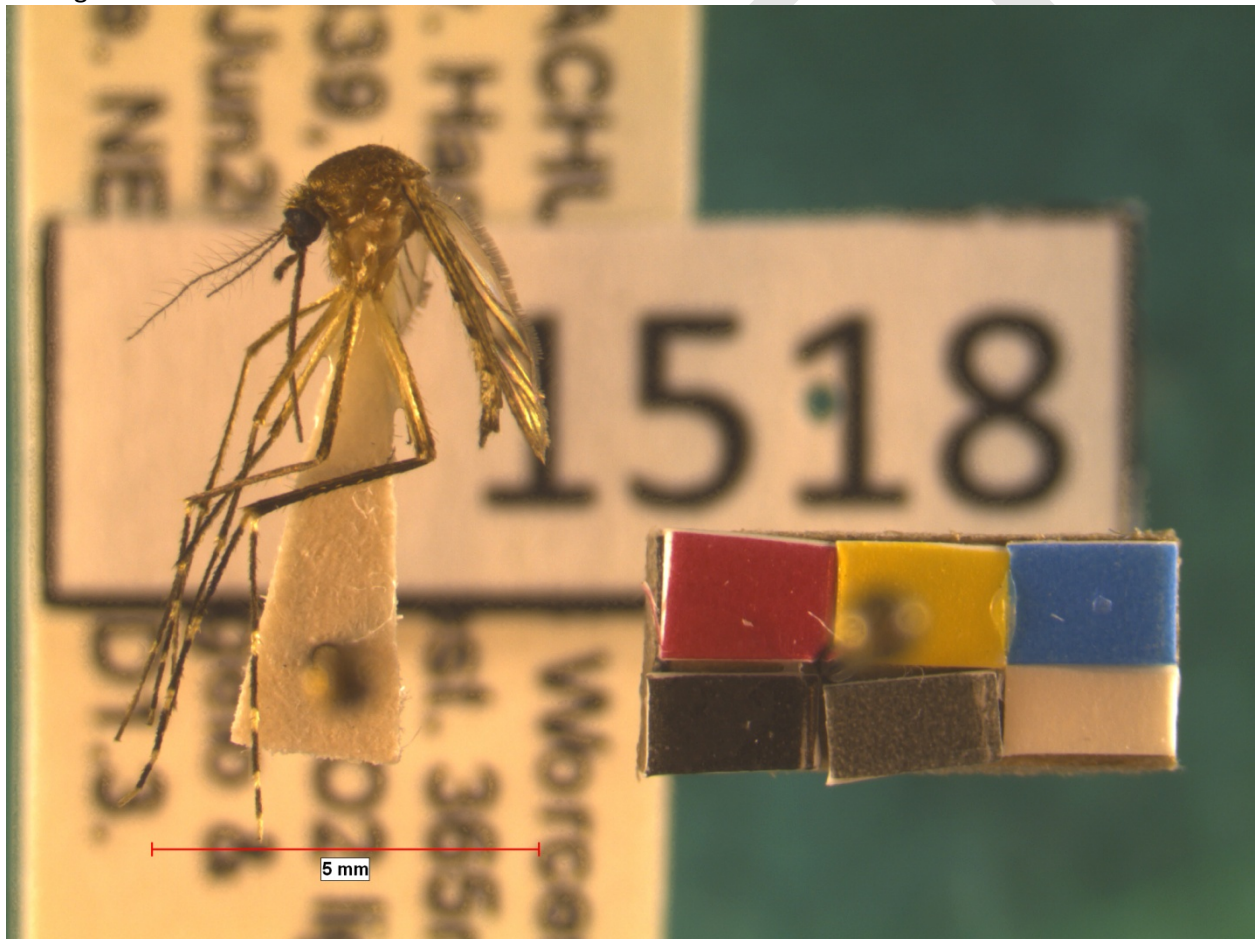


Figure 21

Title: NEON Field and Lab Procedure and Protocol: FSU Mosquito Protocol	Author: C Gibson	Date: 4/29/2012
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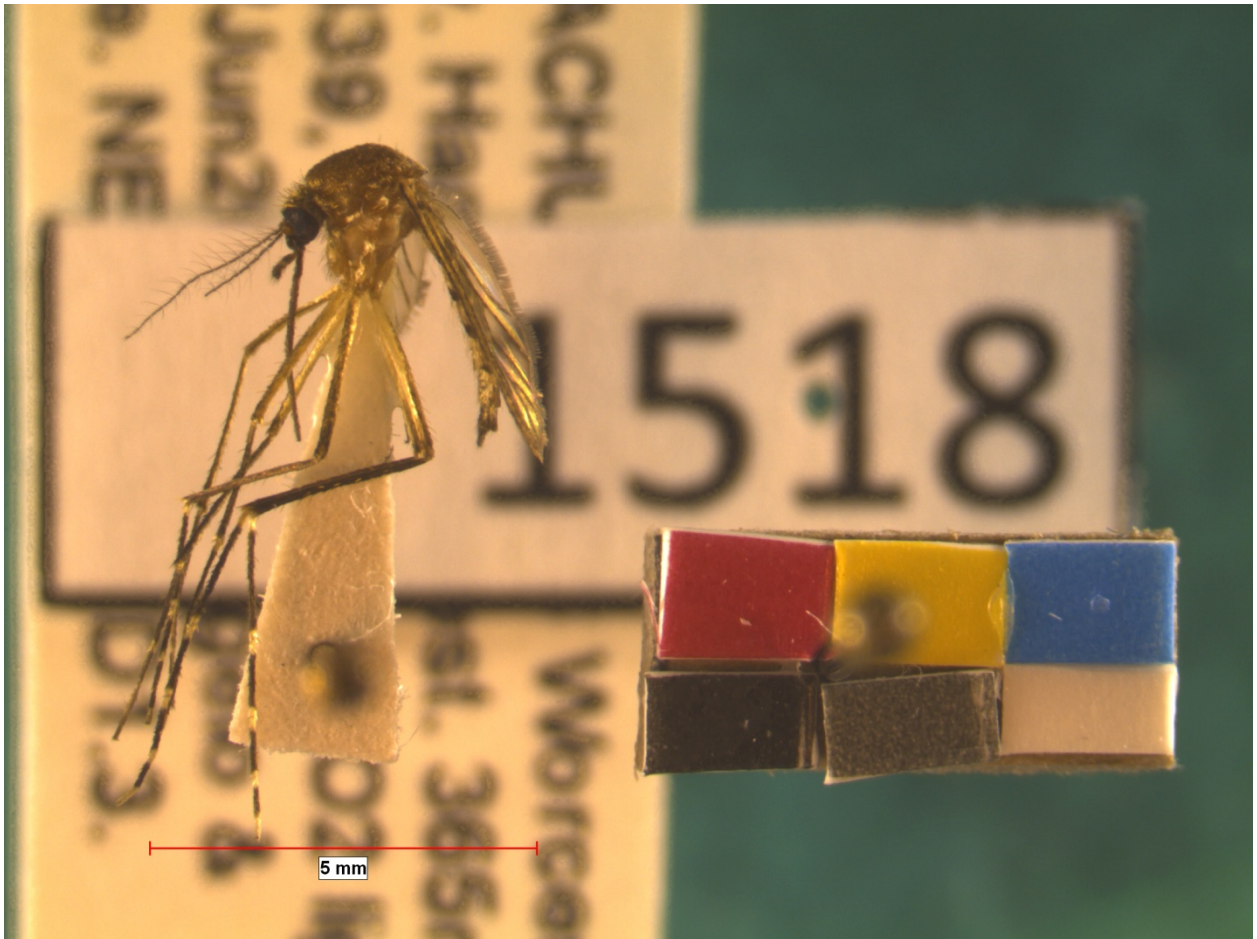


Figure 22

DRAFT

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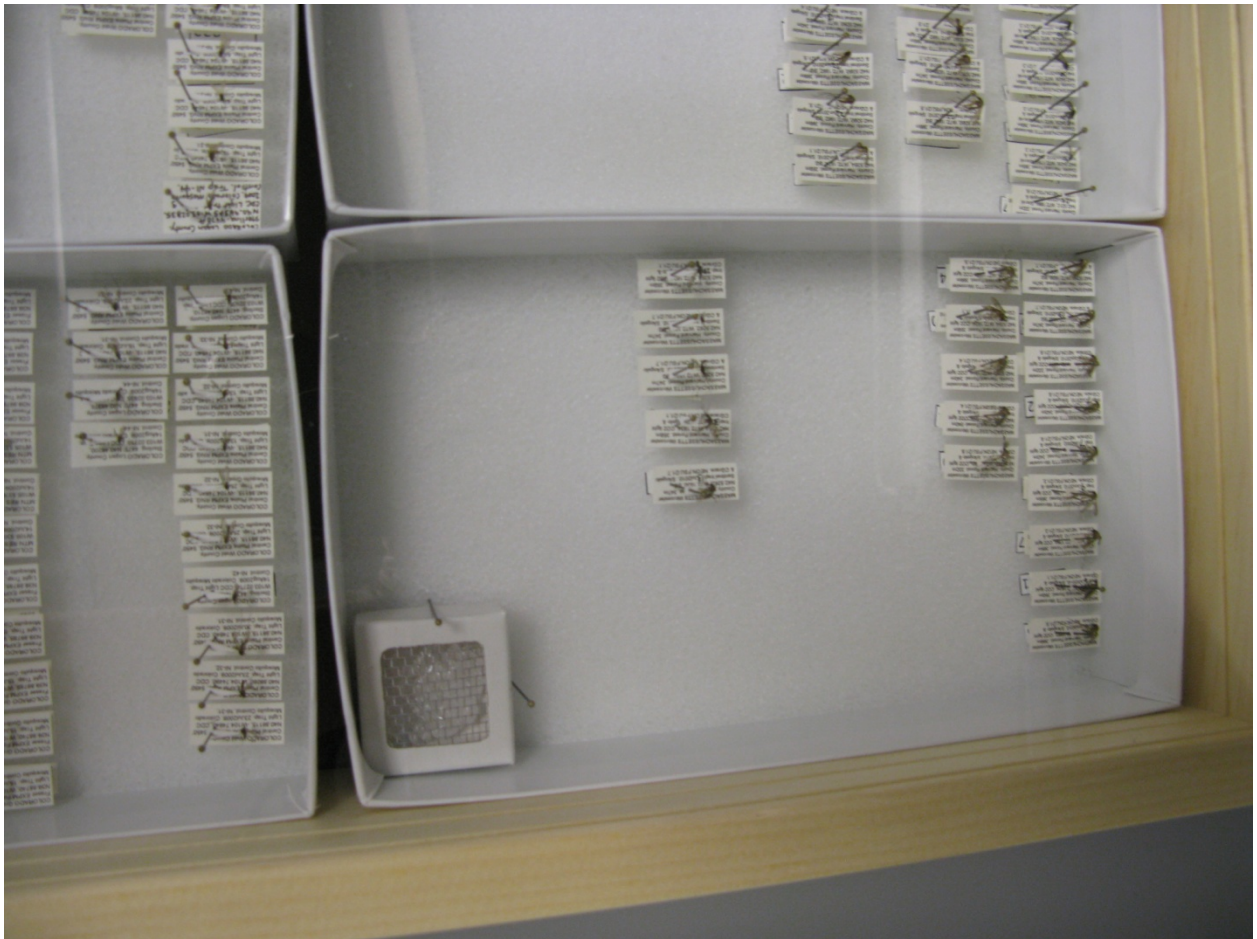


Figure 23

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

Figure 24

<i>Title:</i> NEON Field and Lab Procedure and Protocol: FSU Mosquito Protocol	<i>Author:</i> C Gibson	<i>Date:</i> 4/29/2012
<i>NEON Doc. #:</i> NEON.DOC.01049		<i>Revision:</i> B_DRAFT



Figure 25

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