Welcome to Part Three: Life and Task 3-1. In Part Two you learned more about what people in your community think about mosquitoes. Now the team will begin learning more about the life of the mosquito. For the tasks later in Part Three, it will be beneficial to collect and observe live mosquito eggs, larvae, and pupae in your research site. You may have already started doing this in Task 1-4. However, if you have not, the collection process can take some time. So it’s a good idea to build and set out the traps and then monitor them daily while you are working on the other tasks in Part Three.

Objective

In this task, the team will be focusing on the following questions from the question map. How can we monitor the distribution of mosquitoes?

Monitoring the local mosquitoes can help the team determine where mosquitoes are distributed across the community. Teams will also have the opportunity to participate in a larger Citizen Science project called The Invasive Mosquito Project. This project involves collecting samples from teams over a much larger area. This data will be used to make larger distribution maps of mosquitoes. Participation in the Citizen Science project is voluntary, but participate if you can.

1. Go to the Task 3-1 folder and get the Collecting Mosquito Eggs step-by-step guide for setting up collection cups, Egg Collection procedure, Live Collection instructions, Citizen Science- Invasive Mosquito Project background information, and Invasive Mosquito Project collection form.

2. As a team, use the step-by-step guide and egg collection procedure to set up egg collection cups in your research site. If you're experimenting with the collection design, outline your experimental design. Read the safety notes in the procedure and collection notes before placing cups outside.
3. Mark your research site map from Task 2-1 with the location of your team’s cups.

4. Monitor the cups daily. Refill with water as needed. Be careful of any eggs or larvae in the container.

5. Leave the cups out for seven to fourteen days. Then follow the collection procedure to observe any eggs, larvae, or pupae.

6. Use the Invasive Mosquito Project Collection record form to collect data. Use this even if the team is not participating in the Citizen Science project.

7. If you're participating in the Citizen Science project, mail some of the eggs according to the procedure and collection notes. Save some eggs for others tasks later in this part.

8. If you have found a different Citizen Science project involving mosquito eggs to participate in, do that!

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**Research Tip**

More information about identifying and storing collected eggs and larvae is included in Tasks 3-4 and 3-5, if you need it now.

**Citizen Science Tip**

The more people that participate in Citizen Science projects, the more we all will learn from them. Think about how you could gather information about your community to be part of these global projects.

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Hooray! You completed Task 3-1. Check it off the task list. *Go to Task 3-2!*
Task 3-1 Collecting Mosquito Eggs

Materials and Procedure
General Materials

- Scissors
- Pliers or other sharp object
- Permanent marker
- Scotch tape
- Water
Oviposition Cups

You will need TWO cups. Any material will work.

The interior of the cups must be a dark color.

If you do not have dark cups, you can use tape, fabric, or another material to make the inside of the cups dark.
Oviposition Surface/Substrate

You will need a surface for the mosquito eggs to cling to.

Here are some examples of materials that can be used:

- Paper towel
- Wooden tongue depressor
- Popsicle stick
- Cotton fabric
Step 1: Prepare Oviposition Cups

Label each cup with:
• Its name (A or B)
• Its location (sun or shade)
• Today’s date

Use the pliers (or other sharp object) to poke 2-3 small holes in each cup.

The holes should be about 2/3 of the way up from the bottom of the cup.

The holes will drain excess water.
Step 2: Prepare Oviposition Surface/Substrate

**Option 1: Paper towel or fabric**

Measure and cut 2 strips of material.

They should be about 2/3 of the height of the cup.

Label the corner of each strip the same way you labeled the cups.

**Option 2: Popsicle stick or tongue depressor**

You will need two sticks.

Label the tips of each stick the same way you labeled the cups.
Step 3: Assemble Collection Device

Option 1: Paper towel or fabric

Roll each strip so that the label is on the inside and place it inside the corresponding cup.

The material should sit about 1/3 of the way from the bottom of the cup. Secure with tape if necessary.

Option 2: Popsicle stick or tongue depressor

Place each stick inside the corresponding cup.
Step 4: Placing the Trapping Cups (Sun vs. Shade)

Fill the cups about 2/3 of the way up with water. Excess water will drain out of the holes.

Place Cup A in a sunny location and Cup B in a shady location.

Mark the location of your cups on your research map you created in Task 2-1.

Using the Egg Collection form, document:
• The presence of plants within a 5 foot radius of cup.
Step 4: Collecting Eggs

Tip: If you live in a windy area, you can place a washer, rock, or other heavy object in the bottom of the cup to weigh it down.
Step 5: Experiment Notes

• In this experiment, you will collect data on whether eggs are found in a particular container or not (sun vs. shade).

• The Response variable is the presence of eggs in a particular container or not.

• The Explanatory variable is the location (sun vs. shade)

• Create a hypothesis about which container you think will capture more eggs.
Step 6: Collecting Eggs

- Leave the cups out for 7 days, refilling to 2/3 level as necessary. Check daily.
- After 7 days, take the germination surface out of the cup to collect your eggs. Check the water in the cup for any larva moving around.
- If you find larva in the water, move them to a container, such as the setup option for an Emergence Chamber described in Task 3-4.
- Use one of those setups so you can safely observe them over the next few weeks.
- Complete the rest of the collection form.
- If you are participating in the Invasive Mosquito Project, mail your eggs according to the directions in the egg collection procedure.
- Even if you are participating in the Invasive Mosquito Project, save some of the eggs and place them into either the Adult mosquito trap in the Emergence Chamber setup described in task 3-4. This will allow you to safely observe first instar larvae hatching from eggs and further develop into fully mature larvae and pupae inside of the container.
Including Experimental Design in Egg Collection (optional)

• The egg collection experiment is a great opportunity to explore and test the many different variables in the design.

• These experiments can help your team evaluate and determine which collection setups work the best in your location.

• As you work through this guide, think about how you could setup an experiment to explore different variables in this setup.

• Think about how you could control these variables to determine their effect on mosquito egg capture.

• Use the following guidance to design an experiment of your own.

• This is not required, but an option if you are interested in exploring!
Including Experimental Design (optional)

There are a variety of variables you can test in this setup. As you learn more about the basic collection procedure think about how you could design an experiment to test these variables and their effect on mosquito egg collection.

- Cup Size
- Cup color
- Cup interior color
- Cup material
- Cup Opacity
- Water color or composition (add food coloring, organic matter such as soil or plant matter, fish food, sugar, yeast)
- Hole placement
- Amount of cup that is covered or open
- Germination surface (wood, paper towel, cotton, wood wrapped in paper towel or cotton)
- Water vs. no water
- Cup placement (shade, sun, partial sun, inside vs. outside)
Including Experimental Design in Egg Collection (optional)

Use the following questions to help you design your experiment.

• State your question.
• State the purpose of your experiment.
• State your Hypothesis.

• List your variables.
  • What is your independent variable?
  • What is your dependent variable(s)?
  • What is your controlled variable(s)?
  • Make sure you are conducting a fair test by only changing one variable per experiment.

• Design your Experiment.
  • What materials will you use?
  • Write out the procedure for your experiment.
  • Provide any pictures or drawings to illustrate your setup.
  • What will your control setup include?
  • Will you conduct multiple trials?

• Conduct Experiment and Share results with the team.
Variables— Conducting additional Experiments

You can test different *variables* of the egg collector to see which features make for the most effective design.

Suggestion 1:
Change the coverage of the germination paper in the cup. But, keep the amount of water, size of cup, and color inside all constant.

Suggestion 2:
Change the color of the inside of the cup. But, keep the amount of water, size of cup, and color inside all constant.

Suggestion 3:
Change the size of the cup. But, keep the amount of water, the color inside, and the coverage of the germination paper all constant.

These are a few suggestions, but feel free to get creative and think about what other variables can be changed. Remember to only change one variable at a time when conducting more tests. Keep all other variables constant. This way you can determine what variables are making certain traps more effective in collecting eggs.

- Cup material
- Cup Opacity
- Water color (add food coloring or organic matter)
- Hole placement
- Interior material composition (paper/cotton/polyester/plastic)
- Amount of cup that is covered or open
Others ways to collect mosquito larvae

You can use any vessel that holds standing water to collect mosquito larvae. Here is a list of some ideas:

- Empty food containers
- Buckets
- Bird baths
- Tires
- Tarps
- Wheelbarrows
- Toys

***Just make sure if you place a collection vessel outside to check it regularly so eggs are not given enough time to grow into adults.
Collected Eggs Example
Just remember they are very small!
Active Surveillance - Others ways to collect mosquito larvae

Collect live samples
1. Go outside where your team meets or around your house and look for live mosquito larva + pupae in puddles, water filled containers, and anywhere that water naturally pools.

2. Look for things that wiggle around when the water or container is disturbed.

3. Use a net, spoon, eye dropper, turkey baster, or other device to move the larva into a container for observation. Observation containers can be plastic bags, clear plastic bottles or glass jars.

4. If taking the containers with live larva or pupae inside a building, make sure they cannot escape if they turn into adult mosquitoes.

5. If you are able to view the collected larva or pupae under a microscope, do it! Describe what you see.

• ***Just make sure if you place a collection vessel outside to check it regularly so eggs are not given enough time to grow into adults.
Citizen Science in United States

• If you are collecting mosquito eggs in the United, participate in the Invasive Mosquito project if you can. Find more information here to participate:
  • http://www.citizenscience.us/

Citizen Science in Australia

• If you are collecting mosquito eggs in Australia, participate in the Zika Mozzie Seeker project if you can. Find more information here to participate:
Active Surveillance - Others ways to collect mosquito larvae

Collect live samples
1. Go outside where your team meets or around your house and look for live mosquito larva + pupae in puddles, water filled containers, and anywhere that water naturally pools.

2. Look for things that wiggle around when the water or container is disturbed.

3. Use a net, spoon, eye dropper, turkey baster, or other device to move the larva into a container for observation. Observation containers can be plastic bags, clear plastic bottles or glass jars.

4. If taking the containers with live larva or pupae inside a building, make sure they cannot escape if they turn into adult mosquitoes.

5. If you are able to view the collected larva or pupae under a microscope, do it! Describe what you see.

• ***Just make sure if you place a collection vessel outside to check it regularly so eggs are not given enough time to grow into adults.
Task 3-1 Collecting Mosquito Eggs

Use the included resources for background, procedures, collection forms, and a step-by-step guide for setting up and monitoring mosquito egg collection cups. These resources include:

- Invasive Mosquito Project
  - Egg Collection Notes
  - Egg Collection Procedure and Protocol
  - Collection Record Form
- Step-by-step guide to making collection cups and monitor eggs
  - Includes optional experimental design activities for the team, if you’re interested

Information on Invasive Mosquito Project—Optional Citizen Science Project

The Invasive Mosquito Project is a citizen science project that anyone can participate in. It is aimed at teaching research teams all over the world how to collect and monitor invasive mosquito species that live in containers. By doing this monitoring, we can determine where invasive mosquito species, as well as native species, are distributed across the world and define at-risk human and animal populations based on this distribution. This citizen science project provides anyone interested the protocols and opportunity to collect real data and contribute to a national mosquito species distribution study.

Participation in the Invasive Mosquito Project is completely voluntary. So no pressure at all. This is something we are recommending you participate in, but it is up to your team to decide. However, even if you decide not to participate in the Invasive Mosquito Project, we recommend the team still use the same collection protocols from this project to collect and study mosquitoes in your local space on your own.

So, let’s learn more about some protocols for collecting mosquito eggs and adults in your research site. Find more information here to participate: http://www.citizenscience.us/
Invasive Mosquito Project

Collection notes

Egg Collection: In order to get a census of invasive mosquito populations, students are asked to collect eggs and send the eggs to the USDA. Doing so will give the USDA a good figure of invasive mosquito population distributions across the United States which can help with public health.

Procedure: See “Egg Collection Procedure” handout

Placement: Each student will set out 2 cups, one labeled “A” and the other labeled “B”. Cup A is to be placed in a sunny location and Cup B is to be placed in a protected shady location. On the egg collection paper, students are encouraged to write about the surroundings of the cup (such as flowering vegetation nearby, shade sources, and cover plants, trees, etc).

Safety: Aedes mosquitoes feed throughout the day, therefore students should wear long sleeves, long pants, and insect repellent when near the oviposition cups. These precautions will limit exposure to potential mosquito bites and encourage good behavior and personal protection. Also, students should only place cups around their homes—traveling is not required for this process and will reduce the risk of travel-related injury.

Collection: After a week of letting the oviposition cups sit outside, the collected eggs will be dried with the egg paper and sent to the USDA in Manhattan, Kansas. If desired, ¼ of the eggs may be raised to adulthood as part of a second lesson plan where students will learn more about scientific protocols and the life stages of mosquitoes.

Counting and Shipping: When all the eggs have been collected, students should count the number of eggs on each paper and record on their personal record sheet as well as contribute to class data for shade vs. sun egg collection. Students may input their information from their personal recording sheets into the website so it may be viewed by everyone. Students should observe the difference between eggs (a magnifying glass or microscope will be useful for this) and record information with class data. Class data can be basis for discussion (why one location might have more eggs than another). When observations and discussion have ended, send 3/4th of slightly damp paper (paper should not crinkle) and eggs to a local collaborator (within the same city) who can do the identifications or the USDA in Manhattan, Kansas (USDA-ABADRU – Invasive Mosquito Project, 1515 College Ave., Manhattan, KS 66502). Please be careful when shipping eggs; do not send them to other locations because these are invasive species and can colonize locations quickly and easily. If desired, ¼ of the eggs can be reared to adulthood as part of a 2nd lesson that will also discuss in more detail the mosquito life-cycle, mosquito adaptations, transovarially transmitted mosquito diseases, and scientific protocol.
Materials

Materials participant must provide:

- Oviposition cups (ideally, interior of cup should be black, red, or dark colored)
- Germination paper (brown or dark paper towel)
- Water
- Tape
- Permanent Marker

Procedure

1. With a permanent marker, label germination paper (paper towels) with location information, date, cup name (A or B) and water level
2. Take germination paper and place ends together as to make a large tube with the paper
3. Place the rolled paper in the oviposition cup
4. Label the cup “A” on the exterior and be sure the properly labeled paper is in the cup
5. Punch drainage holes in the cup at 2/3 of the way to prevent over filling with water during rain or when refilling. Then fill the cup 2/3 of the way with water.
6. Repeat steps 1-3 one more time for the second cup, but label the cup “B”
7. Place cup A in a very sunny location with full sun most of the day; (the location can be any place on dirt, cement, decks, etc.) and tape or weight it down to prevent it from being knocked over.
8. Place cup B in a permanently shady and protected location such as under a roof or in a bush.
9. Check on the cups’ water levels once a day
   a. If the water has evaporated, refill to the 2/3 level
   b. The cup should not fill with water because of the drainage holes, but if it has, carefully dump the water so it returns to the 2/3 level (be careful of any eggs in the container)
10. Leave cups out for seven days
11. After seven days, take germination paper out of the cup and let it air dry with the eggs (this will prevent them from hatching). The paper should be slightly damp where the paper does not crinkle. Fold paper in thirds and place into a ziplock bag.
12. Take paper to class on scheduled day and count eggs and record datasheet to email to USDA

Safety

To avoid contact with the egg-laying mosquitoes, avoid going near oviposition cups when possible, except to add water. Participants should wear long sleeves, pants, and mosquito repellent when near the cups. For the safety of the participants and their collections, cups should be placed near the home. Teachers might also find a location near their classroom for additional cups. When observations and discussion have ended, send 3/4th of dried paper and eggs to a local collaborator (within the same city) who can do the identifications or the USDA in Manhattan, Kansas (USDA-ABADRU – Invasive Mosquito Project, 1515 College Ave., Manhattan, KS 66502). Please be careful when shipping eggs; do not send them to other locations because these are invasive species and can colonize locations quickly and easily.
# Invasive Mosquito Project

## Collection Record Form

<table>
<thead>
<tr>
<th>School Name:</th>
<th>Teacher’s Name:</th>
</tr>
</thead>
<tbody>
<tr>
<td>School’s Street Address:</td>
<td>Teacher’s Email:</td>
</tr>
<tr>
<td>School District Number:</td>
<td>Date Cup was Placed (YYYY/MM/DD):</td>
</tr>
<tr>
<td>City/State/Zip Code:</td>
<td>Date Cup was Retrieved (YYYY/MM/DD):</td>
</tr>
<tr>
<td>County:</td>
<td>Collector Name(s):</td>
</tr>
</tbody>
</table>

### How mosquito eggs, larvae, or pupae were collected (circle one or many)

<table>
<thead>
<tr>
<th>Eggs</th>
<th>Larvae/Pupae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open Water</td>
<td>Contained Water</td>
</tr>
</tbody>
</table>

### Location of cup (circle one)

<table>
<thead>
<tr>
<th>Oviposition cup</th>
<th>Dipper cup</th>
<th>Turkey baster</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shade</td>
<td>Sun</td>
<td></td>
</tr>
</tbody>
</table>

### What mosquito stage was collected? (circle one or many)

<table>
<thead>
<tr>
<th>Eggs</th>
<th>Larvae</th>
<th>Pupae</th>
<th>Adults</th>
</tr>
</thead>
</table>

### How many? (circle one or many)

<table>
<thead>
<tr>
<th>Eggs</th>
<th>Larvae</th>
<th>Pupae</th>
<th>Adults</th>
</tr>
</thead>
</table>

### What species of mosquito?

### Are you sending the mosquito(s) to be identified (circle one)?

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

### Number of individuals of each mosquito species collected

### Biotic Factors

Presence of Plants Within 5-Foot Radius of Cup (circle one or many):

- Trees
- Bushes
- Grass
- Flowers

### Abiotic Factors

Temperature (°F) of Day Cup was Retrieved:

Weather Conditions of Day Cup was Retrieved:

Temperature (°F) Day Before Cup was Retrieved:

Weather Conditions Day Before Cup was Retrieved:
Principal Characters for Identifying Mosquitoes of General Importance

<table>
<thead>
<tr>
<th>ANOPHELES</th>
<th>AEDES</th>
<th>CULEX</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EGGS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laid singly</td>
<td>Has floats</td>
<td>Laid singly</td>
</tr>
<tr>
<td><strong>LARVAE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest parallel to water surface</td>
<td>No air tube</td>
<td>Head rotated 180° when feeding</td>
</tr>
<tr>
<td><strong>PUPAE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pupae differ slightly</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ADULTS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maxillary palp as long as proboscis</td>
<td>Maxillary palp shorter than proboscis</td>
<td>Abdominal tip pointed</td>
</tr>
</tbody>
</table>

New Jersey Agriculture Experiment Station Publication SA220-SM-86

Mosquitoes in Your Life
Donald J. Sutherland, Research Professor in Entomology and Wayne J. Crans, Associate Research Professor in Entomology
Invasive Mosquito Project Link
http://www.citizenscience.us/imp/
Team News Articles for Task 3-1

Lee Cohnstaedt USDA News Article 1

Lee Cohnstaedt News Article 2