INQUIRY & INVESTIGATION

Determining Mosquito Distribution from Egg Data: The Role of the Citizen Scientist

NABT BioClub RECOMMENDATION

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Abstract

Nationwide science classes, from elementary through secondary, are placing a larger emphasis on inquiry and authentic experiences. The opportunity for community members (students, teachers, or interested individuals) to collect real data and contribute to a research project is the definition of citizen science. Recent disease outbreaks of mosquito-transmitted pathogens (West Nile, eastern equine encephalitis, dengue, chikungunya, and Zika) demonstrate the need to educate children and adults about the public health risks posed by mosquitoes. This lesson plan has students determine mosquito species and subsequent disease risk around their house and in their community by collecting mosquito eggs and rearing a portion of them to adults. The students identify adult mosquitoes and associated health risks. Furthermore, students and teachers have the option to participate in a national mosquito-species-distribution study by submitting mosquito eggs and adults to the U.S. Department of Agriculture. The data generated by participant submissions will be available to all mosquito submitters, making each student and school part of a larger project. This lesson plan has three objectives beyond the citizen science experience: (1) clarify the individual's role in protecting individuals, communities, and pets from illness; (2) raise awareness of pathogens transmitted by mosquitoes; and (3) participate in a national program to gather mosquito distribution data. The lesson and the associated supplementary material (available at http://www.citizenscience.us) can be used for middle to high school classes, as well as Advanced Placement classes, because the materials and presentations can be easily modified to classroom needs.

Key Words: Aedes aegypti; Aedes albopictus; *mosquito surveillance; citizen science; epidemiology; invasive species; public health.*

○ Why Study Mosquitoes?

In the early 1900s, mosquito-transmitted malaria was prevalent throughout the southern and central United States. Improved infrastructure, housing, and increased mosquito control efforts interrupted the disease transmission cycle and extirpated the pathogen by 1951 (Centers for Disease Control and Prevention [CDC], 2010) (for definitions of three main terms, see Table 1).

In the early 1900s, mosquito-transmitted malaria was prevalent throughout the southern and central United States.

Although human malaria transmission was eliminated from the United States, other mosquito-transmitted pathogens still circulate, such as the encephalitis viruses (West Nile, eastern equine, western equine, La Crosse, and St. Louis encephalitis viruses; U.S. Geological Survey, 2015) and pose a risk to humans, their animals (mainly horses and birds), and wildlife (Figure 1). Also of major concern are mosquitoes that transmit canine heart worm or filarial worms to animals. Three mosquito-transmitted viruses recently introduced to North America – West Nile, chikungunya, and Zika – have highlighted the importance of mosquito control programs and public health education to detect introduced pathogens and reduce disease transmission. One large knowledge gap this project targets is mapping the ever changing and expanding geographic distribution of mosquito species in the United States.

Mosquito monitoring generally consists of trapping mosquitoes and determining the types of species present (species composition). Only a few mosquito species are able to transmit specific pathogens; therefore, transmission is possible only if a vector-competent mosquito species is present. Using mosquito monitoring, scientists can identify the species composition and determine the risk of disease transmission in an area. The more mosquitoes trapped and counted, the better the chance of detecting a vector-competent species. This offers the perfect opportunity for student participation in a citizen science project.

This lesson is designed to target two invasive, container-breeding mosquito species (*Aedes aegypti* and *Ae. albopictus*) associated with the dengue, chikungunya, and Zika viruses. Dengue virus (DENV) is a major public health problem in tropical and subtropical countries throughout the world. The disease causes fever, severe pain, and internal bleeding (hemorrhaging). Although DENV is generally not endemic in the United States, there has been virus circulation in the Florida Keys, where *Ae. aegypti* is present, and in Hawaii, where *Ae. albopictus* is the most common container mosquito species. Chikungunya virus

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Table 1. Definitions of three basic terms.

Pathogen	Causative agent of disease. May be a virus or parasite.
Disease	Possible results of infection by a pathogen.
Vector	Insect species capable of transmitting a pathogen that may cause disease.

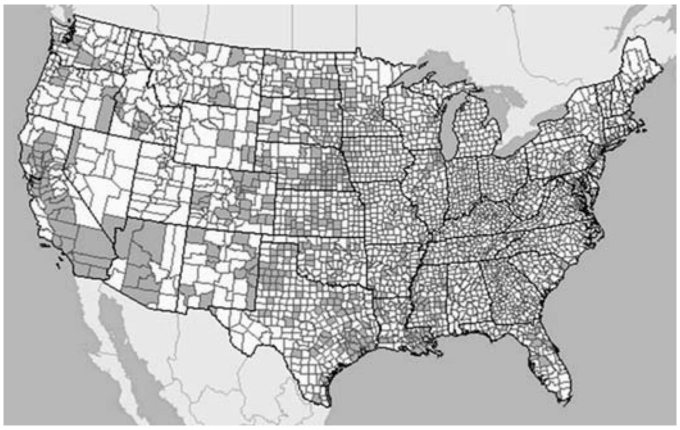

Figure 1. Distribution of positive West Nile cases in 2014. Counties shaded in gray reported at least one case, and counties in white had none (source: http://diseasemaps.usgs.gov/).

(CHIKV) is a virus recently introduced to the Western Hemisphere, and in the summer of 2014 there was transmission in Florida. Furthermore, CHIKV has a high symptomatic frequency, which means that a large percentage of individuals have clinical symptoms if they are infected (CDC, 2015a). Zika virus (ZIKAV) causes mild symptoms (fever and rash) but has been linked to microcephaly in newborns and in 2016 has resulted in several travel warnings to South and Central American countries. The human population at risk of DENV, CHIKV, and ZIKAV transmission in the United States remains unknown because the distribution of the two main mosquito vector species is unknown. The last complete survey of *Ae. aegypti* was by Morlan and Tinker (1965), and a list of reports of *Ae. albopictus* was compiled by Eisen and Moore (2013). More information on their distributions can be found on the CDC website (CDC, 2015b).

Participating in this lesson's mosquito survey will update the distribution data for both species, as well as for other mosquito species that lay their eggs in containers. The lesson emphasizes critical-thinking skills as students use the data collected to assess personal risks from mosquito-borne diseases. Mosquito control and abatement districts funded by local, state, and federal taxes help reduce the risk and burden of these diseases by killing larval and adult mosquitoes. Without these essential services, the number of illnesses would be much higher. However, the best methods to prevent mosquito-transmitted pathogens remain individual actions such as wearing long sleeves, using repellents, and putting screens on windows and doors.

Interpreting data and connecting it to the "real world" is occasionally a hard concept for students to grasp. This inexpensive learning activity introduces students to national datasets and long-term data. The experiment can be run each year to see the year-to-year changes in mosquito abundance and distribution. Furthermore, the lesson covers a variety of branches of biological study and how they all connect in a real-life issue that is pertinent to students' lives (Table 2). Students have individual responsibility for planning and carrying out their own mosquito egg collection, and collaborative work occurs in the classroom during discussions of the collected and supplied data. This lesson allows students to experience obtaining, evaluating, and communicating valuable information as part of a national invasive-mosquito-project study. Students benefit from the fact that this lesson takes place as part of a national program in a number of ways: they have access to the national datasets to compare with their local data; answers to various questions posed by the data collected cannot be found by simply searching on the Internet and must be logically thought out; and the data collected will be visible results of their efforts – an often gratifying reward. Students are not only informed of the global problems in this lesson, but are also educated and participate in a solution to the issue.

In this lesson, students will be exposed to and experience entomological specimen-collection techniques. Practical skills of following protocol and understanding safety concerns are emphasized within the lesson. Safety lessons and mosquito identification can be supported by local or state mosquito control programs, which generally have public education programs integrated into their insect control missions (for a list of local mosquito control agencies, see http://www.mosquito.org). Local biologists can also help with mosquito species identifications. Contributing to national datasets enables students to view and use the datasets, which can be useful for comparing current and previous years' results and constructing explanations for observed change. Quantitative data are collected and are able to be analyzed and explained. With knowledge learned within the lesson, students construct explanations for the causes of different species distributions on a local and national level. Arguably the most important aspect of this activity is providing the students with background information. Investigation without context is meaningless. This lesson can be taught as a lecture or for higher-level classes as a case study/scientific study to learn real-world methods. The materials (narrative, PowerPoint slide, quizzes, etc.) provided in the supplementary material online should be thought of as information sources for both students and teachers. The materials can be used as written or modified into alternative activities based on the classroom level and needs. Alternatively, students can use the material to make presentations to share with the class. One possibility is to provide small work groups with topics that they research and share via whiteboards with the rest of the class. (The teacher can still oversee and guide the work, guaranteeing that the information is covered, but the lesson becomes more interactive.)

○ The Invasive Mosquito Project

Background information will be provided to students in the form of lecture and information sheets on CHIKV and mosquito species (the PowerPoint presentations and fact sheets are provided by the U.S. Department of Agriculture and the CDC; e-mail **Invasive.Mosquito.Project@gmail.com** or download the files at **www.citizenscience.us** under "The Invasive Mosquito Project"). Mosquito specimens and additional supplementary information may be available from local mosquito control and abatement districts. We recommend that 90–135 minutes be allotted for providing background information to students (see Table 3). Using the provided information, students

Table 2. ***Next Generation Science Standards*****: an overview of the scientific practices, crosscutting concepts, and disciplinary core ideas found in this project.**

Science & Engineering Practices	
• Create or revise a simulation of a phenomenon, designed device, process, or system. (HS-LS4-6) • 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) • Evaluate the claims, evidence, and reasoning behind currently accepted explanations or solutions to determine the merits of arguments. (HS-LS2-6) • Evaluate the evidence behind currently accepted explanations or solutions to determine the merits of arguments. (HS-LS2-8)	• Asking questions (for science) and defining problems (for engineering) • Developing and using models • Planning and carrying out investigations • Analyzing and interpreting data • Using mathematics and computational thinking • Constructing explanations (for science) and designing solutions (for engineering) • Engaging in argument from evidence • Obtaining, evaluating, and communicating information
Crosscutting Concepts	
• Cause and Effect: The significance of a phenomenon is dependent on the scale, proportion, and quantity at which it occurs. (HS-LS2-1) • 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) • Stability and Change: Much of science deals with constructing explanations of how things change and how they remain stable. (HS-LS2-6)	• Patterns • Cause and effect: Mechanism and explanation • Scale, proportion, and quantity • Systems and system models • Stability and change
Disciplinary Core Ideas	
• (HS-LS2-8) LS4.C: Adaptation • Changes in the physical environment, whether naturally occurring or human induced, have thus 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)	• HS-LS2-1. Use mathematical and/or computational representations to support explanations of factors that affect carrying capacity of ecosystems at different scales. • HS-LS2-2. Use mathematical representations to support and revise explanations based on evidence about factors affecting biodiversity and populations in ecosystems of different scales. • HS-LS2-6. Evaluate the claims, evidence, and reasoning that the complex interactions in ecosystems maintain relatively consistent numbers and types of organisms in stable conditions, but changing conditions may result in a new ecosystem. • HS-LS2-7. Design, evaluate, and refine a solution for reducing the impacts of human activities on the environment and biodiversity. • HS-LS2-8. Evaluate the evidence for the role of group behavior on individual and species' chances to survive and reproduce. HS-LS2-1. Use mathematical and/or computational representations to support explanations of factors that affect carrying capacity of ecosystems at different scales.

will consider biotic (e.g., presence of plants and predators) and abiotic (e.g., temperature, water level, relative humidity, shade/sun) factors while planning the egg collection. The procedure states where each cup will be placed in relation to sun and shade and suggests other factors that can be recorded by students for use in after-collection discussion.

After the presentation of background information and egg-collection planning, students are asked to place oviposition cups around their homes to collect eggs laid by container-dwelling

Table 3. Basic procedure.

1. Present information to the class.
2. Prepare oviposition cups and place them outdoors.
3. Wait ≥7 days, then collect eggs if present. If no eggs are present, leave cups in place.
4. Analyze collected data.
5. Hatch eggs (optional). Remember the safety measures.

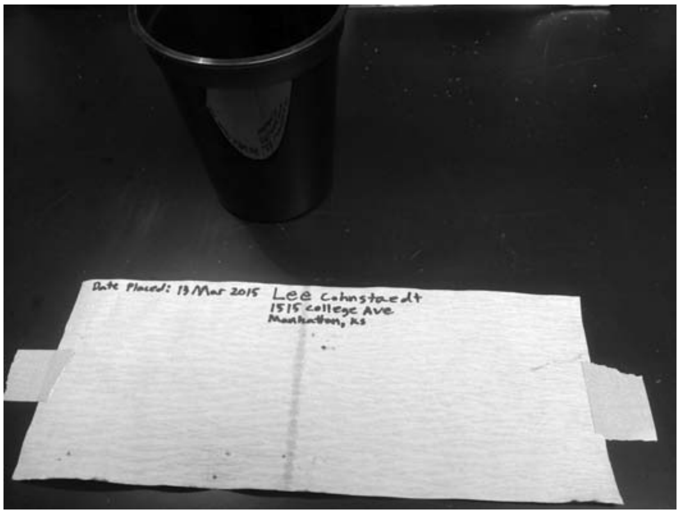

Figure 2. Label the paper lining with collection location, collector's name, and date of placement.

mosquitoes. After obtaining a piece of germination paper (can be substituted with the brown paper towels commonly found in most science classrooms), students should first label the paper with collection location, the collector's name, and date of placement. The oviposition cups are then assembled by students by placing germination paper within a 16 oz. cup and filling the cup with water, submerging about two-thirds of the paper (Figures 2 and 3). Holes should be punched in the cup at the desired waterline to prevent overfilling. Students in the pilot study used rocks or tape to secure the cup in a predetermined location so that wind would not blow it over. The cups must be a dark color, preferably black or dark red. Evaporation is a factor. Monitor the cups daily to ensure that at least two-thirds of the paper remains submerged, because the mosquitoes will lay eggs on the paper, not on the water. Students are advised against going near the placed cups during the day, because that is when the targeted species of mosquitoes (*Ae. aegypti* and *Ae. albopictus*) feed.

After seven days the germination paper and water will be examined for eggs. Examples of eggs are shown in Figures 4 and 5. If eggs are not present, leave the experiment in place another week. In areas with low mosquito abundance and/or during certain times of the year such as late fall and early spring, mosquito abundance and activity may be very low because of temperature. If eggs are present on the paper, collect them by removing the paper and letting it *air dry*. The removed paper can be replaced with a new one, for as long as the teacher and student continue collecting data. After the paper is *completely dry*, place it in a sandwich bag and seal it before bringing it to class. Qualitative and quantitative observations of the collected eggs are made by students and recorded. Data can also be compared with class collections from previous years.

○ Data Analysis

In the classroom, a microscope can be used to make more detailed observations about the eggs. High-power microscopes will show complicated dimples that form the chorion patterns in the eggs, and lower-power magnification will be helpful when counting the eggs. Practical observation skills can be further developed. Count the eggs and record the number along with the location, date of

Figure 3. Oviposition cup with paper lining. Place rocks or weight at the bottom of the cup to prevent it from being knocked over and punch a hole in the cup two-thirds of the way up to allow excess water to drain. Remember that in warm, dry places the water will evaporate quickly and require frequent refilling.

Figure 4. *Aedes* eggs are separate from each other and are laid on the sides of containers.

Figure 5. Mosquitoes in genus *Culex* lay their eggs in rafts on the water surface. Egg rafts should not be dried. If they remain in the water, they will hatch in two or three days.

collection (set date and number of days outside), sun or shade, and biotic factors (e.g., flowers, plants, and trees near the oviposition cups). After the observations are done, cut a quarter of the paper off and place in a container covered with netting and filled with water to hatch. Egg rafts can remain in the water and will hatch (see Figure 5). Remember that these are live animals: within a week the eggs will hatch, and if fed small amounts of fish food within 7–10 days they will become blood-seeking mosquitoes. *Remember to keep the container covered at all times.* If classes want to identify the mosquitoes to species, they can do it with picture keys provided online by Walter Reed Biosystematics Unit (2015). To confirm adult mosquito identification, kill the mosquitoes by placing them in a freezer for at least 24 hours; the dead adults can be carefully packed and shipped to the U.S. Department of Agriculture (USDA) Arthropod-Borne Animal Diseases Research Unit at this address: USDA-ABADRU – Invasive Mosquito Project, 1515 College Ave., Manhattan, KS 66502. The remaining three-fourths of *dried* paper with attached mosquito eggs can be placed in a plastic bag and shipped as well. Alternatively, teachers or students can contact local or regional mosquito control district members and request help with the identifications. A biologist or entomologist at a local university may be able to help as well. Contact information can be found at the American Mosquito Control Association website (http://www.mosquito.org).

Fill in the datasheets in the lesson plan packet. Additional information, datasheets, and procedures can be downloaded at http://www.citizenscience.us. The information submitted to the database should include the school's name, street address, district number, and state; the teacher's name; and collection dates, sun or shade, date the cup was retrieved, number of eggs, and number of adults of each mosquito species collected (the datasheet containing this information can be mailed to invasive.mosquito.project@gmail.com).

For in-class data analysis, some questions are proposed in Table 4 that may be helpful; however, student creativity should be encouraged, and many questions are possible with the data – for example, where to target control measures, and correlations with environmental factors

Table 4. Data analysis questions regarding entomological risk, at various habitat scales.

Scale	Data Analysis Questions
Local level	Are there more eggs in the sun or in the shade cup? Are there more species of mosquitoes in one cup? Which mosquito species prefer which habitats? Does one habitat (disturbed areas, ponds, crops, etc.) near a house place that house at increased risk compared to other houses? If you are looking to remove mosquito habitats (pools of water), where would you look? Could tree holes, tires, plastic containers, and birdbaths be important?
City level	Is the chance of being fed upon by specific mosquito species the same between class members' houses? Does one house have more mosquito eggs or more mosquito species, which indicates more mosquitoes in the area? Are some areas closer to mosquito habitat around the town? Therefore, do certain houses or parts of the city have to be more careful and take extra precautions? Would these be a good place to target municipal mosquito control programs?
State level	Are all the schools in the state similar? How are they different? Does temperature or rainfall make a difference? Is there a difference in timing when the eggs are found throughout the state? Are the mosquito species the same throughout the state? Are certain parts of the state more at risk than others?

(temperature, rainfall, lakes, ponds, and agriculture). Additional risk assessments can be made at various venues throughout the city, which can help with city planning in the event of a disease outbreak.

Data analysis and safety are emphasized and are the main focus of this lesson. Therefore, a study description should be sent home with the students. This will help educate the parents about the purpose of the study as well as about the risks of mosquitoes in their surroundings. The purpose of the egg collection is to educate students and introduce them to various scientific methods, as well as to gather data about mosquito species distributions in the United States. Data analysis starts with the students practicing proper record keeping. Data collected in this project can be easily observed and explained and may span many years if multiple classes conduct the same studies. National data will be consolidated and stored by the USDA from the students and from the participating mosquito control or public health agencies. The data will be compiled and shared with all mosquito contributors. Students and teachers can monitor the website to discuss data and collection methods. To make the class more interactive, students can be required to post comments, ideas, or respond to comments on the website. National datasets allow students to observe data on a national scale and draw conclusions from their data on a continental scale. Ultimately the goal is to relate the mosquito species back to current or possible disease distributions presented in the lectures.

○ Conclusion

This lesson introduces students to mosquito monitoring and the public health risks mosquitoes can pose. The student analysis teaches (1) scientific inquiry and processes, (2) knowledge and application of biological and environmental concepts and principles, and (3) understanding science as an endeavor that addresses human and animal health.

With this project, students are able to participate in field research in a real-world setting. They gain firsthand experience in gathering and recording scientific data and recognizing its importance and implications. Being a part of this nationwide project allows students to gather data on the vectors in their area and contribute to a national study tracking population distributions. Through the data they gather and analyze, students contribute to our knowledge of species distributions in the United States. They also learn about mosquitoes and associated health risks and, hence, to protect themselves, their family, and their pets from mosquito-transmitted pathogens. The data collection and in-class discussions teach students that mosquitoes act as vectors of major disease-causing pathogens and how these diseases are spread. With this information, students can educate others about public and individual health and safety.

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