Continuing the Role of the Citizen Scientist: Larval & Pupal Collections for National Mosquito Distribution Surveys

Julie Tseouras, William Walton, Roselyn Schimerlik, Lee W. Cohnstaedt

Abstract

New introductions to invasive mosquitoes, Aedes aegypti and Aedes albopictus, increase the risk for vector-borne diseases such as dengue, chikungunya, and Zika in the United States. Tracking these new introductions is more important than ever. This lesson plan focuses on the collection of mosquito larvae and pupae before the onset of summer with a focus on insect development. Students will observe the immature mosquitoes grow and metamorphose into adults. Novel aspects include collecting larvae and pupae around the home/school, observing the mosquito life cycle by safely rearing them from immature stages (larvae and pupae) to adults, and learning important background information on mosquito biology and pathogens that mosquitoes can transmit. The lesson describes new tools to use with the Invasive Mosquito Project, an international citizen science–based mosquito surveillance program exploring mosquitoes and the pathogens they may transmit to interested community members (including students and teachers) and their companion animals. This project is a stand-alone or follow-up lesson plan to the mosquito egg collection lesson used prior to the onset of winter (see Cohnstaedt et al., 2016). Students are able to participate in a hands-on tutorial to build a sealed emergence chamber to safely raise mosquitoes. Long-term data sets can also be used by teachers and students for further classroom discussions on the risks of mosquito-borne illnesses nationwide. This lesson further focuses on how individuals must play an active role in protecting their communities and pets from illness and increasing awareness of the dangerous pathogens mosquitoes can transmit and the importance of mosquito management. Materials from this lesson plan (available at http://www.citizenscience.us) can be adapted for each classroom but are best-suited for middle school to high school classes, as well as Advanced Placement classes.

Key Words: Aedes aegypti; yellow fever mosquito; Aedes albopictus; Asian tiger mosquito; citizen science; invasive species; mosquito surveillance; public health; epidemiology; environmental science.

Mosquitoes & Vector-Borne Diseases

Aedes aegypti, the yellow fever mosquito, has been an important vector monitored in the United States for many years. Aedes aegypti was introduced from Africa to the New World during European colonization (Nelson, 1986). The first confirmed outbreak of a vector-borne disease in the New World dates to 1648, when yellow fever struck the Yucatan (Powell & Tabachnick, 2013). However, transmission of yellow fever virus by Aedes aegypti was not confirmed until 1900 (Nelson, 1986). Today, with the advent of a global economy and ease of international travel, new mosquito species are introduced frequently within the United States (Figure 1), which means an ever-changing landscape of disease risks.

Although yellow fever was eradicated from the United States, other mosquito-transmitted pathogens present a risk to humans, animals (mainly horses and birds), and wildlife every year. These pathogens include the encephalitis viruses West Nile, eastern equine encephalitis, western equine encephalitis, La Crosse encephalitis, St. Louis encephalitis, and dengue (Centers for Disease Control and Prevention, 2022). Mosquito control programs and public health education focusing on these mosquito-transmitted viruses stress the importance of quickly detecting known mosquito vector species and virus transmission. The Invasive Mosquito Project (IMP), started in 2016, aims to help map the ever-changing and expanding geographic distribution of mosquito species within the United States (Cohnstaedt et al., 2016).

Surveying for mosquito distributions involves trapping mosquitoes and identifying the species present (species composition) in the community. Correct species identification is essential to determining risk of certain vector-borne diseases because only a few mosquito species are capable of transmitting viral pathogens. The IMP egg collection lesson plan was published in The American Biology Teacher (Cohnstaedt et al., 2016) as a way of monitoring these vector-competent mosquito species while involving interested community members (including students and teachers) as participants in a citizen science project will help motivate students to be active participants in the scientific community.
project to collect real data and contribute to a research project with the U.S. Department of Agriculture (USDA). IMP targets *Aedes aegypti* and *Aedes albopictus*, which are invasive, container-inhabiting mosquitoes associated with diseases such as dengue, chikungunya, and Zika. The geographic distribution of these two mosquito vectors remains uncertain and dynamic, with us relying on models that calculate these mosquitoes’ potential geographical ranges (Figure 2 and Figure 3). As long as the distribution of *Aedes aegypti* and *Aedes albopictus* remains unknown, people remain at risk of virus transmission in the United States.

In 1999, an outbreak of mosquito-transmitted West Nile virus, involving *Culex pipens* and *Culex restuans*, in New York City demonstrated that exotic viruses can be introduced to the United States (Nash et al., 2001). As of January 2020, there have been 51,649 West Nile virus cases throughout the United States. Cases per state have ranged from a total of 1 (Puerto Rico and Hawaii) to 7,015 cases (California).

**Why Study Mosquitoes?**

Mosquitoes make terrific subjects for biology lessons because globally they are often pests to people and animals and are potentially the deadliest animal in the world (Gates, 2014). Mosquitoes are a common nuisance and are capable of transmitting pathogens through blood meals, which can result in sickness and possibly death to the host. This lesson aims to teach students about local mosquito species with the ability to transmit harmful pathogens. Mosquitoes can be collected in the field when immature (eggs, larvae, or pupae) and raised to adults in the classroom. By collecting immature mosquitoes, individuals lower their risk of pathogen transmission. Similar to butterflies, the larvae will grow into pupae and then metamorphose into adults in as few as 10 days. The entire mosquito life cycle can be studied inexpensively using a covered cup of water. Teachers can then discuss vector-borne diseases and the epidemiology of malaria, eastern equine encephalitis, St. Louis encephalitis, La Crosse encephalitis, West Nile, dengue fever, yellow fever, chikungunya, and Zika. Maps of these viruses by the Centers for Disease Control are available at https://wwwn.cdc.gov/arbonet/Maps/ADB_Diseases_Map.

**Figure 1.** Mosquito introductions into the United States over the last 200 years. Each dot represents a species’ first introduction. The black dot represents where two species have the same introduction location in Houston, Texas.

**Figure 2.** Records of *Aedes aegypti* and *Aedes albopictus* findings throughout North America.

**Figure 3.** Records of *Aedes aegypti* and *Aedes albopictus* findings worldwide.
Table 1. Next Generation Science Standards: an overview of the scientific practices, crosscutting concepts, and disciplinary core ideas found in the Invasive Mosquito Project.

| Science & Engineering Practices | Invasive Mosquito Project |
|---------------------------------|---------------------------|
| **Using Mathematics and Computational Thinking** | |
| Create or revise a simulation of a phenomenon, designed device, process, or system (HS-LS4-6). | Build an emergence chamber to rear mosquitoes to adults. |
| Use mathematical and/or computational representations to support explanations of factors that affect carrying capacity of ecosystems at different scales (HS-LS2-1). | Quantify the abundance of mosquitoes capable of transmitting vector-borne diseases in a community and posing a risk to public health. |
| Use mathematical representations to support and revise explanations based on evidence about factors affecting biodiversity and populations in ecosystems of different scales (HS-LS2-2). | Examine population differences from collected larvae/pupae based on differences in location (school/home, near water / drier areas). |
| **Constructing Explanations and Designing Solutions** | |
| Design, evaluate, and refine a solution to a complex real-world problem, based on scientific knowledge, student-generated sources of evidence, prioritized criteria, and tradeoff considerations (HS-LS2-7). | Collect larvae/pupae, identify mosquito species, and determine if they are potential vectors. |
| **Engaging in Argument from Evidence** | |
| Evaluate the claims, evidence, and reasoning behind currently accepted explanations or solutions to determine the merits of arguments (HS-LS2-6). | Test different hypothesis around what environmental conditions are best for larval/pupal rearing. |
| Evaluate the evidence behind currently accepted explanations or solutions to determine the merits of arguments (HS-LS2-8). | Use collection data to understand mosquito control within/around school/home. Identify local mosquito control. Compare students’ mosquito abundance from larval/pupal collection to mosquito control collections. |
| **Disciplinary Core Ideas** | |
| **LS4.C: Adaptation** | |
| Changes in the physical environment, whether naturally occurring or human induced, have contributed to the expansion of some species, the emergence of new distinct species as populations diverge under different conditions, and the decline—and sometimes the extinction—of some species (HS-LS4-6). | Identify larval habitats of invasive species of mosquitoes around homes/schools and how these aid colonization of new environments. |
| **LS2.C: Ecosystem Dynamics, Functioning, and Resilience** | |
| Anthropogenic changes (induced by human activity) in the environment—including habitat destruction, pollution, introduction of invasive species, overexploitation, and climate change—can disrupt an ecosystem and threaten the survival of some species (HS-LS2-8). | Identify new habitat sources where larvae/pupae of invasive species are found that have resulted from human activity, such as discarded trash and bird baths. Understand how this interaction can help colonization of invasive species. |
| **Crosscutting Concepts** | |
| **Scale, Proportion, and Quantity** | |
| The significance of a phenomenon is dependent on the scale, proportion, and quantity at which it occurs (HS-LS2-1). | Use data from mosquito collections to map and show scale of invasive species in the environment. |
| Using the concept of orders of magnitude allows one to understand how a model at one scale relates to a model at another scale (HS-LS2-2). | Compare collection data from different collection sites (home/school, rural/urban areas, backyards/parks, etc.) |
| **Stability and Change** | |
| Much of science deals with constructing explanations of how things change and how they remain stable (HS-LS2-6). | Use collection data from several years to build maps that show how mosquitoes change from year to year. |
This lesson plan includes biological principles in multiple fields of biology (Table 1). Field collection of immature mosquitoes in urban habitats can be used to teach aspects of ecology, biology, and public health. Rearing mosquitoes in the lab/classroom demonstrates complex biological concepts, such as habitat utilization, resource allocation, metamorphosis, and species identification. The purpose of this lesson is not to delve into any one field in detail but rather to allow students to make connections between fields of biology while seeing organisms complete their entire life cycle (hatching from an egg to laying eggs) multiple times in a month.

O The Invasive Mosquito Project: Larval & Pupal Collections Lesson Plan

Caution Statement
In this lesson, students will never be in contact with live adult mosquitoes. Bites from adult mosquitoes may place students at risk. Only larval and pupal collections will be conducted outdoors. Ensure the emergence containers are well sealed to prevent contact with adults that are raised in the classroom.

Project Overview
In this lesson, students will collect larval and pupal mosquitoes from water sources around their schools and homes for mosquito surveillance. Students will raise the immature mosquitoes, watch them grow in sealed containers, and observe larval change into pupae and ultimately metamorphose and emerge as adults. Lectures and information sheets, providing background information on mosquito species and vector-borne diseases, are provided on the IMP website. (The narrative, PowerPoint presentations, fact sheets, and quizzes are provided by the U.S. Department of Agriculture and the CDC at http://www.citizenscience.us/imp/resources.php.) A time allotment of 90–135 minutes is suggested to cover the background information with students (see Table 2). Supplies needed for this project include a small cup of water (in which to place immature mosquitoes), fish food, dipper cup or turkey baster, panty hose, and an empty ice cream container.

Identify Mosquito Habitat
Mosquito eggs may be laid in various locations depending on the species (Figure 5A and Figure 5B). Common larval mosquito habitat around buildings can be any object that holds water for more than 5–7 days. These sources may include discarded cups and trash, bird baths, fountains and ponds, tree holes (rotted holes in trees that hold water), and even bottle caps. Cemeteries (e.g., flower vases in headstones) are another location that typically have immature mosquito habitats. It is important to remember that immature stages (larvae and pupae) are only found in water.

Water can be in many locations, and students are encouraged to think creatively to find it. For example, is a blocked gutter or corrugated drain holding water? What about a broken sprinkler head? The obvious locations are found quickly, but less obvious ones are harder to find. Send us an email and picture at Invasive.Mosquito.Project@gmail.com if you think you have found a new cryptic or hidden habitat.

Larval/Pupal Collection
When collecting larvae, wear long sleeves and long pants, with pants tucked into socks, to prevent being bitten by adult mosquitoes in the field. To collect larvae and pupae from water, use a scoop (e.g., dipper cup) or suction (e.g., turkey baster). Remember, they will see your shadow and dive into the container when they see you coming. Move fast or be creative to get them all out. They will rise to the surface to use their siphons to breathe, so you may have to come back a few times to get them all if you miss them the first time. Place the immature mosquitoes and a pinch of fish food in a small cup with water. If they will be raised to adulthood, emergence containers will need to be constructed either by the teacher or the students.

Constructing the Emergence Container
A simple emergence container can be made from a small plastic cup, an empty ice cream container, and tight-meshed material, such as pantyhose (Figure 4A). Other similarly sized containers will work as well. Be sure there is enough visibility to see the mosquitoes but that the mesh holes are very small so mosquitoes cannot escape. Cut out the top of the ice cream container and use just the rim. Place the small cup with water, food, and mosquitoes (Figure 4B) inside the ice cream container (Figure 4C) and then place the mesh material on top. Use the rim of the ice cream container lid to keep the meshed material in place (Figure 4D). Place a rubber band vertically around the container to secure the lid to the container. The container is then inspected by the teacher for any large holes.

Table 2. Lesson plan procedures.

1. Present background information, including PowerPoint presentations, fact sheets, and quizzes, to the class.

2. Collect supplies needed for larval/pupal collection and possible emergence chamber.

3. Collect immature mosquitoes around homes or local areas. Place collections into a small plastic cup with some fish food. Label cups with date and location.

4. Bring collection cups into classrooms. Record the results in each cup.

5. If rearing mosquitoes to adults, place collection cups into emergence chambers.
   (a) Construct emergence chambers. A separate emergence chamber should be used for each location.
   (b) Review the mosquito safety measures.
   (c) After most adults emerge, place emergence chamber in a freezer for 24 hours to kill off mosquitoes.
   (d) After freezing, allow water to thaw and remove adults.

6. Identify genus of collected larvae and adults and record observations.

7. Record data collection into IMP website.

8. Discuss collections as a class and what the findings mean for local government agencies and vector-borne disease risk.
Rearing Mosquitoes

Place the small cup with water, immature mosquitoes, and a pinch of fish food inside the emergence container. The smaller the larvae, the less food they need. Be careful, too frequent feeding or too much food will contaminate the water and cause bacterial overgrowth, which will kill the larvae. Students can observe as larvae get larger after each molt and eventually become pupae. After the first pupa is observed, stop feeding the mosquitoes and let the other larvae pupate and later emerge. Pupae do not feed. Again, stop feeding after you see pupae, and never open the container after the first pupa is observed! Always double check the container for holes. Any holes that are found can be sealed with tape. If an adult mosquito dies and falls on the water prior to freezing them, don’t worry you can still identify it. After most, if not all, of the adults emerge from the pupae, place the emergence container in a freezer for 24 hours to kill the mature and immature mosquitoes. This ensures that safe observations can be made with microscopes or hand-held lenses. After freezing, thaw the frozen water and remove the adults that are floating on the water. If students choose to keep the adult mosquitoes alive, apple slices can be placed on the top of the emergence container to provide sugar and water to the adults until more emerge.

Safety Precautions

While collecting larvae and pupae outdoors, students are advised to avoid the two hours before and after sunrise and sunset (the crepuscular hours) when mosquitoes are most prevalent. Mosquito larvae and pupae may be raised in a communal container within the classroom to conserve space. Adult mosquitoes must always be frozen for at least 24 hours before being handled so students are not exposed to live adults. Teachers must check the mosquito emergence container regularly for holes.

Figure 4a. Materials for an emergence container.

Figure 4b. Small cup placed inside an ice cream container.

Figure 4c. Small cup with water, food, and various stages of immature mosquitoes.

Figure 4d. Mesh material is placed on top, and the lid rim of the ice cream container is applied to keep the meshed material in place.
**Mosquito Identification**

Teachers have two options for choosing how to proceed on mosquito identification. The first option allows students to identify the larvae and pupae to genus level using the NASA GLOBE Observer application (https://observer.globe.gov/about/get-the-app), which was developed in consultation with the Invasive Mosquito Project. The NASA GLOBE program's Mosquito Habitat Mapper allows students to track their locations and works in coordination with the IMP. The app leads participants through pictorial keys to identify the larvae and pupae to genus level. For the second option, classes raise the immature mosquitoes within sealed containers until adult emergence and then identify the adults. The adult mosquitoes can be identified using an online morphological key provided by the Walter Reed Biosystematics Unit (2018), comparing them to pictures, consulting a local expert, or using phone apps such as the iNaturalist Mosquito Identification app (available on their website, https://www.inaturalist.org/projects/mosquito-identification, and on Google Play and Apple Store). Additionally, species identification can be confirmed by shipping the specimens to the USDA (USDA Center for Grain and Animal Health Research, 1515 College Ave., Manhattan, KS 66502).

Mosquito larvae and pupae are very distinct. The light-colored larvae have small head capsules and distinct siphons (Figure 5C and Figure 5D). Siphons are tube-like structures through which mosquitoes breathe, like a snorkel. Larvae also have a distinct wriggling motion when moving through water. Larvae feed on decomposing organic matter, bacteria, and protozoa. Each larva molts four times, and after completing four larval instars, it becomes a pupa (Figure 5E and Figure 5F). The nonfeeding pupa is also quite distinct, appearing as a small black comma that moves in a tumbling motion when disturbed. The pupa will emerge as an adult mosquito (Figure 5G and Figure 5H). Adult mosquitoes will mate, search for blood meals that contain nutrients required to make eggs, and look for aquatic habitats to then lay eggs.

**Table 3. Life cycle of *Culex* and *Aedes* mosquitoes.**

|                 | **Culex Mosquitoes** | **Aedes Mosquitoes** |
|-----------------|----------------------|----------------------|
| **Eggs**        | Figure 5A            | Figure 5B            |
| **Larvae**      | Figure 5C            | Figure 5D            |
| **Pupae**       | Figure 5E            | Figure 5F            |
| **Adults**      | Figure 5G            | Figure 5H            |
Data Analysis

Data analysis is one of the main foci of this lesson plan. The purpose of larval/pupal collection is to teach students about real-world data collection methods (and numerous other scientific methods). Students will record observations, such as location and number of immature mosquitoes collected, collection technique, and date of collection. For example, ponds typically have a larger number of mosquitoes than artificial containers around a house, as this source of water is larger than a container in a home’s backyard. However, this is not always the case. Larger larvae indicate older instars, suggesting that the water has been present for a longer period of time. If pupae are collected, these will be counted as well, but keep in mind, the pupae are about to metamorphose into adults (1–3 days, depending on the temperature). Students should notice the differences in locomotion between larvae and pupae. After the adults have been frozen and the genera identified (individual species are generally harder to differentiate), a hypothesis can be formed as to why a certain location had more mosquitoes than another. (Factors may include foliage, fauna, nutrients in the water, larval predators, and available standing water).

Classroom Discussion

A particular emphasis of this lesson is for students to become more comfortable sharing their information with others. After making observations about local data, students should discuss in class their ideas as to why some species of mosquitoes are concentrated in certain areas more than others. Active participation provides a good indicator of understanding and interest. The collections and rearing contribute to a national, long-term data collection project by the USDA to determine areas in the United States that are particularly prone to mosquito-borne diseases by finding mosquito population distributions. By encouraging students to discuss the data collected in regard to public and individual safety, they will be more comfortable sharing their results with their families and community. Participating in this project will raise students’ awareness about diseases spread by mosquitoes and their consequences. Additionally, aiding in a national project will help motivate students to be active participants in the scientific community.

Conclusion

The larval and pupal collection builds on the previous egg collection lesson published in The American Biology Teacher in 2016. This lesson plan targets a different time of year (when there may be fewer egg-laying females present) and offers new tools. Again, students are given the opportunity to identify the mosquitoes that pose a public health threat to their community through mosquito monitoring (Cohnstaedt et al., 2016). The data analysis reinforces that students learn about the scientific procedures important for collecting data to help identify the risks posed to human and animal health. With this additional collection plan, students get the opportunity to experience science in their own environments and communities. Through the data collected, students can begin to understand the differences of mosquito species’ composition throughout the United States. Class discussions educate students on the vectors associated with current disease-causing pathogens and how mosquitoes can facilitate these diseases in an area. Once students are aware of the risks within their community, they will be able to play a vital role in protecting the health of not only themselves but also those around them.

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JULIE TSECOURAS is a PhD candidate and WILLIAM WALTON was a professor (deceased) in the Entomology Department at the University of California, Riverside. Roselyn SCHIMMERLIK is an early childhood development educator (retired) at Oregon State University. LEE W. COHNSTAEDT (Lee.Cohnstaedt@ars.usda.gov) is a research epidemiologist in the Agricultural Research Service of the U.S. Department of Agriculture.